Impact of insulin resistance on vasculature and kidney

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Key points

- Besides the classical insulin target tissues insulin acts on most human organs/cells including arterial vasculature and kidney
- In insulin-resistant states like obesity or type 2 diabetes not only the classical insulin effects are impaired but also the effects on vasculature and kidney
- Insulin stimulates its own delivery to target cells by PI3 kinase-mediated and NO-stimulated feed-forward actions on the vasculature involving increased capillary recruitment and endothelial transcytosis effects which are all impaired in insulin resistance
- Insulin resistance affects kidney function in many aspects such as alterations in hemodynamics, podocyte and tubular function
- Insulin´s actions on renal sodium handling is preserved in insulin-resistance contributing to sodium retention and arterial hypertension
- Renal and vascular insulin-resistance can be improved by an integrated approach including life-style and/or pharmacological agents

Abstract

Insulin resistance is a systemic disorder affecting many organs and insulin-regulated pathways. It is characterized by a reduced insulin action in spite of increased insulin concentrations (hyperinsulinemia). The vasculature and the kidney are non-classical insulin target organs with distinct functions, partly differing from the insulin effects on classical target organs. Insulin causes vasodilation by enhancing endothelial NO production via activation of the PI3 kinase pathway. In insulin resistant states this pathway is impaired and the MAPK/ERK pathway, with increased endothelin-1 production, overrides the NO action, resulting in vasoconstriction. While the insulin action on perivascular fat tissue and the subsequent effects on the vascular wall are not fully understood, the hepatokine fetuin-A released by the fatty liver, may provoke pro-inflammatory effects of the perivascular fat. The strong association of salt-sensitive arterial hypertension with insulin resistance indicates an involvement of the kidney in the insulin resistance syndrome. The functional relevance of the insulin receptor bearing renal tubular cells and podocytes has been studied in conditional cell-specific insulin receptor knockout models in mice revealing decisive roles of insulin signaling in podocyte viability and tubular function. Renal sodium transport is preserved in insulin resistance and contributes to salt-sensitivity of blood pressure in hyperinsulinemia. Therapeutically, renal and vascular insulin resistance can be improved by an integrated holistic approach aiming at restoring overall insulin sensitivity and improving insulin signaling.

Introduction

The development of valid determination of plasma insulin concentrations in the 1960s revealed disproportionate hyperinsulinemia in apparently healthy obese individuals, thereafter termed insulin resistance. With the discovery of the insulin receptor (IR) in the early 1970s[1] the mechanisms of reduced insulin action could be studied on a molecular basis in both humans and laboratory animals. Insulin resistance has been shown to occur in the classical insulin-responsive organs liver, skeletal muscle and white adipose tissue [2-4], particularly in association with obesity and the metabolic syndrome [5]. This condition is accompanied by impaired glucose tolerance and dyslipidemia with high triglyceride plasma concentrations. Insulin effects other than those affecting metabolism have been described in non-classical insulin-responsive organs such as pancreatic β–cell survival, endothelium-dependent vasodilation in the vasculature and renal sodium transport [6]. Unexpectedly, insulin has been shown to influence brain function to regulate glucose metabolism and food-seeking behavior as reviewed recently [7]. In the present review we describe insulin´s action on the vasculature and the kidney, including molecular mechanism of insulin signal transduction and its impairment in the insulin resistant states, of the otherwise healthy obese individual with normal kidney function. The development of insulin resistance in chronic kidney disease and uremia is covered elsewhere [8].

Dysregulation of insulin signaling in peripheral tissues

Insulin is the central hormone in the control of glucose and lipid metabolism. It mediates its biological effects via binding to IR consisting of two extracellular insulin binding α-subunits and two intracellular tyrosine kinase β subunits. Alternate splicing of the single insulin receptor gene results in the two IR isoforms A and B [9,10]. Insulin may also bind to the highly homologous IGF-1 receptor or insulin/IGF-1 receptor heterodimers albeit with reduced affinity [11]. The insulin-mediated receptor activation leads to activation and autophosphorylation of the receptor tyrosine kinase [12] and initiates different cascades of phosphorylation events. The two major arms of the activated insulin receptor signal

transduction are the insulin receptor substrate (IRS) – PI3-kinase (PI3K) – Akt/PKB pathway and the Grb2 – SOS – Ras – MAPK pathway (Figure 1A). IRS isoforms are intracellular adaptor proteins that are recruited to and phosphorylated on tyrosine residues by the activated insulin receptor initiating the recruitment and activation of PI3K [13,14]. Activated PI3K phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-triphosphate (PIP3), which as a membrane-anchored lipid second messenger activates 3-phosphoinositide-dependent protein kinase 1 (PDK1) and recruits Akt/PKB. The serine/threonine kinase Akt/PKB is a central node in regulating the biological effects of insulin, since it phosphorylates and activates essential downstream kinases (glycogen synthase kinase 3 (GSK3), mTORC1, ribosomal protein S6 kinase), transcriptional regulators such as Forkhead box O family members (FoxO), sterol regulatory element binding protein (SREBP), peroxisome proliferator-activated receptor γ coactivator 1 (PGC1α), and the GTPase-activating protein Akt substrate 160 KDa (AS160) [15]. Subsequently, a network of metabolic regulators is controlled on the level of enzymatic activity or gene expression leading to enhanced glucose uptake, glucose storage, lipid synthesis, protein synthesis and suppression of gluconeogenesis and lipolysis. PDK1 also activates atypical protein kinases C PKCζ and PKCλ/ί contributing to insulin-stimulated glucose uptake and lipid synthesis [16]. The insulin-induced Grb2-SOS-Ras pathway leads to activation of MAPK ERK1/2 and is a major control mechanism of cell proliferation and differentiation. Other non-metabolic effects of insulin mediated via the PI3K/Akt/PKB pathway include inhibition of apoptosis and promotion of cell survival [17].

Insulin signal transduction must be tightly controlled to avoid severe metabolic and proliferative perturbations. The negative regulators are often activated by insulin as feedback mechanism to inhibit the signaling pathway on the critical nodes insulin receptor/IRS or Akt/PKB. Dysregulation of these regulators by chronic hyperactivation contributes to the development of insulin resistance (figure 1B). Negative regulators are phosphotyrosine and phosphoserine/threonine protein phosphatases (PTP1B, PP2A,B,C) [18,19], lipid phosphatases controlling PIP3 levels (PTEN, SHIP) [20,21], and adaptor proteins of the

insulin receptor and IRS (Grb, SOCS) [22,23]. Another well-studied inhibitory mechanism of the insulin signaling pathway is the serine/threonine phosphorylation of the insulin receptor and IRS via the insulin-mediated activation of serine/threonine kinases, predominantly by c-Jun amino-terminal kinase (JNK), IkB kinase (IKK), PKC, S6K1, and ERK (Fig. 1B) [24-28].

In the insulin resistant state, the cellular response to insulin is reduced and the activation of the insulin signaling pathway requires higher concentrations of insulin. Important contributors to insulin resistance are hyperinsulinemia, hyperglycemia, inflammation, excess of lipids, mitochondrial dysfunction, and ER stress [29]. Serine/threonine kinases are activated by the elevated plasma levels of insulin, glucose or free fatty acids, by lipids or intermediates of lipid metabolism (ceramides, diacylglycerol), by increased concentrations of cytokines, ER stress and increased levels of reactive oxygen species. Hyperactivation of protein phosphatases [30], increased expression of adaptor proteins, and enhanced O-N-acetylglucosamine-modification of insulin signaling molecules [31] are also related to the development of insulin resistance. As a consequence, the modified proteins show an impaired interaction with their signaling partners and altered affinities, or enhanced protein degradation.

Regulation of the insulin signaling cascade and its dysregulation leading to insulin resistance are well-described in the classical insulin target tissues skeletal muscle, liver and adipose tissue, which play an important role in the regulation of glucose and lipid metabolism. However, the insulin receptor is ubiquitously expressed and functional insulin signaling is found in other peripheral, non-classical insulin target tissues, including the vasculature [32] and the kidney [33]. Binding of insulin to its receptor is found in the cells of the glomerulum, in podocytes [34], in mesangial cells [35], endothelial and epithelial cells [36], and the tubulus [37]. This serves diverse functions ranging from glucose uptake as energy fuel to regulation of glomerular function, gluconeogenesis and tubular transport. In this review, we describe the metabolic and non-metabolic effects of the insulin signaling cascade in the vasculature and the kidney, as well as causes and consequences of insulin resistance in these tissues.

Organ cross-talk in the insulin-resistant state

Insulin resistance is a hallmark of obesity, metabolic syndrome and type 2 diabetes mellitus. It indicates a condition in which target cells show an attenuated response to insulin which is compensated by increased insulin secretion. Insulin resistance affects many organs and cellular insulin-regulated pathways, however, the extent of insulin resistance can vary considerably. Insulin resistance affects also organ crosstalk by altered metabolic or hormonal signals promoting organ dysfunction and disease (Fig. 2). In the kidney, obesity and insulin-resistance is an important risk factor for a decline of glomerular filtration rate (GFR) as well as the onset and progression of chronic kidney disease (CKD) [38-42]. Particularly visceral obesity, e.g. as estimated by increased waist circumference, was found to be a stronger predictor of end-stage renal disease than the elevated body mass index (BMI) [43]. Increased visceral obesity may cause CKD by promoting metabolic diseases. However, studies indicate that even in the absence of the well-known risk factors hypertension or diabetes, obesity per se may be harmful to the kidney by causing hyperfiltration [44-46]. In this respect it was shown that insulin resistance, which often accompanies visceral obesity, is a strong marker of incident CKD, independent of other risk factors, including age and fasting plasma glucose [47]. Thus, insulin resistance itself and/or factors promoting insulin resistance may early on play a role in the development of CKD [48].

But what are the major organs and pathophysiological mechanisms that are involved in both, the development of insulin resistance and impaired kidney function? Defects in skeletal muscle, white adipose tissue and liver resulting in impairment of insulin signaling have been shown to induce hyperglycemia, predominantly by impairing glucose disposal and increasing hepatic glucose production [49]. Recent evidence suggests that impaired brain insulin signaling [7] and an altered gut microbiome [50] are involved in this process. Because the onset and progression of CKD may also be independent of glycemia, other mechanisms like humoral signals of metabolic tissues may be relevant for the early stages of impaired renal function. In this respect the expansion of visceral adipose tissue, which is being infiltrated by immune cells, is involved in this process [51]. Pro-inflammatory cytokine

signaling not only induces insulin resistance, but also impairs kidney function [52]. Furthermore, the infiltration of expanded visceral adipose tissue by immune cells and the pro-inflammatory cytokine signaling in adipose tissue decreases the secretion of the anti-inflammatory adipokine adiponectin. This protein is considered important for sustaining whole body insulin sensitivity and is thought to have beneficial effects on the kidney [53]. Furthermore, the expansion of adipose tissue results in increased production of the adipokine leptin. This adipokine is thought to induce proteinuria and type IV collagen expression in the kidney by increased production of TGF-ß1 and, thereby, to contribute to the development of glomerulosclerosis [54,55]. In addition, increased visceral obesity may be linked with the onset and progression of CKD via hyperinsulinemia, inappropriate activation of the renin-angiotensin-system and oxidative stress in the kidney. The resulting pathology includes impaired pressure/natriuresis relationship, increased salt sensitivity of blood pressure, aldosterone excess, glomerular hypertension, endothelial dysfunction and vasoconstriction as well as matrix expansion [42,56].

Furthermore, we have recently proposed a novel concept to explain how nonalcoholic fatty liver disease (NAFLD) affects metabolism. When lipids accumulate in the liver, it is not only that hepatic glucose production increases and that dyslipidemia develops, but proteins with signaling properties in other tissues (hepatokines) are also being differently regulated [57-59]. Among these, fetuin-A is the best studied hepatokine. Its expression is increased in NAFLD [60-63] and fetuin-A is well known to inhibit insulin signaling [64]. We could show that fetuin-A induces cytokine expression in monocytes and adipose tissue [65] and we and others found fetuin-A to predict incident diabetes [66,67] as well as cardiovascular disease [68,69]. Recently in animals as well as *in vitro* it was shown that fetuin-A serves as an adaptor protein for saturated fatty acids, allowing them to activate toll-like receptor 4. Thereby, fetuin-A induces inflammatory signaling and insulin resistance [70], which are important factors driving the development of type 2 diabetes and CVD. We could show that these animal data and *in vitro* findings can be translated to humans [71,72].

Fetuin-A may also be involved in the pathogenesis of CKD. In advanced CKD and in end stage renal disease fetuin-A may inhibit ectopic calcification, thus protecting the kidney [73]. However, in early stages of CKD, when ectopic calcification is not yet relevant, the pro-inflammatory effects of fetuin-A may prevail to a large extent. Of note, elevated fetuin-A levels had been found in women with normal glucose tolerance but with albuminuria; this relationship was independent of well-known predictors of albuminuria [74].

Effects of insulin on the vasculature and vascular insulin resistance

Insulin acts on the vasculature and causes endothelium-dependent vasodilation. This was first suggested by hypotensive episodes following s.c. or i.v. administration of insulin [75] although this effect was difficult to distinguish from hypoglycemia-induced counter-regulation. In pioneering experiments in the early 1990's Baron et al. [76] found an insulin-mediated increase of flow into the skeletal muscle of the leg during a hyperinsulinemic-euglycemic clamp. Compared to lean individuals, the dose-response curve of insulin was gradually right-shifted and flatter in insulin-resistant obese humans and type 2 diabetic patients [77,78]. Since both, the dose-response characteristic and the time course for glucose disposal paralleled the insulin-mediated vasodilatory effects, the authors speculated that insulin's effect on the blood flow may contribute to, or even limit, insulin's effects on glucose uptake of the skeletal muscle. Detailed analysis revealed that insulin-mediated vasodilation in skeletal muscle occurs in two phases [79]: After a few minutes through dilation of the terminal arterioles the number of perfused capillaries is increased without changes in blood flow (Fig. 3A). Within 30 minutes insulin induces the relaxation of larger resistance arterioles, thus increasing the overall limb blood flow with a maximum flow after about 2 hours. Thus in vivo the response to insulin is an integration of both capillary recruitment and increased total blood flow and both insulin actions are reduced and retarded in insulin resistant states like obesity and type 2 diabetes [80]. In a recent clinical study insulin applied during a euglycemic-hyperinsulinemic insulin clamp improved endothelial function at each of three

levels of the arterial tree in healthy controls, but not in obese insulin resistant subjects, emphasizing the close link between metabolic and vascular insulin resistance in humans [80]:. Previous studies examining the mechanism behind the hemodynamic effects of insulin showed that application of the NO synthase inhibitor L-N-monomethyl-L-arginine abrogated the insulin-induced vasodilation in skeletal muscle, suggesting a major role for insulin-stimulated NO generation [81]. The early finding that the insulin receptor is expressed on vascular endothelium at all levels of the arterial tree further supported an insulin-stimulated, NO-mediated vasodilation [82]. Independent from endothelium insulin may also cause vasodilation by acting directly on vascular smooth muscle cells (VSMC) [83].

Insulin-mediated endothelial transcytosis

Although insulin promotes its own delivery to the skeletal muscle microvasculature, it still has to cross the tight endothelial barrier to access the interstitium for action on the target cells. Several key experiments in animals showing that the metabolic insulin action closely correlates with the interstitial concentration of insulin, more so than with the plasma levels, suggest that the trans-endothelial transport is the rate-limiting step in insulin availability at target tissues. Previous studies provided evidence for a specific, saturable trans-endothelial insulin transport [84]. A recent profound study showed that in microvascular capillaries insulin is transported through the endothelium via clathrin-dependent entry and vesicle-mediated exocytosis across endothelial cells, thus by-passing the lysosomal degrading pathway (Figure 3B) [85]. This trans-endothelial insulin transport is promoted by insulin-induced endothelial NO production (Fig. 3B), indicating that insulin enhances its own transport [86]. These findings explain why whole-body insulin-induced glucose uptake is maximal after more than one hour, while insulin-induced glucose uptake in isolated adipocytes or skeletal muscle cells occurs within minutes. They also explain the reduced and retarded insulin-stimulated glucose uptake in insulin resistant subjects, where the endothelial insulin effects are impaired (endothelial dysfunction). In summary, insulin stimulates its own delivery to the target tissue via capillary recruitment, increase of

blood flow and, finally its own transcytosis. All of these processes are impaired in insulin resistant states (Fig. 3A,B).

Insulin and endothelial NO signaling

Insulin's hemodynamic effects have been identified in different organs, indicating an ubiquitous NO-mediated vasodilatory action. How does insulin promote endothelial NO production? After binding to endothelial IR, insulin activates PI-3K, PDK1 and Akt/PKB (Fig. 1A) leading to phosphorylation of endothelial NO-Synthase (eNOS) at Ser^{1177}, enhanced eNOS activity and increased NO production [87] (Fig. 4A). As was shown for classical insulin target cells, insulin does not only activate the PI3K pathway, but insulin also stimulates the MAPK/ERK pathway, as outlined in Figure 1A. In endothelial cells activation of this pathway induces the expression and secretion of endothelin 1, stimulating both, vasoconstriction, and VSMC proliferation. In the insulin resistant state, when the PI3K pathway is impaired, and the subsequent NO production is reduced, the MAPK/ERK pathway is not affected, or even enhanced (see below), resulting in an imbalance of both pathways towards vasoconstriction, VSMC proliferation and eventually hypertension [88] (Fig.4B).

Recently the importance of APPL1 (adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1) has been reported for the regulation of the vascular tone in endothelial cells [89]. The scaffold protein APPL1 is a major component in the signal transduction of adiponectin, a known insulin sensitizer in classical insulin target tissues [90]. Transgenic overexpression of APPL1 prevented the age- and obesity-induced impairment of insulin-mediated vasodilation and reversed obesity-induced augmentation of insulin-evoked ET-dependent vasoconstriction. By contrast, deletion of APPL1 caused a selective impairment in the PI3-K/Akt/eNOS pathway and augmented ERK1/2 signaling cascade in vascular endothelium, leading to decreased NO availability and enhanced ET-1 production, respectively. Thus the balance between vasodilatory and vasoconstrictory actions of insulin is shifted towards endothelial dysfunction (Fig.4B). In addition to its function as scaffold protein, APPL1 blocks the binding of the Akt/PKB inhibitor TRB3, thus, supporting the NO production at two intervention

points of the PI3K pathway (Fig. 4A). Activated Akt/PKB phosphorylates Raf-1 at the inhibitory Ser^{259}, thereby, reducing the MAPK /ERK cascade. Hence, adiponectin controls insulin-mediated vasoreactivity via APPL1 and AMPKα2 [91]. Besides AMPK activators, glucagon-like peptide 1 induces PKA-mediated NO production (Fig. 4) which in turn stimulates muscle microvascular recruitment [92]. Accordingly, clinical trials revealed that GLP-1 receptor agonist treatment consistently reduced diastolic and systolic blood pressure up to 5 mm Hg [93].

Lessons from mouse models

To prove the causal involvement of the insulin signal transduction in insulin-mediated vasodilation a vascular endothelial cell IR knockout mouse was generated [94]. Unexpectedly, in these mice the arterial blood pressure was not increased, but tended to be lower. They had normal glucose homeostasis. Since both, eNOS and ET-1 mRNA expression were reduced by approximately 50%, the reduction of both, the pro-vasodilatory and the pro-vasoconstrictive effect may have outweighed each other, resulting in no net effect. Of note, mice lacking eNOS displayed hypertension, insulin-resistance, fasting hyperinsulinemia, hyperlipidemia, and a 40% lower insulin-stimulated glucose uptake than control mice [95].

To further reveal the role of the insulin signaling cascade in regulating the vasculature tone mice with deleted IRS-1 were generated that showed elevated plasma triglyceride levels and an increased blood pressure [96]. A relevant role of IRS-1 for the regulation of human blood pressure was recently demonstrated. Carriers of the IRS-1 Gly972Arg mutation had higher blood pressure and lower plasma nitrate/nitrite levels [97]. Endothelial cells from donors with this mutation had lower eNOS expression/activity indicating a significant role of endothelial IRS-1 in regulation of the vascular tone in humans.

To ultimately answer the question whether insulin-mediated capillary recruitment and its trans-endothelial transport is related, or functions independently, the group of Kadowaki [98] have generated

knock out mice specifically lacking endothelial IRS-2, which is the major IRS isoform in endothelial cells. In these mice insulin signaling was impaired, as demonstrated by a decreased AKT phosphorylation after 60 minutes and a lack of Ser^{1177} phosphorylation of eNOS. Quantitative analysis of the data indicated that about 50% of the glucose uptake by skeletal muscle was mediated by the endothelium via insulin-mediated vasodilation and its own transport, similar to values from human subjects [98]. The observed insulin-mediated vasodilation and insulin delivery in skeletal muscle was not observed in the liver of the endothelial IRS-2 knock out mouse. This difference can be explained by the difference of the capillary structure. While capillaries in skeletal muscle have occluded conjugations in between the non-fenestrated endothelial cells, the sinusoid endothelium of the liver capillaries permits essentially free access of insulin and, thus, direct and faster action on hepatocytes. Therefore, the considerations that are valid for the skeletal muscle and adipose tissue do not apply for organs/organelles in which no tight endothelium is present, e.g. in renal glomeruli with fenestrated endothelium.

Are these insulin effects on the vasculature limited to skeletal muscle? Kadowaki's group addressed this question and investigated whether the insulin effects on vascular endothelium are also present in the pancreatic islets. Using the same mice they could show that the absence of IRS-2 in endothelial cells impairs the islet blood flow, similar to the extent that was seen in skeletal muscle [99]. Since pharmacological stimulation of islet blood flow in these animals almost completely restored insulin secretion, insulin-induced and endothelium-mediated increase in blood flow may regulate insulin distribution, in concert with other classical metabolic and hormonal stimuli. Since IRS-2 is the major IRS isoform expressed in endothelial cells and hyperinsulinemia leads to the down-regulation of IRS-2 in endothelial cells, impaired insulin action may be one of the mechanisms responsible for the decrease and/or the observed delay of insulin secretion in obese and type 2 diabetic subjects.

Impact of vascular insulin-resistance on renal hemodynamics

In rats, insulin induces NO-mediated vaso-relaxation in interlobular arteries, afferent and efferent arterioles [100]. In healthy subjects, insulin increased renal blood flow as analyzed by clearance of para-

aminohippuric acid during a hyperinsulinemic-euglycemic clamp [101]. This effect was abrogated by application of the NO synthase inhibitor L-N-monomethyl-L-arginine indicating insulin-stimulated NO generation in the renal vasculature [101]. It is expected that reduced NO signaling, that is characteristic for the insulin-resistant state, is also present in the renal vasculature. Indeed, it has been shown that renal vessels from the insulin-resistant Zucker rat failed to dilate in response to insulin and acetylcholine indicating endothelial dysfunction [102]. In addition, there was a blunted myogenic vasoconstriction upon pressure, pointing to alterations in VSMC of the media. Endothelial dysfunction of renal vessels would lead to increased reno-vascular resistance and ultimately reduced renal blood flow. In a study with patients ranging from healthy to those with metabolic syndrome and type 2 diabetes, renal resistive index (RI) as index of reno-vascular resistance was gradually increased [103]. In that study, plasma concentration of adiponectin, which is known for its insulin-sensitizing effects, was an independent protective predictor, showing a negative correlation with RI.

Increased reno-vascular resistance due to reduced insulin-stimulated NO production would reduce glomerular filtration rate (GFR). However, in obese patients with metabolic syndrome or overt diabetes GFR is often increased. It is likely that mediators other than insulin account for glomerular hyperfiltration, which is also found in type 1 diabetes mellitus and defines early diabetic nephropathy. Reduced tubulo-glomerular feedback and dilation of afferent arteriole, owing to the increased reabsorption of sodium along with glucose, is thought to be one of the principal mechanism for glomerular hyperfiltration [104].

Role of perivascular and renal sinus fat disposition

As schematically outlined in Figure 5 arteries of different size (except the cerebral vasculature), including small arterioles, are coated by adipose tissue. For long time it was thought that arteries were embedded in this perivascular fat tissue (PVAT) to protect and physically support the blood vessels. Recent studies have identified PVAT as an endocrine compartment releasing adipokines and other factors with vasodilatory activity. These factors may directly act on the vessel wall, because particularly in smaller arteries and microvessels there is no anatomical barrier between adventitia and PVAT [105,106]. PVAT is not uniformly distributed along a specific vascular bed and PVAT differs in function and phenotype within the regions of a given blood vessel and also between the different PVAT compartments e.g. epicardial, periaortal, peritibial and renal sinus PVAT [107]. Furthermore, PVAT differs from other fat compartments like subcutaneous and visceral fat [108]. PVAT is not an innocent bystander, since these fat cells express and secrete both, pro-inflammatory cytokines like IL-6, IL-8, TNF-α and monocyte chemoattractant protein-1 (MCP-1) and the anti-inflammatory and insulin-sensitizing adiponectin and the regenerative factor hepatocyte growth factor (HGF). Extensive ex vivo studies with human PVAT showed that fetuin-A, a hepatokine released by the fatty liver, triggers expression and secretion of pro-inflammatory cytokines [105,109]. Possibly functionally very important, but not finally characterized, is the adipocyte-derived relaxing factor (ADRF) showing anti-contractile effects on the vascular wall. This factor exerts its relaxing effects on vascular smooth muscle cells also in the absence of endothelium, indicating an independent regulation of the vascular tone by PVAT. Several reports attempting to identify the chemical nature of ADRF found evidence for low molecular weight compounds like H_2S and the palmitate methyl ester as candidates [110].

Microvascular dysfunctions have been demonstrated to occur very early, even before clinical manifestation of vascular lesions. Therefore, Yudkin *et al.* have proposed that PVAT may be the cause of microvascular dysfunctions [111,112]. A significant association between endothelial dysfunction and insulin resistance in young healthy first degree relatives of patients with type 2 diabetes was found,

independent of the classic cardiovascular risk factors [113]. The quantity of PVAT surrounding human brachial artery is inversely associated with insulin sensitivity, but is not correlated with local endothelial dysfunction, as determined by flow-mediated dilation [114].

Only few studies have investigated the role of perirenal sinus adipose tissue for metabolic health and specifically for renal function [115]. In a subcohort of the Framingham Heart Study PVAT quantity around the renal sinus was associated with hypertension and negative outcome for the patients [116]. Furthermore, the amount of fat within this compartment predicted the outcome of diabetic renal disease. An independent study found that para- and perirenal fat thickness was an independent predictor of chronic kidney disease, increased renal resistance index and hyperuricemia in patients with type 2 diabetes[117]. Renal sinus fat mass is associated with an increased albumin excretion rate during exercise in healthy individuals who were at increased risk of type 2 diabetes [118]. In insulin resistant individuals with fatty liver, the GFR was found to correlate inversely with increased renal sinus fat [105].

Cellular dysregulation of the insulin signaling cascade in the kidney

Besides its putative actions on renal vasculature, insulin can act on virtually all other renal cell types such as mesangial cells, podocytes and tubular epithelial cells. Both isoforms of the insulin receptors are widely expressed in the kidney [33]. Unique to the kidney, insulin can access these target cells both from the lumen (in the case of podocytes) and from the basolateral side (in the case of tubular cells) as shown in a study with radiolabelled insulin [119]. Studies published during the last years have focused on insulin signaling in these particular cells and revealed a variety of effects, ranging from glucose uptake, regulation of ion transport, prevention of apoptosis etc.

Glomerular endothelial and mesangial cells

Glomerular endothelial cells express the IR and respond to insulin binding with increased NO generation in analogy the extrarenal vasculature [33]. Because of its fenestration, the glomerular endothelium is, however, freely permeable to insulin, in that it can cross the basement membrane and enter bowman´s space and tubulus. Therefore, insulin-mediated endothelial NO signaling is not a limiting factor for the transport of insulin to sub-endothelial cells, such as the VSMC (Fig. 3B), suggesting that changes in insulin plasma concentrations readily affect renal mesangial cells and podocytes. Insulin-mediated NO generation could in vivo contribute to the increase in GFR after a meal [120]. NO may also be an important player of the cross-talk between the podocytes and mesangial cells, that are adjacent to each other, separated only by the glomerular basement membrane. It has been shown that in glomerular endothelial cells insulin signaling also does not stimulate glucose uptake or remodeling of the actin cytoskeleton[33].

In mesangial cells, insulin is a potent survival factor that confers protection from apoptotic stimuli via stimulation of the PI3K/Akt pathway (Fig. 1A) [121]. Increased MAPK signaling by insulin has been shown to stimulate the large conductance Ca^{2+}-activated K^+ channels (BK_{Ca} channels), resulting in mesangial cell relaxation, and possibly increased proliferation [122]. Impaired insulin signaling in mesangial cells may be associated with reduced GFR, as was shown in a cross-sectional study that included 670 individuals and investigated the effects of the Gly(972)Arg variant of the human insulin receptor substrate 1 (IRS-1) gene [123]. Mesangial cells transfected with this variant exhibited attenuated insulin-stimulated phosphorylation of IRS-1 and Akt. Silencing of the IR in mesangial cells resulted in formation of homodimeric IGF1-receptors and increased IGF1 signaling [124], that may lead to cell growth and proliferation. In mesangial cells from rats with STZ-induced diabetes, insulin reversed the mitogenic action of IGF-1 by regulating STAT5/SOCS2 expression [125]. When mesangial cells were kept in insulin deficient media, Kong *et al.* noted increased IGF1 signaling, resulting in increased cell proliferation and enhanced synthesis of fibronectin and collagen IV [126]. Furthermore, mesangial cells overexpressing GLUT 1 produced fibronectin and collagen IV in the absence of insulin, indicating that elevated glucose

utilization induces mesangial matrix production [127]. Altogether, these studies suggest that reduced insulin signaling in mesangial cells could contribute to mesangial cell hypertrophy, proliferation and matrix deposition, characteristic for early diabetic nephropathy.

The essential role of insulin in both endothelial and mesangial cells *in vivo* remains to be elucidated as cell-specific knockout models targeting the IR in these cells are lacking. They have proven to be an important tool in defining the role of insulin signaling, particularly in non-classical insulin target organs.

Podocytes

Podocytes are insulin-sensitive cells and respond to insulin stimulation with an increase in PI3K and MAPK signaling, resulting in glucose uptake via GLUT4 similar to skeletal muscle cells [34]. Besides energy uptake, this seems to serve adapative changes in the podocytes in response to increased insulin secretion and hyperfiltration after a meal [128]. The insulin effect is dependent on expression of the transmembrane protein nephrin, which is an essential constituent of the slit diaphragm and is involved in the regulation of the actin cytoskeleton of the podocytes [128]. Nephrin allows the insertion of GLUT1- and GLUT4-containing vesicles into the plasma membrane by interacting with the vesicular SNARE protein VAMP2 (Vesicle-associated membrane protein 2). As nephrin expression is reduced with progression of diabetic nephropathy and albuminuria, insulin signaling is expected to be reduced and, thus, to negatively affect the filtration barrier. Insulin´s action on podocytes also includes up-regulation of the ion channels TRPC (transient receptor potential cation channel, subfamily C) and BK_{Ca} channels [129,130]. In podocytes isolated from insulin-resistant obese db/db mice insulin failed to phosphorylate PKB and this was associated with reduced viability of the cells [131]. The strongest evidence for the *in vivo* relevance of insulin on podocyte function comes from mouse models with a deletion of the IR in the podocyte [132]. Starting at 8 weeks of age, these knockout mice developed albuminuria caused by foot process loss and severe podocyte damage. After 13 weeks of age, light microscopy revealed podocyte loss, glomerulosclerosis and mesangial expansion, features characteristic of human diabetic nephropathy. As a mechanism, the authors found severe impairment of the remodeling of the actin

cytoskeleton, leading to loss of podocyte architecture and morphology. All these changes occurred in the absence of hyperglycemia, underscoring the role of normal insulin signaling and glucose uptake for podocyte health. Another mechanism could be related to dysfunction of the endoplasmatic reticulum (ER) that is induced by the absence of insulin signallng [133]. This has been found to impair PI3K-dependent translocation of transcription factor spliced X-box binding protein-1 (sXBP1) into the nucleus resulting in maladaptive ER-stress signalling.

Tubular epithelial cells

Long before the advent of the current cell biologic approach to investigate insulin signaling in the kidney, insulin effects on renal tubular function have been studied in animals since the 1970s, using microperfusion (ex vivo) or micropuncture (in vivo) methods. The results clearly showed a stimulatory effect of acutely administered insulin on tubular sodium reabsorption in the proximal tubule and Henle´s loop of rabbits and dogs [134-136]. In addition, acutely administered i.v. insulin stimulated reabsorption of phosphate, potassium and water [136,137]. In contrast, chronic infusion of insulin over five days in rats reduced sodium excretion only transiently on the first day, an effect that disappeared thereafter, but was associated with an increase in blood pressure [138]. Extensive studies in dogs under different conditions such as insulin resistance, chronic norepinephrine infusion, reduced kidney mass, high-salt intake or chronic angiotensin II infusion could not demonstrate that chronic insulin administration causes sustained sodium retention or hypertension [139]. Much of the controversy whether insulin was anti-natriuretic or not was solved when it became clear that hyperglycemia was required to induce a sodium-retaining effect of hyperinsulinemia, indicating that the euglycemic, hyperinsulinemic clamp was not the appropriate model to study the pathogenesis of hypertension in hyperglycemic diabetic patients [139]. By inducing sustained hyperglycemia and hyperinsulinemia at the same time, formerly resistant dogs developed sodium retention during chronic insulin administration that was sufficient to antagonize the natriuresis caused by hyperglycemia [140].

Distal tubule

In cells expressing the epithelial sodium channel (ENaC), insulin was found to stimulate Na^+ transport via up-regulation of ENaC membrane abundance, which was dependent on PI3K activity [141]. Increased PI3K signaling by insulin leads to activation of mTORC2 and the serum- and glucocorticoid kinase 1 (SGK1), which are essential serine-threonine kinases involved in the regulation of ENaC in the distal tubule (Fig. 6). SGK1 is heavily up-regulated by aldosterone on a transcriptional and activity level via K-Ras2a-activation of PI3K and PDK1. SGK1 increases ENaC membrane expression by inhibiting the ubiquitin ligase Nedd4-2 (neuronal precursor cells expressed developmentally down-regulated) mediated internalization and degradation[142]. Elevated glucose concentration also increases SGK1 gene expression and this may be one of the critical factors underlying the stimulation of sodium retention in type 2 diabetes [143]. Increased SGK1 expression by hyperglycemia would also explain the development of sodium retention upon chronic insulin infusion in the above mentioned study in dogs [140].

Surprisingly, mice with deletion of the IR in the distal tubule, driven by the Ksp (Kidney specific) cadherin promoter, displayed hypertension and impaired natriuresis, following a sodium load [144]. As an explanation, the authors suggested that reduced renal NO signaling, by absent insulin signaling, could account for this finding. In a subsequent study, the authors confirmed that reduced insulin signaling in those mice conferred salt sensitivity of blood pressure by reducing renal NOS expression [145]. In another mouse model with a deletion of the IR in the collecting duct using an aquaporin-2 promoter, the same group found that ENaC activity was reduced and associated with reduced blood pressure [146]. The stimulating effect of insulin on ENaC currents was confirmed in isolated split-open tubules, an effect that was blunted in mice with IR deletion and abrogated by inhibition of PI3K and mTOR [147]. Altogether, the previous and also current data confirm that insulin is per se an anti-natriuretic hormone in the distal tubule.

Proximal tubule

Insulin signaling in the proximal tubule might affect renal gluconeogenesis that accounts for up to 25% of the glucose released into the circulation [148]. Reduced insulin signaling would lead to disinhibition of gluconeogenesis that would in turn contribute to hyperglycemia. In mice with a deletion of the insulin receptor in the proximal tubule, driven by the glutamyltransferase promoter, fasting glucose was indeed elevated and the activity of glucose-6-phosphatase, the enzyme catalyzing the final step of gluconeogenesis and responsible for release of glucose into the circulation, was increased in renal cortex homogenates [149]. These data confirmed the findings of an earlier study in insulin-resistant diabetic Zucker rats, which had disinhibited renal gluconeogenesis due to increased mRNA expression and activity of rate-limiting gluconeogenic enzymes [150]. Another key function of the proximal tubule is the complete reabsorption of the filtered glucose load (160-180 g per day) that is accomplished by two sodium-coupled glucose transporters (SGLT1 and 2) with SGLT2 contributing to 97% of the renal glucose reabsorption [104]. SGLT2-mediated transport is up-regulated by insulin and involves phosphorylation of SGLT2 via IR signaling [151]. Up-regulation of SGLT2 activity by insulin may serve to minimize glucose losses after a meal. The renal expression of SGLT2 in animal models of diabetes does not follow a clear pattern and is dependent on the model [152]. However, it has been established for long time that glucose reabsorption is increased in patients with type 2 diabetes [153], arguing against an insulin-resistance of SGLT2 up-regulation in type 2 diabetes.

Disturbances of insulin-regulated renal functions in humans

Relevance of IR deletion for understanding of human insulin resistance

Mouse models involving the deletion of IR are thought to represent the most extreme form of insulin resistance. However, the validity of the results with regards to understanding insulin resistance in humans is not always clear. First, even in conditions of severe insulin resistance in humans a complete disruption of insulin receptor signaling does not occur. Secondly, the models are often equivocal and do not fully agree with the complex situation in humans; some findings are even unexpected and

conflicting. Mice with a constitutive deletion of the IR are massively hyperglycemic and die of diabetic ketoacidosis within few days after birth, despite developing hyperinsulinemia [154,155]. In contrast, humans with genetic deficiency of the IR exhibit fasting hypoglycemia, retarded growth and develop ketoacidosis only after a meal [156]. Mice with specific deletion of the IR in the skeletal muscle, a classical insulin target tissue that is thought to be insulin resistant from the early stages of insulin resistance in humans, were normoglycemic and not hyperinsulinemic [157]. However, they had increased fat cell mass and obesity, suggesting redirection of energy and storage of fat similar to the human situation. Deletion of the hepatic IR resulted in severe fasting hyperglycemia, disinhibition of gluconeogenesis and glucose intolerance [158]. These changes resemble the human situation as the fatty liver is of central importance to insulin resistance [62].

Accordingly, data from mice with deletion of the IR in various renal cell types have to be interpreted with great caution. Contrary to the expectation that reduced insulin signaling may cause natriuresis, the first published mouse model using an IR deletion under the Ksp promoter showed reduced sodium excretion [144]. Subsequent studies with a more defined model involving the deletion of ENaC in the distal tubule fitted better with the role of insulin in promoting sodium retention [146,147]. Unlike in humans with diabetic nephropathy, the deleterious effect of IR deletion in podocytes has been shown to occur under normal glucose and insulin concentrations in otherwise healthy and insulin-sensitive mice. In addition, these mice did not have other typical features of diabetic nephropathy, such as enlarged kidneys, mesangial hypercellularity or Kimmelstiel–Wilson lesions, despite severe podocytopathy [132]. In conclusion, genetic IR knock out models only highlight single distinct aspects of human insulin resistance, either within an organ, or in the whole organism, and represent only a small piece of the complex mosaic underlying systemic insulin resistance.

Insulin resistance of the kidney

Despite the compelling evidence that the kidney is an insulin responsive organ with insulin regulating different functions, it is not clear whether the kidney in general or in parts is affected by insulin,

resistance similar to the classical target organs such as liver, skeletal muscle or white adipose tissue. The human kidney abundantly expresses the IR isoform B which is the isoform found in the classically insulin-responsive organs [10] and glucose uptake is insulin-dependent in some (such as podocytes), but not all cells (tubulus, mesangial cells). Studying rats with insulinopenic and insulin-resistant diabetes identified that insulin signaling, as represented by Akt phosphorylation, was reduced only in isolated glomeruli, while tubuli were not affected [159]. In glomeruli, the phosphorylation of other downstream effectors of insulin, such as eNOS and GSK 3α, were similarly reduced. However, there was no change of MAPK phosphorylation, indicating resistance only of the IRS-1/PI-3K driven arm of insulin signaling (Fig. 1A). The authors found reduced protein expression of IRS-1 that could be restored by inhibition of PKCß. Altogether, this important study has shown that within the kidney glomeruli, but not tubuli, can develop insulin resistance. However, the study could not elaborate whether this is confined to glomerular endothelial cells only, or also involves podocytes and mesangial cells. The study also did not address if insulin sensitivity was retained equally in the whole tubulus or only in parts.

Tubular sodium transport

If the tubulus epithelium is not affected by insulin resistance, then hyperinsulinemia will lead to a stimulation of renal sodium transport and promote sodium retention and salt-sensitive hypertension, features that are frequently encountered in obese patients with the metabolic syndrome. Indeed, insulin infusion during a euglycemic, hyperininsulinemic clamp induced a similar reduction of sodium excretion in obese adolescents [160] or in patients with type 2 diabetes, compared to control subjects, while the effect on peripheral glucose uptake was reduced in insulin-resistant obese and diabetic patients only [161]. The site of salt retention during hyperinsulinemia is the distal tubule and involves stimulation of ENaC by PI3K/SGK1 (Fig. 6). However, it can also involve the proximal tubule, where the stimulatory effect of insulin on sodium transport has been shown to be preserved in type 2 diabetic rat models and in patients [162,163]. This was associated with preserved expression of IRS-2 and Akt phosphorylation, whereas expression of IRS-1 was reduced in the renal cortex. Transcription factors

such as forkhead box class O1 (FoxO1) and sterol regulatory element-binding protein 1 (SREBP 1), that are involved in the down-regulation of IRS-2 in the fatty liver, were not changed, indicating subtle differences in altered insulin signaling during insulin-resistance between liver and the kidney.

It must be emphasized that in addition to hyperinsulinemia, hyperglycemia stimulates sodium transport in the proximal and distal nephron by transcriptional up-regulation of SGK1 which is then activated by insulin- and PI3K-dependent phosphorylations at consecutively two sites [142,143,164]. Altogether, both hyperinsulinemia and hyperglycemia converge to enhance sodium transport in the distal tubule and promote net sodium retention that is able to override osmosis-driven diuresis by hyperglycemia in the proximal tubule [140].

Renal gluconeogenesis

Besides periportal hepatocytes, only proximal tubular cells are capable of gluconeogenesis, which accounts for up to 25% of the glucose released into the circulation, and is suppressed by insulin [165]. In patients with type 2 diabetes both, renal and hepatic gluconeogenesis, was found to be increased, thus, contributing to hyperglycemia during the fasting state (IFG) [166]. This finding implies that the proximal tubule is resistant to the suppressive action of insulin on gluconeogenesis, as it is the case in the liver. This is in agreement with animal models of insulin-resistant rats and mice with specific deletion of the IR in the proximal tubule, showing disinhibition of gluconeogenesis [149,150]. In contrast, tubular sodium transport seems to be spared from insulin-resistance that can only explained by differences in intracellular signaling such as alterations of IRS-1 and IRS-2 expression [163] and/or involvement of SGK1, regulating a variety of sodium channels. Whereas reduced IRS-1 expression may be responsible for disinhibition of gluconeogenesis [162] (and also reduced NO production [159]), preserved IRS-2 expression (independent of reduced IRS-1) seems to maintain stimulation of sodium transport in the proximal tubule. This evidence is supported by a study in IRS-1 and IRS-2 knockout mice showing attenuated stimulation of sodium transport exclusively in mice with deletion of IRS-2 [167].

Possible therapeutic approaches to overcome renal and vascular insulin resistance

Effects of weight loss

The strong association of obesity and insulin resistance suggests that lifestyle changes, which result in weight loss and increased muscle mass, restore whole-body insulin sensitivity. Indeed, lifestyle intervention in individuals with both, impaired and also normal glucose tolerance, improved insulin sensitivity, as estimated from the relation of glucose and insulin plasma concentration, and was associated with a reduction in total-, visceral- and ectopic fat mass [168]. MR studies showed that visceral adipose tissue and hepatic lipids can be significantly reduced during lifestyle intervention with their baseline values being predictive factors for an improvement of insulin sensitivity [169,170]. Similar to the improvement of whole body insulin sensitivity, renal and vascular insulin resistance should similarly improve with lifestyle intervention. However, studies focusing on the insulin sensitivity of renal and vascular function are rare and can barely discriminate between systemic and organ-specific effects. The available lifestyle intervention studies with renal endpoints showed reduction of glomerular hyperfiltration and albuminuria, suggesting normalization of afferent vasodilatation, filtration pressure and possibly podocyte dysfunction [171]. They are also thought to improve salt sensitivity and to reduce blood pressure [172], suggesting altered tubular sodium handling. With regard to vascular insulin resistance, lifestyle intervention improved macro- and microcirculation, as indicated by increased flow-mediated vasodilation in the brachial artery and insulin-induced vasodilation in cutaneous vessels [173,174]. However, weight loss after lifestyle intervention is not uniformly associated with improved insulin-resistance and there is a substantially high proportion of patients not responding to lifestyle intervention that might be related to persistence of visceral obesity and, more importantly, liver steatosis [170,175]. Compared to lifestyle intervention, bariatric surgery mostly results in a large loss of body weight, and hence, more effectively improves insulin sensitivity, but this treatment can only be considered in patients with morbid obesity [176].

Pharmacological treatment

Metformin and PPARγ agonists. Pharmacologically, insulin-resistance can be improved with metformin, and with thiazolidindiones (TZD) the latter of which activate the peroxisome proliferator activated receptor gamma (PPARγ). Whereas metformin is increasingly being recommended in the very early stages of insulin resistance and pre-diabetes, treatment with thiazolidindiones, such as pioglitazone, is indicated in patients with overt diabetes [177]. They lower blood glucose concentration predominantly by stimulation of lipogenesis in the white adipose, while reducing hyperinsulinemia. This has brought up their attribute to be insulin-sensitizers. Whereas metformin has predominantly effects on the liver, pioglitazone acts on both, the vasculature and the kidney, by a direct effect on various cell types such as endothelium, VSMC, podocytes or tubular cells. Pioglitazone treatment improves endothelial function and lowers blood pressure, most probably by a direct vasodilating effect [178]. In the kidney, several studies found a significant reduction of albuminuria, which according to a metaanalysis, amounted to 25 to 65%, depending on spot urine samples or collected urine samples [179]. In the randomized controlled DREAM trial involving 5269 patients with prediabetes (IFG and IGT), rosiglitazone treatment reduced renal outcomes, predominantly by reducing albuminuria (12.3 vs. 15.0 %, HR 0.82 [0.69–0.98]) [180]. Reduction of progression of renal function, as defined by GFR loss ≥30 %, was reduced with borderline significance (3.1 vs 4.0%, HR 0.77 [0.58–1.04]). However, rosiglitazone increased risk for developing heart failure (0.53 vs. 0.08%; HR 7.04 [1.60 –31.0]), which is the most severe adverse effect of TZD treatment, that has limited its wide-spread use. This complication is caused by stimulation of sodium retention by the kidney, involving activation of ENaC and up-regulation of SGK1 [181].

A clinical important effect of TZDs is their ability to improve liver steatosis and dysregulated hepatokine production, not only via actions on adipose tissue, but also via direct effects on the liver. In this respect pioglitazone was found to suppress mRNA and protein expression of fetuin-A in Fao hepatoma cells. Interestingly, rosiglitazone, but not metformin, also inhibited fetuin-A expression. In addition, GW

9662, an inhibitor of peroxisome proliferator-activated receptor (PPAR) gamma, reversed pioglitazone-induced suppression of fetuin-A [182]. These data suggest that thiazolidinedione derivatives may have common characteristics with regard to fetuin-A suppression, possibly through PPAR gamma activation. In agreement with these studies pioglitazone, but not metformin or exercise, decreased fetuin-A levels during a 6 month intervention, although there were similar effects in the improvement of insulin sensitivity between the three groups [183].

Insulin. One important pathophysiological factor which causes insulin resistance is the elevated plasma glucose itself. The association of increased blood glucose levels with insulin resistance is called glucotoxicity, or more accurately glucolipotoxicity [184,185]. As a result of chronic hyperglycemia, insulin signaling is inhibited, which can be seen also in type 1 diabetes [186]. Glucolipotoxicity is not only associated with insulin resistance but also with beta cell dysfunction. Therefore, one therapeutic concept of treating hyperglycemia in type 2 diabetes is to reverse glucotoxicity by insulin treatment, resulting in recovery of residual β-cell function and improvement of insulin resistance. The ORIGIN trial has examined early insulin treatment with a long-acting insulin in patients with dysglycemia including pre-diabetes and type 2 diabetes in a randomised controlled intervention study [187]. It has been shown that diabetes can be prevented with early insulin treatment [188]. In addition, early insulin therapy significantly reduces the incidence of eye and kidney disease in patients with a baseline HbA1c >6.4% [189].

SGLT2 inhibitors. SGLT2 inhibitors might be a promising new pharmacological approach to overcome insulin-resistance. These new drugs inhibit proximal tubular glucose reabsorption in an insulin-independent manner and induce glucose loss that amounts to 50 – 150 g [190] in diabetic patients, depending on the level of hyperglycemia. Although SGLT2 inhibitors lack a systemic effect, recent studies in humans and animals suggest that this new class of antidiabetic drugs improves insulin sensitivity. In a small study with 12 patients with type 2 diabetes, treatment with the SGLT2 inhibitor dapagliflozin improved peripheral glucose uptake, as analyzed with a euglycemic, hyperinsulinemic

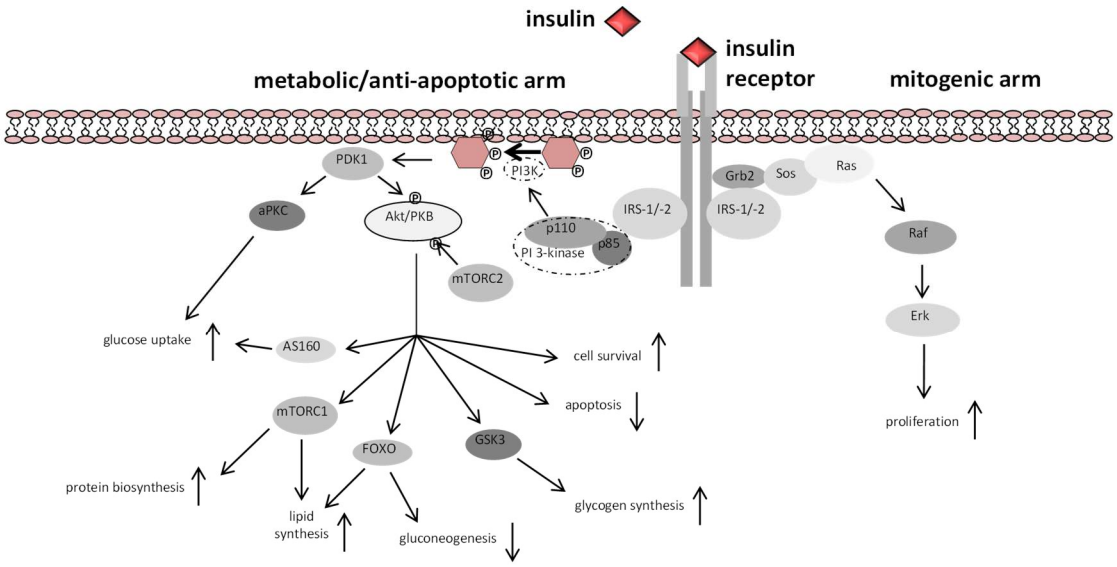
clamp, while it also reduced plasma insulin concentrations [191]. The effects of dapagliflozin occured rapidly, within two weeks of treatment, well before induction of significant weight loss occurred. The authors of that study explain these findings with the immediate correction of hyperglycemia by SGLT2 inhibition, leading to regression of glucotoxicity. In a larger study including 66 patients with type 2 diabetes the effects of SGLT2 inhibition on insulin secretion and peripheral glucose uptake were detectable even after a single dose of the SGLT2 inhibitor empagliflozin, which is best explained by beneficial effects of immediately lowering plasma glucose concentration and, thereby, reducing glucotoxicity [190]. However, the contribution of the insulin sensitizing effects to the overall beneficial effect of SGLT2 inhibitors needs to be examined and confirmed in large outcome trials, taking into account measures of insulin sensitivity such as the HOMA (homeostasis model assessment of insulin resistance), or others. In addition to possibly improving insulin sensitivity, SGLT2 inhibitors have shown beneficial effects on cardiovascular outcomes [192] and, most recently, on renal end points, by hitherto poorly-defined mechanisms. Altogether, SGLT2 inhibitors emerge as a new therapeutic approach to treat patients with insulin resistance and manifest type 2 diabetes.

Conclusion

Renal and vascular insulin resistance develops as part of the complex mosaic of systemic insulin resistance and affects organ-specific functions and cross-talk, alike. Dysregulation of insulin-regulated pathways in the kidney and vasculature culminate and sustain pathophysiological alterations found in metabolic syndrome, such reduced endothelial function, increased sodium retention and renal gluconeogenesis. These changes occur early in the course of the development of type 2 diabetes and significantly determine subsequent renal and vascular damage. To prevent micro- and macro-angiopathic diseases, intervention at the insulin resistant state is required to halt progression, or even reverse pathophysiological alterations. Identification of molecular mechanisms promoting renal and vascular insulin resistance is warranted, to better understand the organ cross-talk involving classical and non-classical insulin-responsive organs.

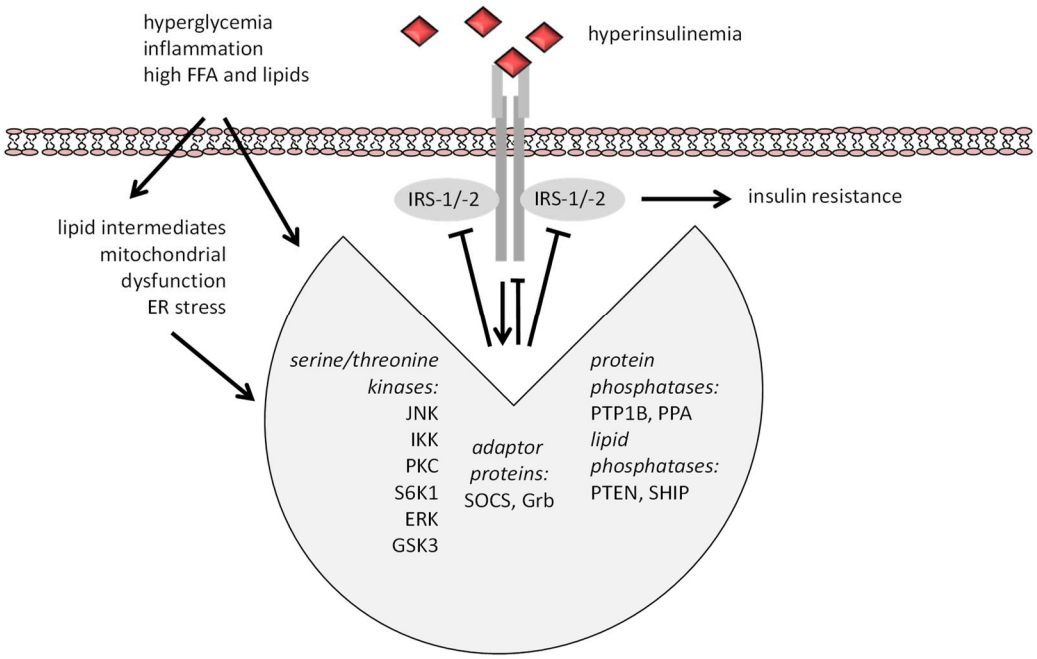
Figures with legends

Figure 1A



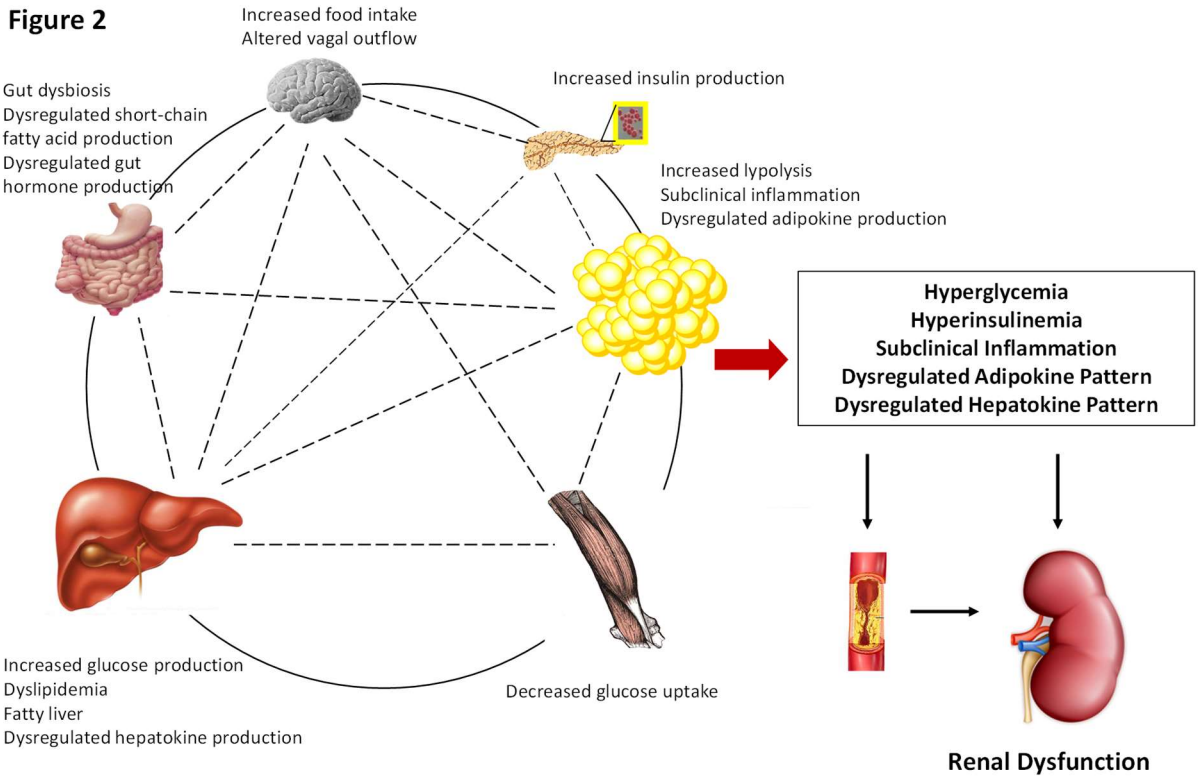
Scheme of the insulin signaling pathway. Binding and activation of the insulin receptor leads to recruitment of IRS isoforms and subsequently to activation of the PI3-kinase-Akt/PKB pathway (left arm) and regulates glucose and lipid metabolism, protein biosynthesis, cell survival and apoptosis. Activation of the Ras/Erk pathway increases proliferation (right arm).

Figure 1B



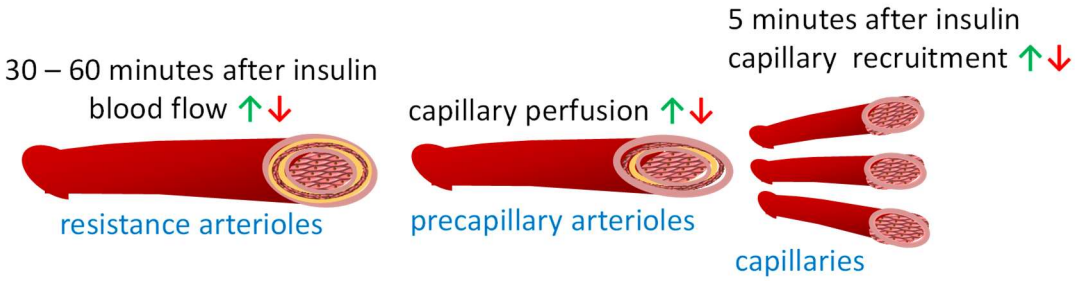
Negative regulation of the insulin signaling pathway. Hyperglycemia, hyperinsulinemia, high plasma free fatty acids (FFA) and inflammation activate via several mechanisms serine/threonine kinases, adaptor proteins and phosphatases resulting in a chronically reduced cellular response to insulin

Figure 2



Organ cross-talk in the insulin-resistant state. Insulin exerts its metabolic actions classically on liver, fat and skeletal muscle. Non-classical insulin actions include the brain, gut, pancreas, vasculature and the kidney. The physiological organ cross-talk is impaired in the insulin resistant state

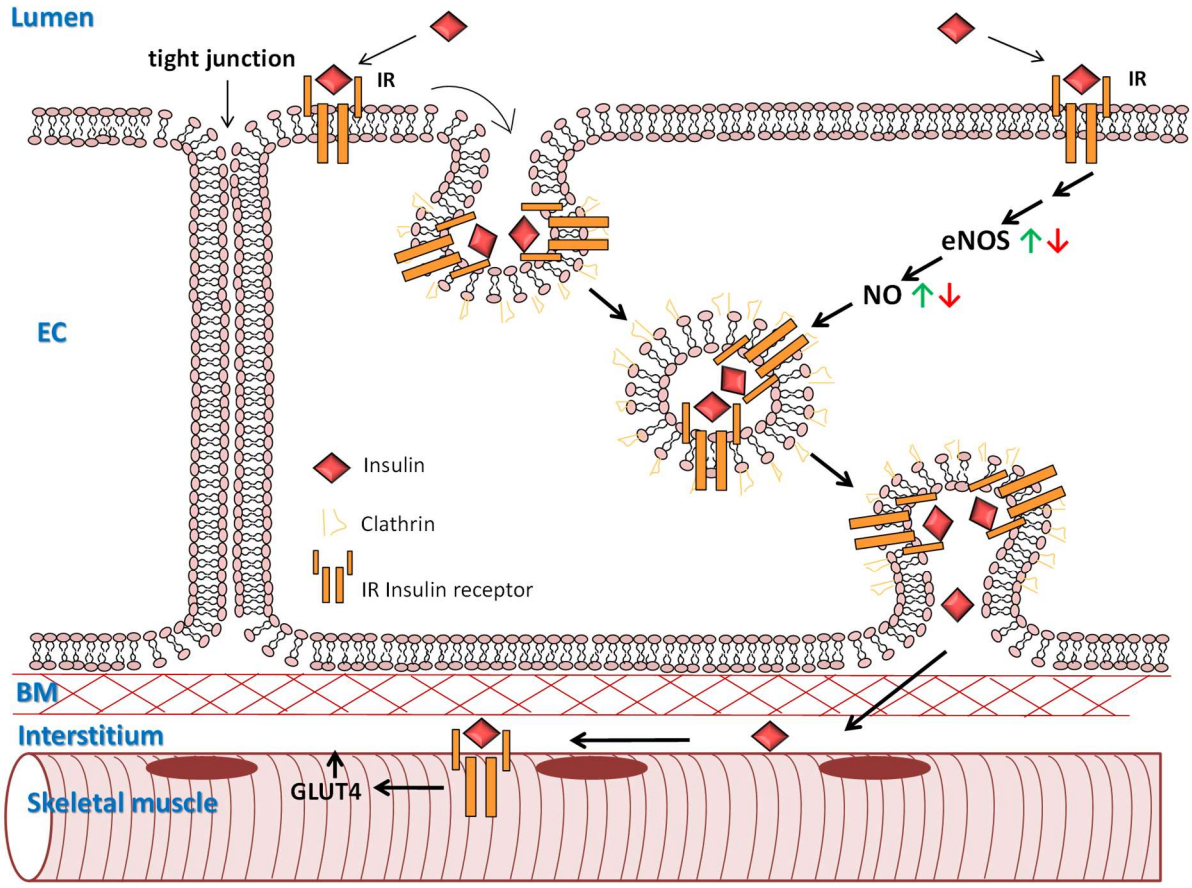
Figure 3A



Physiologic and impaired insulin effects on the arterial vasculature

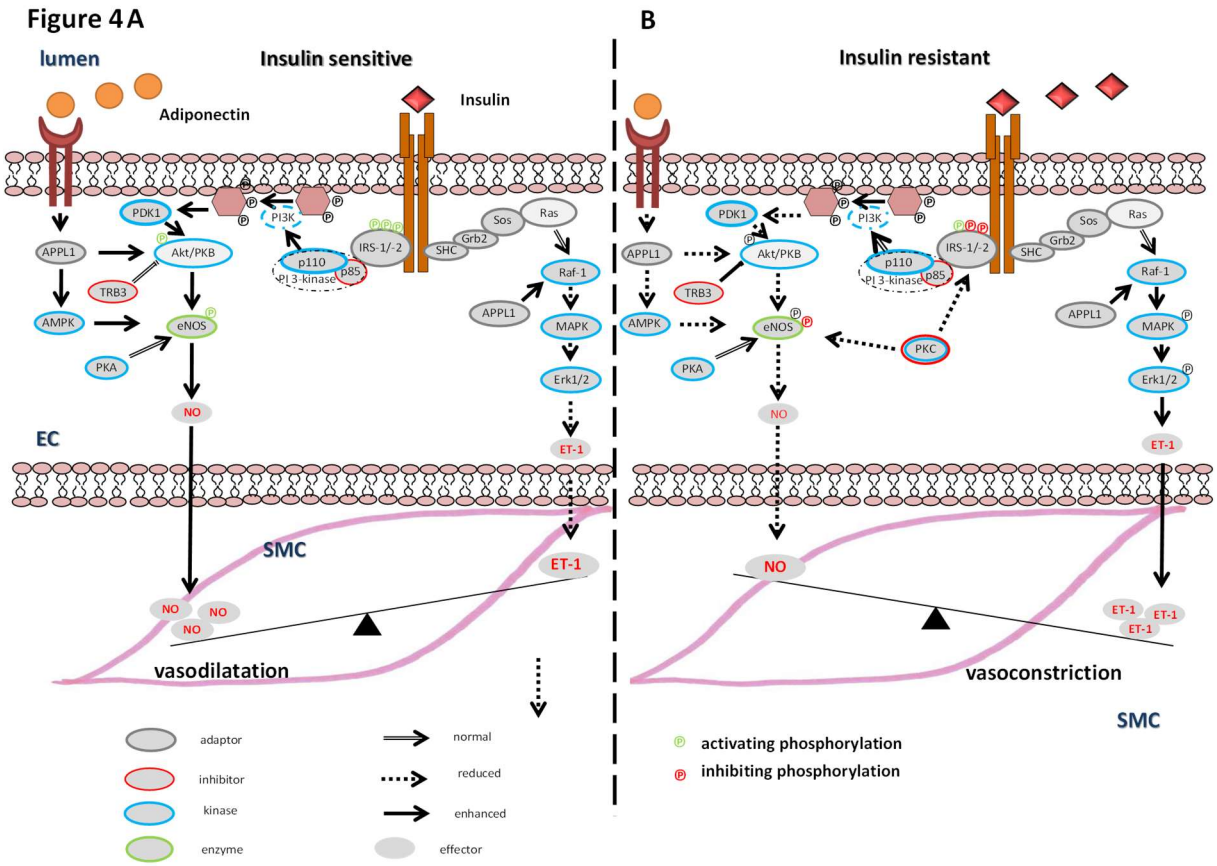
Insulin acts at all three levels of the vascular tree. In the skeletal muscle, after a few minutes, insulin induces capillary recruitment by vasodilation of the precapillary arterioles, followed by relaxation of the larger resistance arterioles, leading to an enhanced blood flow (green arrows). Accordingly, insulin improves endothelial function in conduit arteries. These actions of insulin are reduced in obese subjects and patients with type 2 diabetes(red arrows).

Figure 3B



Insulin stimulates its own trans-endothelial transport by clathrin-dependent NO-mediated endo- and transcytosis. In skeletal muscle and in adipose tissue the non-fenestrated microvascular endothelial monolayer forms a tight barrier, restricting free access of plasma constituents to the subendothelial interstitium and, if not activated, preventing the interaction of blood cells with the endothelium. For delivery to subendothelial target cells insulin binds to its receptor (IR) on the endothelial plasma

membrane and the resulting complex is clathrin-dependently engulfed, transported through the endothelial cell and released by vesicle-mediated exocytosis. After passage through the basement membrane (BM) insulin stimulates glucose-uptake at the target cells. Transcytosis of insulin is stimulated by NO which in turn is generated by insulin-stimulated activation of eNOS (green arrows). All these insulin actions are impaired in insulin resistant subjects (red arrows).



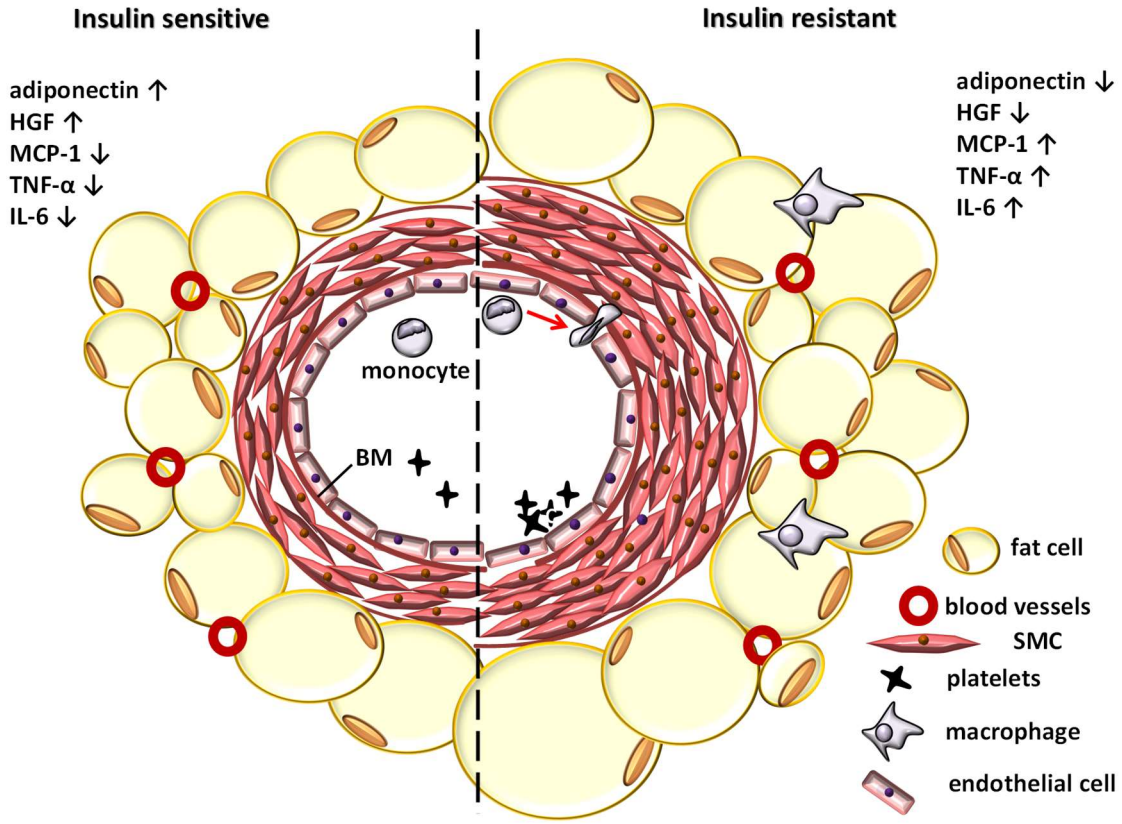
Physiologic and impaired insulin signal transduction in endothelial cells
Insulin plays an essential role in maintaining vascular function by regulating both endothelial NO (left arm) and endothelin-1 (ET-1) production (right arm) acting on vascular smooth muscle cells (VSMC) causing vasodilation and vasoconstriction, respectively. Insulin resistance induces endothelial dysfunction

A Binding of insulin to its cognate receptor stimulates the PI3K-Akt axis of the cascade via IRS-1/2 as shown in detail in Figure 1A. Activated Akt phosphorylates Ser^{1177} of eNOS for NO-dependent vasodilation. Ser^{1177} may also be phosphorylated by activated AMP-kinase (AMPK) or protein kinase A (PKA). The insulin sensitizing hormone adiponectin supports insulin action by activating AMPK via the adaptor protein phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1). APPL1 competes with the Akt inhibitor TRB3 both actions further stimulate eNOS activity and subsequent NO production. Since insulin-mediated stimulation of the MAPK/ERK axis is reduced by APPL1-meditated inhibition of Raf-1 ET-1 production and action is overbalanced by NO resulting in vasodilation. (enhanced actions = green arrow; reduced actions = red arrow), MAPK = mitogen-activated protein kinase; ERK = extracellular signal-regulated kinase

B In insulin resistant state post-receptor interferences reduce insulin signal transduction. Various metabolic and inflammatory factors inhibit the action of IRS1/2 through serine phosphorylation (Fig. 1B) leading to reduced activation of the PI3 kinase-Akt axis and NO production. Furthermore, since adiponectin levels are reduced in insulin resistant and obese subjects phosphorylation of APPL1 Ser^{401}

and subsequently its function is reduced further shifting the insulin signal from the PI3 kinase-Akt axis to the MAPK/ERK axis which is independent of IRS1/2. Since the inhibitory effect of APPL1 on RAF-1 is reduced the MAPK/ERK axis is even enhanced. Furthermore, activated PKC in insulin resistant individuals (see Fig. 1B) phosphorylated the inhibitory Ser^{495} of eNOS further reducing NO production. Together the reduced NO production and increased ET-1 causes vasoconstriction of the small artery. Furthermore since the expression of adhesion molecules is under the control of the MAPK/ERK axis overexpression of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin is stimulated.

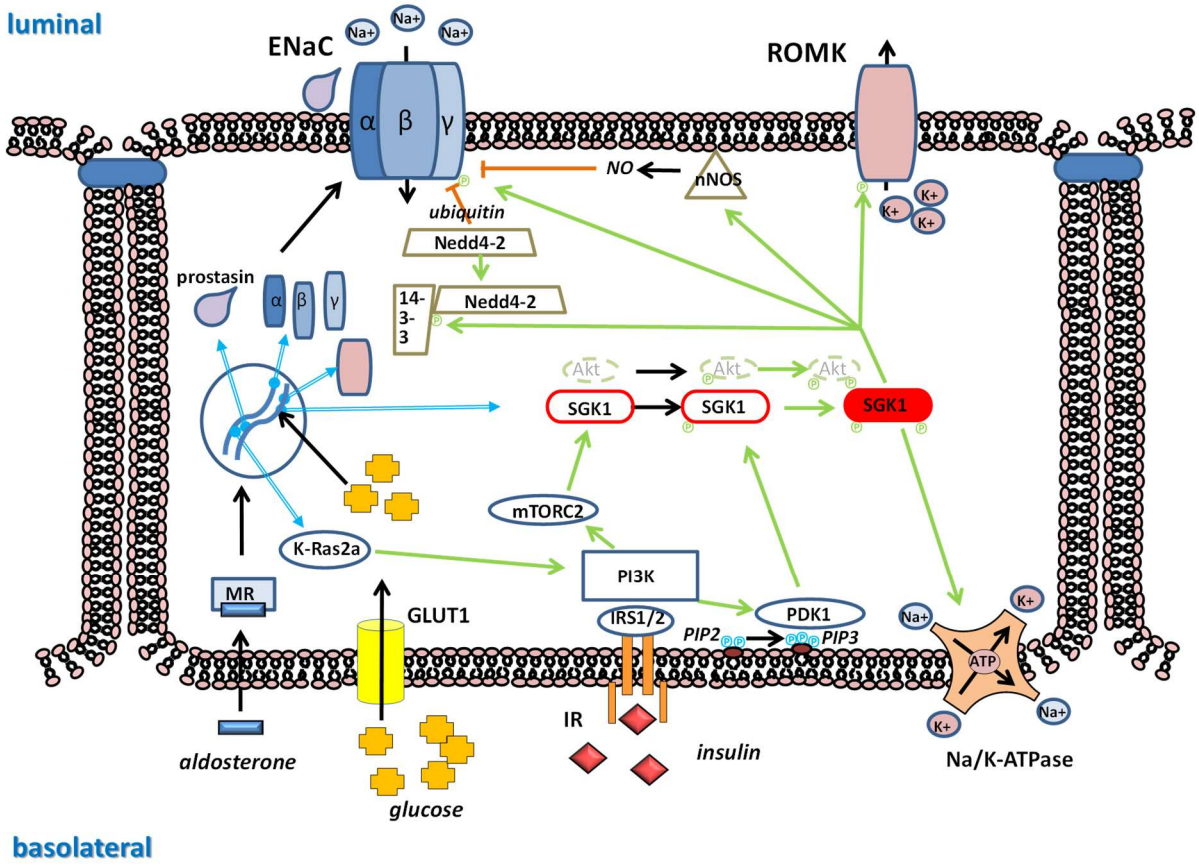
Figure 5



Perivascular adipose tissue influences vascular function

Large and smaller arteries are surrounded by adipose tissue. In healthy insulin sensitive individuals adiponectin levels are increased and the perivascular fat cells secrete vasodilating factors e.g. ADRF which are still ill-defined. In the insulin resistant state perivascular fat cells increase in size and in number and secrete more proinflammatory factors like IL-6 and TNF-α together with monocyte chemoattractive factors (MCP-1) and less regenerative factors like hepatocyte growth factor (HGF) particularly under the influence of fatty liver derived fetuin-A. Furthermore these proinflammatory factors increase endothelial permeability and enhance insulin resistance by inhibition of the IRS-PI3K-Akt- axis of insulin signal transduction (see Fig. 1). Factors like MCP-1 foster the attraction and infiltration of monocyte/macrophages into the vascular wall and the surrounding adipose tissue and in turn may further increase the proinflammatory state (vicious cycle). The activated endothelium expresses adhesion factors thus attracting leukocytes and activing platelets.

Figure 6



Insulin signaling in the principal cell of the aldosterone-sensitive distal nephron.

Serum-and Glucocorticoid-Inducible Kinase 1 (SGK1) is the central effector of both insulin and aldosterone. SGK1 expression is rapidly induced on a transcriptional level by aldosterone/MR and also by hyperglycemia. It is activated after sequential phosphorylation at Ser^{422} by mTORC2 [164] and Thr^{256} by PDK1. Active SGK1 increases ENaC membrane abundance and currents via direct and indirect effects, among them inhibition of the ubiquitin ligase Nedd4-2. It also stimulates renal outer medullary potassium channel (ROMK) and the Na/K-ATPase. Akt1 is of minor importance as effector of insulin, although it is similarly phosphorylated and has the same downstream targets. (MR=mineralocorticoid receptor)..

References

1 Cuatrecasas, P. The insulin receptor. *Diabetes* **21**, 396-402 (1972).

2 Olefsky, J. M. Insulin binding, biologic activity, and metabolism of biosynthetic human insulin. *Diabetes care* **4**, 244-247 (1981).

3 Kahn, C. R., Neville, D. M., Jr. & Roth, J. Insulin-receptor interaction in the obese-hyperglycemic mouse. A model of insulin resistance. *The Journal of biological chemistry* **248**, 244-250 (1973).

4 Groop, L. C. *et al.* Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. *The Journal of clinical investigation* **84**, 205-213, doi:10.1172/jci114142 (1989).

5 Prager, R., Wallace, P. & Olefsky, J. M. In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. *The Journal of clinical investigation* **78**, 472-481, doi:10.1172/jci112599 (1986).

6 Rask-Madsen, C. & Kahn, C. R. Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. *Arteriosclerosis, thrombosis, and vascular biology* **32**, 2052-2059, doi:10.1161/atvbaha.111.241919 (2012).

7 Heni, M., Kullmann, S., Preissl, H., Fritsche, A. & Haring, H. U. Impaired insulin action in the human brain: causes and metabolic consequences. *Nature reviews. Endocrinology* **11**, 701-711, doi:10.1038/nrendo.2015.173 (2015).

8 Koppe, L. *et al.* Insulin resistance in chronic kidney disease: new lessons from experimental models. *Nephrology Dialysis Transplantation* **29**, 1666-1674, doi:10.1093/ndt/gft435 (2014).

9 Kellerer, M. *et al.* Distinct alpha-subunit structures of human insulin receptor A and B variants determine differences in tyrosine kinase activities. *Biochemistry* **31**, 4588-4596 (1992).

10 Seino, S. & Bell, G. I. Alternative splicing of human insulin receptor messenger RNA. *Biochemical and biophysical research communications* **159**, 312-316 (1989).

11 Belfiore, A., Frasca, F., Pandini, G., Sciacca, L. & Vigneri, R. Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocrine reviews* **30**, 586-623, doi:10.1210/er.2008-0047 (2009).

12 Kasuga, M., Karlsson, F. A. & Kahn, C. R. Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. *Science (New York, N.Y.)* **215**, 185-187 (1982).

13 Backer, J. M. *et al.* Phosphatidylinositol 3'-kinase is activated by association with IRS-1 during insulin stimulation. *The EMBO journal* **11**, 3469-3479 (1992).

14 Sun, X. J. *et al.* Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* **352**, 73-77, doi:10.1038/352073a0 (1991).

15 Taniguchi, C. M., Emanuelli, B. & Kahn, C. R. Critical nodes in signalling pathways: insights into insulin action. *Nature reviews. Molecular cell biology* **7**, 85-96, doi:10.1038/nrm1837 (2006).

16 Farese, R. V., Sajan, M. P. & Standaert, M. L. Atypical protein kinase C in insulin action and insulin resistance. *Biochemical Society transactions* **33**, 350-353, doi:10.1042/bst0330350 (2005).

17 Manning, B. D. & Cantley, L. C. AKT/PKB signaling: navigating downstream. *Cell* **129**, 1261-1274, doi:10.1016/j.cell.2007.06.009 (2007).

18 Brady, M. J. & Saltiel, A. R. The role of protein phosphatase-1 in insulin action. *Recent progress in hormone research* **56**, 157-173 (2001).

19 Elchebly, M. *et al.* Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science (New York, N.Y.)* **283**, 1544-1548 (1999).

20 Lazar, D. F. & Saltiel, A. R. Lipid phosphatases as drug discovery targets for type 2 diabetes. *Nature reviews. Drug discovery* **5**, 333-342, doi:10.1038/nrd2007 (2006).
21 Vinciguerra, M. & Foti, M. PTEN and SHIP2 phosphoinositide phosphatases as negative regulators of insulin signalling. *Archives of physiology and biochemistry* **112**, 89-104, doi:10.1080/13813450600711359 (2006).
22 Emanuelli, B. *et al.* SOCS-3 is an insulin-induced negative regulator of insulin signaling. *The Journal of biological chemistry* **275**, 15985-15991 (2000).
23 Holt, L. J. & Siddle, K. Grb10 and Grb14: enigmatic regulators of insulin action--and more? *The Biochemical journal* **388**, 393-406, doi:10.1042/bj20050216 (2005).
24 Copps, K. D. & White, M. F. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia* **55**, 2565-2582, doi:10.1007/s00125-012-2644-8 (2012).
25 Fritsche, L. *et al.* Insulin-induced serine phosphorylation of IRS-2 via ERK1/2 and mTOR: studies on the function of Ser675 and Ser907. *American journal of physiology. Endocrinology and metabolism* **300**, E824-836, doi:10.1152/ajpendo.00409.2010 (2011).
26 Neukamm, S. S. *et al.* Phosphorylation of serine 1137/1138 of mouse insulin receptor substrate (IRS) 2 regulates cAMP-dependent binding to 14-3-3 proteins and IRS2 protein degradation. *The Journal of biological chemistry* **288**, 16403-16415, doi:10.1074/jbc.M113.474593 (2013).
27 Weigert, C. *et al.* Interplay and effects of temporal changes in the phosphorylation state of serine-302, -307, and -318 of insulin receptor substrate-1 on insulin action in skeletal muscle cells. *Molecular endocrinology (Baltimore, Md.)* **22**, 2729-2740, doi:10.1210/me.2008-0102 (2008).
28 Weigert, C. *et al.* The phosphorylation of Ser318 of insulin receptor substrate 1 is not per se inhibitory in skeletal muscle cells but is necessary to trigger the attenuation of the insulin-stimulated signal. *The Journal of biological chemistry* **280**, 37393-37399, doi:10.1074/jbc.M506134200 (2005).
29 Boucher, J., Kleinridders, A. & Kahn, C. R. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harbor perspectives in biology* **6**, doi:10.1101/cshperspect.a009191 (2014).
30 Kowluru, A. & Matti, A. Hyperactivation of protein phosphatase 2A in models of glucolipotoxicity and diabetes: potential mechanisms and functional consequences. *Biochemical pharmacology* **84**, 591-597, doi:10.1016/j.bcp.2012.05.003 (2012).
31 Vaidyanathan, K. & Wells, L. Multiple tissue-specific roles for the O-GlcNAc post-translational modification in the induction of and complications arising from type II diabetes. *The Journal of biological chemistry* **289**, 34466-34471, doi:10.1074/jbc.R114.591560 (2014).
32 Potenza, M. A., Addabbo, F. & Montagnani, M. Vascular actions of insulin with implications for endothelial dysfunction. *American journal of physiology. Endocrinology and metabolism* **297**, E568-577, doi:10.1152/ajpendo.00297.2009 (2009).
33 Hale, L. J. & Coward, R. J. The insulin receptor and the kidney. *Current opinion in nephrology and hypertension* **22**, 100-106, doi:10.1097/MNH.0b013e32835abb52 (2013).
34 Coward, R. J. *et al.* The human glomerular podocyte is a novel target for insulin action. *Diabetes* **54**, 3095-3102 (2005).
35 Conti, F. G. *et al.* Studies on binding and mitogenic effect of insulin and insulin-like growth factor I in glomerular mesangial cells. *Endocrinology* **122**, 2788-2795, doi:10.1210/endo-122-6-2788 (1988).
36 Conti, F. G., Elliot, S. J., Striker, L. J. & Striker, G. E. Binding of insulin-like growth factor-I by glomerular endothelial and epithelial cells: further evidence for IGF-I action in the renal glomerulus. *Biochemical and biophysical research communications* **163**, 952-958 (1989).

37 Nakamura, R., Emmanouel, D. S. & Katz, A. I. Insulin binding sites in various segments of the rabbit nephron. *The Journal of clinical investigation* **72**, 388-392 (1983).
38 Ejerblad, E. *et al.* Obesity and risk for chronic renal failure. *Journal of the American Society of Nephrology : JASN* **17**, 1695-1702, doi:10.1681/asn.2005060638 (2006).
39 Fox, C. S. *et al.* Predictors of new-onset kidney disease in a community-based population. *Jama* **291**, 844-850, doi:10.1001/jama.291.7.844 (2004).
40 Kanasaki, K., Kitada, M., Kanasaki, M. & Koya, D. The biological consequence of obesity on the kidney. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **28 Suppl 4**, iv1-7, doi:10.1093/ndt/gft098 (2013).
41 Pinto-Sietsma, S. J. *et al.* A central body fat distribution is related to renal function impairment, even in lean subjects. *American journal of kidney diseases : the official journal of the National Kidney Foundation* **41**, 733-741 (2003).
42 Ritz, E. Metabolic syndrome and kidney disease. *Blood purification* **26**, 59-62, doi:10.1159/000110566 (2008).
43 Kramer, H. *et al.* Waist Circumference, Body Mass Index, and ESRD in the REGARDS (Reasons for Geographic and Racial Differences in Stroke) Study. *American journal of kidney diseases : the official journal of the National Kidney Foundation* **67**, 62-69, doi:10.1053/j.ajkd.2015.05.023 (2016).
44 Chandie Shaw, P. K. *et al.* Central obesity is an independent risk factor for albuminuria in nondiabetic South Asian subjects. *Diabetes care* **30**, 1840-1844, doi:10.2337/dc07-0028 (2007).
45 Cirillo, M. *et al.* Microalbuminuria in nondiabetic adults: relation of blood pressure, body mass index, plasma cholesterol levels, and smoking: The Gubbio Population Study. *Archives of internal medicine* **158**, 1933-1939 (1998).
46 Tozawa, M. *et al.* Influence of smoking and obesity on the development of proteinuria. *Kidney international* **62**, 956-962, doi:10.1046/j.1523-1755.2002.00506.x (2002).
47 Nerpin, E. *et al.* Insulin sensitivity measured with euglycemic clamp is independently associated with glomerular filtration rate in a community-based cohort. *Diabetes care* **31**, 1550-1555, doi:10.2337/dc08-0369 (2008).
48 De Cosmo, S., Menzaghi, C., Prudente, S. & Trischitta, V. Role of insulin resistance in kidney dysfunction: insights into the mechanism and epidemiological evidence. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **28**, 29-36, doi:10.1093/ndt/gfs290 (2013).
49 Saltiel, A. R. & Kahn, C. R. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414**, 799-806, doi:10.1038/414799a (2001).
50 Karlsson, F. H. *et al.* Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **498**, 99-103, doi:10.1038/nature12198 (2013).
51 Odegaard, J. I. & Chawla, A. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. *Science (New York, N.Y.)* **339**, 172-177, doi:10.1126/science.1230721 (2013).
52 Adamczak, M. & Wiecek, A. The adipose tissue as an endocrine organ. *Seminars in nephrology* **33**, 2-13, doi:10.1016/j.semnephrol.2012.12.008 (2013).
53 Sharma, K. *et al.* Adiponectin regulates albuminuria and podocyte function in mice. *The Journal of clinical investigation* **118**, 1645-1656, doi:10.1172/jci32691 (2008).
54 Wolf, G. *et al.* Leptin stimulates proliferation and TGF-beta expression in renal glomerular endothelial cells: potential role in glomerulosclerosis [seecomments]. *Kidney international* **56**, 860-872, doi:10.1046/j.1523-1755.1999.00626.x (1999).
55 Nerlich, A. G., Schleicher, E. D., Wiest, I., Specks, U. & Timpl, R. Immunohistochemical localization of collagen VI in diabetic glomeruli. *Kidney international* **45**, 1648-1656 (1994).

56 Stefan, N. *et al.* Obesity and renal disease: not all fat is created equal and not all obesity is harmful to the kidneys. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*, doi:10.1093/ndt/gfu081 (2014).
57 Stefan, N. & Haring, H. U. The role of hepatokines in metabolism. *Nature reviews. Endocrinology* **9**, 144-152, doi:10.1038/nrendo.2012.258 (2013).
58 Stefan, N., Haring, H. U., Hu, F. B. & Schulze, M. B. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. *The lancet. Diabetes & endocrinology* **1**, 152-162, doi:10.1016/s2213-8587(13)70062-7 (2013).
59 Stefan, N. *et al.* Identification and characterization of metabolically benign obesity in humans. *Archives of internal medicine* **168**, 1609-1616, doi:10.1001/archinte.168.15.1609 (2008).
60 Haukeland, J. W. *et al.* Fetuin A in nonalcoholic fatty liver disease: in vivo and in vitro studies. *European journal of endocrinology / European Federation of Endocrine Societies* **166**, 503-510, doi:10.1530/eje-11-0864 (2012).
61 Lehmann, R. *et al.* Circulating lysophosphatidylcholines are markers of a metabolically benign nonalcoholic fatty liver. *Diabetes care* **36**, 2331-2338, doi:10.2337/dc12-1760 (2013).
62 Stefan, N. & Haring, H. U. The metabolically benign and malignant fatty liver. *Diabetes* **60**, 2011-2017, doi:10.2337/db11-0231 (2011).
63 Stefan, N. *et al.* Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. *Diabetes care* **29**, 853-857 (2006).
64 Auberger, P. *et al.* Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. *Cell* **58**, 631-640 (1989).
65 Hennige, A. M. *et al.* Fetuin-A induces cytokine expression and suppresses adiponectin production. *PloS one* **3**, e1765, doi:10.1371/journal.pone.0001765 (2008).
66 Ix, J. H. *et al.* Fetuin-A and incident diabetes mellitus in older persons. *Jama* **300**, 182-188, doi:10.1001/jama.300.2.182 (2008).
67 Stefan, N. *et al.* Plasma fetuin-A levels and the risk of type 2 diabetes. *Diabetes* **57**, 2762-2767, doi:10.2337/db08-0538 (2008).
68 Fisher, E. *et al.* Association of AHSG gene polymorphisms with fetuin-A plasma levels and cardiovascular diseases in the EPIC-Potsdam study. *Circulation. Cardiovascular genetics* **2**, 607-613, doi:10.1161/circgenetics.109.870410 (2009).
69 Weikert, C. *et al.* Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. *Circulation* **118**, 2555-2562, doi:10.1161/circulationaha.108.814418 (2008).
70 Pal, D. *et al.* Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nature medicine* **18**, 1279-1285, doi:10.1038/nm.2851 (2012).
71 Stefan, N. & Haring, H. U. Circulating fetuin-A and free fatty acids interact to predict insulin resistance in humans. *Nature medicine* **19**, 394-395, doi:10.1038/nm.3116 (2013).
72 Stefan, N., Schick, F. & Haring, H. U. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *The New England journal of medicine* **371**, 2236-2237, doi:10.1056/NEJMc1412427#SA3 (2014).
73 Schafer, C. *et al.* The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *The Journal of clinical investigation* **112**, 357-366, doi:10.1172/jci17202 (2003).
74 Li, M. *et al.* Association between higher serum fetuin-A concentrations and abnormal albuminuria in middle-aged and elderly chinese with normal glucose tolerance. *Diabetes care* **33**, 2462-2464, doi:10.2337/dc10-0595 (2010).
75 Page, M. M. & Watkins, P. J. Provocation of postural hypotension by insulin in diabetic autonomic neuropathy. *Diabetes* **25**, 90-95 (1976).

76 Baron, A. D. Hemodynamic actions of insulin. *The American journal of physiology* **267**, E187-202 (1994).
77 Laakso, M., Edelman, S. V., Brechtel, G. & Baron, A. D. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *The Journal of clinical investigation* **85**, 1844-1852, doi:10.1172/jci114644 (1990).
78 Laakso, M. *et al.* Kinetics of in vivo muscle insulin-mediated glucose uptake in human obesity. *Diabetes* **39**, 965-974 (1990).
79 Kim, J. A., Montagnani, M., Koh, K. K. & Quon, M. J. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* **113**, 1888-1904, doi:10.1161/circulationaha.105.563213 (2006).
80 Jahn, L. A. *et al.* Insulin Enhances Endothelial Function Throughout the Arterial Tree in Healthy But Not Metabolic Syndrome Subjects. *The Journal of clinical endocrinology and metabolism* **101**, 1198-1206, doi:10.1210/jc.2015-3293 (2016).
81 Steinberg, H. O., Brechtel, G., Johnson, A., Fineberg, N. & Baron, A. D. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *The Journal of clinical investigation* **94**, 1172-1179, doi:10.1172/jci117433 (1994).
82 Jialal, I. *et al.* Characterization of the receptors for insulin and the insulin-like growth factors on micro- and macrovascular tissues. *Endocrinology* **117**, 1222-1229, doi:10.1210/endo-117-3-1222 (1985).
83 Montero, D. Hemodynamic actions of insulin: beyond the endothelium. *Frontiers in physiology* **4**, 389, doi:10.3389/fphys.2013.00389 (2013).
84 King, G. L. & Johnson, S. M. Receptor-mediated transport of insulin across endothelial cells. *Science (New York, N.Y.)* **227**, 1583-1586 (1985).
85 Azizi, P. M. *et al.* Clathrin-dependent entry and vesicle-mediated exocytosis define insulin transcytosis across microvascular endothelial cells. *Molecular biology of the cell* **26**, 740-750, doi:10.1091/mbc.E14-08-1307 (2015).
86 Wang, H., Wang, A. X., Aylor, K. & Barrett, E. J. Nitric oxide directly promotes vascular endothelial insulin transport. *Diabetes* **62**, 4030-4042, doi:10.2337/db13-0627 (2013).
87 Symons, J. D. *et al.* Contribution of insulin and Akt1 signaling to endothelial nitric oxide synthase in the regulation of endothelial function and blood pressure. *Circulation research* **104**, 1085-1094, doi:10.1161/circresaha.108.189316 (2009).
88 Muniyappa, R., Iantorno, M. & Quon, M. J. An integrated view of insulin resistance and endothelial dysfunction. *Endocrinology and metabolism clinics of North America* **37**, 685-711, ix-x, doi:10.1016/j.ecl.2008.06.001 (2008).
89 Wang, Y. *et al.* APPL1 counteracts obesity-induced vascular insulin resistance and endothelial dysfunction by modulating the endothelial production of nitric oxide and endothelin-1 in mice. *Diabetes* **60**, 3044-3054, doi:10.2337/db11-0666 (2011).
90 Ryu, J. *et al.* APPL1 potentiates insulin sensitivity by facilitating the binding of IRS1/2 to the insulin receptor. *Cell reports* **7**, 1227-1238, doi:10.1016/j.celrep.2014.04.006 (2014).
91 de Boer, M. P. *et al.* Globular adiponectin controls insulin-mediated vasoreactivity in muscle through AMPKalpha2. *Vascular pharmacology* **78**, 24-35, doi:10.1016/j.vph.2015.09.002 (2016).
92 Dong, Z. *et al.* Protein kinase A mediates glucagon-like peptide 1-induced nitric oxide production and muscle microvascular recruitment. *American journal of physiology. Endocrinology and metabolism* **304**, E222-228, doi:10.1152/ajpendo.00473.2012 (2013).
93 Wang, B. *et al.* Blood pressure-lowering effects of GLP-1 receptor agonists exenatide and liraglutide: a meta-analysis of clinical trials. *Diabetes, obesity & metabolism* **15**, 737-749, doi:10.1111/dom.12085 (2013).

94 Vicent, D. *et al.* The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *The Journal of clinical investigation* **111**, 1373-1380, doi:10.1172/jci15211 (2003).
95 Duplain, H. *et al.* Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation* **104**, 342-345 (2001).
96 Abe, H. *et al.* Hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation in mice lacking insulin receptor substrate-1. *The Journal of clinical investigation* **101**, 1784-1788, doi:10.1172/jci1594 (1998).
97 Huang, C. *et al.* Arg(9)(7)(2) insulin receptor substrate-1 inhibits endothelial nitric oxide synthase expression in human endothelial cells by upregulating microRNA-155. *International journal of molecular medicine* **36**, 239-248, doi:10.3892/ijmm.2015.2192 (2015).
98 Kubota, T. *et al.* Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell metabolism* **13**, 294-307, doi:10.1016/j.cmet.2011.01.018 (2011).
99 Hashimoto, S. *et al.* Insulin receptor substrate-2 (Irs2) in endothelial cells plays a crucial role in insulin secretion. *Diabetes* **64**, 876-886, doi:10.2337/db14-0432 (2015).
100 Hayashi, K. *et al.* Effects of insulin on rat renal microvessels: studies in the isolated perfused hydronephrotic kidney. *Kidney international* **51**, 1507-1513 (1997).
101 Schmetterer, L. *et al.* Renal and ocular hemodynamic effects of insulin. *Diabetes* **46**, 1868-1874 (1997).
102 Hayashi, K. *et al.* Altered renal microvascular response in Zucker obese rats. *Metabolism: clinical and experimental* **51**, 1553-1561, doi:10.1053/meta.2002.36311 (2002).
103 Buscemi, S. *et al.* Intra-renal hemodynamics and carotid intima-media thickness in the metabolic syndrome. *Diabetes research and clinical practice* **86**, 177-185, doi:10.1016/j.diabres.2009.09.015 (2009).
104 Novikov, A. & Vallon, V. Sodium glucose cotransporter 2 inhibition in the diabetic kidney: an update. *Current opinion in nephrology and hypertension* **25**, 50-58, doi:10.1097/mnh.0000000000000187 (2016).
105 Siegel-Axel, D. I. & Haring, H. U. Perivascular adipose tissue: An unique fat compartment relevant for the cardiometabolic syndrome. *Reviews in endocrine & metabolic disorders*, doi:10.1007/s11154-016-9346-3 (2016).
106 Tano, J. Y., Schleifenbaum, J. & Gollasch, M. Perivascular adipose tissue, potassium channels, and vascular dysfunction. *Arteriosclerosis, thrombosis, and vascular biology* **34**, 1827-1830, doi:10.1161/atvbaha.114.303032 (2014).
107 Gil-Ortega, M., Somoza, B., Huang, Y., Gollasch, M. & Fernandez-Alfonso, M. S. Regional differences in perivascular adipose tissue impacting vascular homeostasis. *Trends in endocrinology and metabolism: TEM* **26**, 367-375, doi:10.1016/j.tem.2015.04.003 (2015).
108 Rittig, K. *et al.* The secretion pattern of perivascular fat cells is different from that of subcutaneous and visceral fat cells. *Diabetologia* **55**, 1514-1525, doi:10.1007/s00125-012-2481-9 (2012).
109 Siegel-Axel, D. I. *et al.* Fetuin-A influences vascular cell growth and production of proinflammatory and angiogenic proteins by human perivascular fat cells. *Diabetologia* **57**, 1057-1066, doi:10.1007/s00125-014-3177-0 (2014).
110 van den Born, J. C., Hammes, H. P., Greffrath, W., van Goor, H. & Hillebrands, J. L. Gasotransmitters in Vascular Complications of Diabetes. *Diabetes* **65**, 331-345, doi:10.2337/db15-1003 (2016).
111 Houben, A. J. *et al.* Perivascular Fat and the Microcirculation: Relevance to Insulin Resistance, Diabetes, and Cardiovascular Disease. *Current cardiovascular risk reports* **6**, 80-90, doi:10.1007/s12170-011-0214-0 (2012).

112 Yudkin, J. S., Eringa, E. & Stehouwer, C. D. "Vasocrine" signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. *Lancet (London, England)* **365**, 1817-1820, doi:10.1016/s0140-6736(05)66585-3 (2005).
113 Balletshofer, B. M. *et al.* Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* **101**, 1780-1784 (2000).
114 Rittig, K. *et al.* Perivascular fatty tissue at the brachial artery is linked to insulin resistance but not to local endothelial dysfunction. *Diabetologia* **51**, 2093-2099, doi:10.1007/s00125-008-1128-3 (2008).
115 de Vries, A. P. *et al.* Fatty kidney: emerging role of ectopic lipid in obesity-related renal disease. *The lancet. Diabetes & endocrinology* **2**, 417-426, doi:10.1016/s2213-8587(14)70065-8 (2014).
116 Foster, M. C. *et al.* Fatty kidney, hypertension, and chronic kidney disease: the Framingham Heart Study. *Hypertension* **58**, 784-790, doi:10.1161/hypertensionaha.111.175315 (2011).
117 Lamacchia, O. *et al.* Para- and perirenal fat thickness is an independent predictor of chronic kidney disease, increased renal resistance index and hyperuricaemia in type-2 diabetic patients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **26**, 892-898, doi:10.1093/ndt/gfq522 (2011).
118 Wagner, R. *et al.* Exercise-induced albuminuria is associated with perivascular renal sinus fat in individuals at increased risk of type 2 diabetes. *Diabetologia* **55**, 2054-2058, doi:10.1007/s00125-012-2551-z (2012).
119 Hysing, J., Ostensen, J., Tolleshaug, H., Andersen, K. J. & Kiil, F. Luminal and basolateral uptake and degradation of insulin in the proximal tubules of the dog kidney. *Acta physiologica Scandinavica* **146**, 241-250, doi:10.1111/j.1748-1716.1992.tb09413.x (1992).
120 ter Maaten, J. C. *et al.* Insulin's acute effects on glomerular filtration rate correlate with insulin sensitivity whereas insulin's acute effects on proximal tubular sodium reabsorption correlation with salt sensitivity in normal subjects. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **14**, 2357-2363 (1999).
121 Hiromura, K., Monkawa, T., Petermann, A. T., Durvasula, R. V. & Shankland, S. J. Insulin is a potent survival factor in mesangial cells: role of the PI3-kinase/Akt pathway. *Kidney international* **61**, 1312-1321, doi:10.1046/j.1523-1755.2002.00257.x (2002).
122 Foutz, R. M., Grimm, P. R. & Sansom, S. C. Insulin increases the activity of mesangial BK channels through MAPK signaling. *American journal of physiology. Renal physiology* **294**, F1465-1472, doi:10.1152/ajprenal.00012.2008 (2008).
123 Thameem, F. *et al.* The Gly(972)Arg variant of human IRS1 gene is associated with variation in glomerular filtration rate likely through impaired insulin receptor signaling. *Diabetes* **61**, 2385-2393, doi:10.2337/db11-1078 (2012).
124 Yano, N. *et al.* In vitro silencing of the insulin receptor attenuates cellular accumulation of fibronectin in renal mesangial cells. *Cell communication and signaling : CCS* **10**, 29, doi:10.1186/1478-811x-10-29 (2012).
125 Isshiki, K. *et al.* Insulin regulates SOCS2 expression and the mitogenic effect of IGF-1 in mesangial cells. *Kidney international* **74**, 1434-1443, doi:10.1038/ki.2008.403 (2008).
126 Kong, Y. L. *et al.* Insulin deficiency induces rat renal mesangial cell dysfunction via activation of IGF-1/IGF-1R pathway. *Acta pharmacologica Sinica* **37**, 217-227, doi:10.1038/aps.2015.128 (2016).
127 Weigert, C. *et al.* Evidence for a novel TGF-beta1-independent mechanism of fibronectin production in mesangial cells overexpressing glucose transporters. *Diabetes* **52**, 527-535 (2003).

128 Coward, R. J. *et al.* Nephrin is critical for the action of insulin on human glomerular podocytes. *Diabetes* **56**, 1127-1135, doi:10.2337/db06-0693 (2007).
129 Kim, E. Y., Anderson, M. & Dryer, S. E. Insulin increases surface expression of TRPC6 channels in podocytes: role of NADPH oxidases and reactive oxygen species. *American journal of physiology. Renal physiology* **302**, F298-307, doi:10.1152/ajprenal.00423.2011 (2012).
130 Kim, E. Y. & Dryer, S. E. Effects of insulin and high glucose on mobilization of slo1 BKCa channels in podocytes. *Journal of cellular physiology* **226**, 2307-2315, doi:10.1002/jcp.22567 (2011).
131 Tejada, T. *et al.* Failure to phosphorylate AKT in podocytes from mice with early diabetic nephropathy promotes cell death. *Kidney international* **73**, 1385-1393, doi:10.1038/ki.2008.109 (2008).
132 Welsh, G. I. *et al.* Insulin signaling to the glomerular podocyte is critical for normal kidney function. *Cell metabolism* **12**, 329-340, doi:10.1016/j.cmet.2010.08.015 (2010).
133 Madhusudhan, T. *et al.* Defective podocyte insulin signalling through p85-XBP1 promotes ATF6-dependent maladaptive ER-stress response in diabetic nephropathy. *Nature communications* **6**, 6496, doi:10.1038/ncomms7496 (2015).
134 Baum, M. Insulin stimulates volume absorption in the rabbit proximal convoluted tubule. *The Journal of clinical investigation* **79**, 1104-1109, doi:10.1172/jci112925 (1987).
135 Takahashi, N., Ito, O. & Abe, K. Tubular effects of insulin. *Hypertension research : official journal of the Japanese Society of Hypertension* **19 Suppl 1**, S41-45 (1996).
136 DeFronzo, R. A., Goldberg, M. & Agus, Z. S. The effects of glucose and insulin on renal electrolyte transport. *The Journal of clinical investigation* **58**, 83-90, doi:10.1172/jci108463 (1976).
137 Nizet, A., Lefebvre, P. & Crabbe, J. Control by insulin of sodium potassium and water excretion by the isolated dog kidney. *Pflugers Archiv : European journal of physiology* **323**, 11-20 (1971).
138 Brands, M. W., Hildebrandt, D. A., Mizelle, H. L. & Hall, J. E. Sustained hyperinsulinemia increases arterial pressure in conscious rats. *The American journal of physiology* **260**, R764-768 (1991).
139 Brands, M. W. & Manhiani, M. M. Sodium-retaining effect of insulin in diabetes. *American journal of physiology. Regulatory, integrative and comparative physiology* **303**, R1101-1109, doi:10.1152/ajpregu.00390.2012 (2012).
140 Manhiani, M. M., Cormican, M. T. & Brands, M. W. Chronic sodium-retaining action of insulin in diabetic dogs. *American journal of physiology. Renal physiology* **300**, F957-965, doi:10.1152/ajprenal.00395.2010 (2011).
141 Blazer-Yost, B. L., Esterman, M. A. & Vlahos, C. J. Insulin-stimulated trafficking of ENaC in renal cells requires PI 3-kinase activity. *American journal of physiology. Cell physiology* **284**, C1645-1653, doi:10.1152/ajpcell.00372.2002 (2003).
142 Lang, F., Artunc, F. & Vallon, V. The physiological impact of the serum and glucocorticoid-inducible kinase SGK1. *Current opinion in nephrology and hypertension* **18**, 439-448, doi:10.1097/MNH.0b013e32832f125e (2009).
143 Lang, F. *et al.* Deranged transcriptional regulation of cell-volume-sensitive kinase hSGK in diabetic nephropathy. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 8157-8162 (2000).
144 Tiwari, S. *et al.* Impaired sodium excretion and increased blood pressure in mice with targeted deletion of renal epithelial insulin receptor. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 6469-6474, doi:10.1073/pnas.0711283105 (2008).
145 Li, L., Garikepati, R. M., Tsukerman, S., Tiwari, S. & Ecelbarger, C. M. Salt sensitivity of nitric oxide generation and blood pressure in mice with targeted knockout of the insulin receptor

from the renal tubule. *American journal of physiology. Regulatory, integrative and comparative physiology* **303**, R505-512, doi:10.1152/ajpregu.00033.2012 (2012).
146 Li, L. *et al.* Reduced ENaC activity and blood pressure in mice with genetic knockout of the insulin receptor in the renal collecting duct. *American journal of physiology. Renal physiology* **304**, F279-288, doi:10.1152/ajprenal.00161.2012 (2013).
147 Pavlov, T. S. *et al.* Regulation of ENaC in mice lacking renal insulin receptors in the collecting duct. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **27**, 2723-2732, doi:10.1096/fj.12-223792 (2013).
148 Stumvoll, M., Meyer, C., Mitrakou, A. & Gerich, J. E. Important role of the kidney in human carbohydrate metabolism. *Medical hypotheses* **52**, 363-366, doi:10.1054/mehy.1997.0655 (1999).
149 Tiwari, S. *et al.* Deletion of the insulin receptor in the proximal tubule promotes hyperglycemia. *Journal of the American Society of Nephrology : JASN* **24**, 1209-1214, doi:10.1681/asn.2012060628 (2013).
150 Eid, A. *et al.* Intrinsic gluconeogenesis is enhanced in renal proximal tubules of Zucker diabetic fatty rats. *Journal of the American Society of Nephrology : JASN* **17**, 398-405, doi:10.1681/asn.2005070742 (2006).
151 Ghezzi, C. & Wright, E. M. Regulation of the human Na+-dependent glucose cotransporter hSGLT2. *American journal of physiology. Cell physiology* **303**, C348-354, doi:10.1152/ajpcell.00115.2012 (2012).
152 Vallon, V. *et al.* Knockout of Na-glucose transporter SGLT2 attenuates hyperglycemia and glomerular hyperfiltration but not kidney growth or injury in diabetes mellitus. *American journal of physiology. Renal physiology* **304**, F156-167, doi:10.1152/ajprenal.00409.2012 (2013).
153 Wilding, J. P. The role of the kidneys in glucose homeostasis in type 2 diabetes: clinical implications and therapeutic significance through sodium glucose co-transporter 2 inhibitors. *Metabolism: clinical and experimental* **63**, 1228-1237, doi:10.1016/j.metabol.2014.06.018 (2014).
154 Accili, D. *et al.* Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. *Nature genetics* **12**, 106-109, doi:10.1038/ng0196-106 (1996).
155 Joshi, R. L. *et al.* Targeted disruption of the insulin receptor gene in the mouse results in neonatal lethality. *The EMBO journal* **15**, 1542-1547 (1996).
156 Accili, D. Insulin Receptor Knock-Out Mice. *Trends in Endocrinology & Metabolism* **8**, 101-104, doi:http://dx.doi.org/10.1016/S1043-2760(97)00031-3 (1997).
157 Brüning, J. C. *et al.* A Muscle-Specific Insulin Receptor Knockout Exhibits Features of the Metabolic Syndrome of NIDDM without Altering Glucose Tolerance. *Molecular Cell* **2**, 559-569, doi:http://dx.doi.org/10.1016/S1097-2765(00)80155-0 (1998).
158 Michael, M. D. *et al.* Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* **6**, 87-97 (2000).
159 Mima, A. *et al.* Glomerular-specific protein kinase C-beta-induced insulin receptor substrate-1 dysfunction and insulin resistance in rat models of diabetes and obesity. *Kidney international* **79**, 883-896, doi:10.1038/ki.2010.526 (2011).
160 Rocchini, A. P. *et al.* Insulin and renal sodium retention in obese adolescents. *Hypertension* **14**, 367-374 (1989).
161 Skott, P. *et al.* Effect of insulin on renal sodium handling in hyperinsulinaemic type 2 (non-insulin-dependent) diabetic patients with peripheral insulin resistance. *Diabetologia* **34**, 275-281 (1991).
162 Nakamura, M. *et al.* Stimulatory effect of insulin on renal proximal tubule sodium transport is preserved in type 2 diabetes with nephropathy. *Biochemical and biophysical research communications* **461**, 154-158, doi:10.1016/j.bbrc.2015.04.005 (2015).

163 Nakamura, M. *et al.* Preserved Na/HCO3 cotransporter sensitivity to insulin may promote hypertension in metabolic syndrome. *Kidney international* **87**, 535-542, doi:10.1038/ki.2014.351 (2015).
164 Grahammer, F. *et al.* mTORC2 critically regulates renal potassium handling. *The Journal of clinical investigation*, doi:10.1172/jci80304 (2016).
165 Gerich, J. E. Role of the kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: therapeutic implications. *Diabetic medicine : a journal of the British Diabetic Association* **27**, 136-142, doi:10.1111/j.1464-5491.2009.02894.x (2010).
166 Meyer, C. *et al.* Abnormal renal and hepatic glucose metabolism in type 2 diabetes mellitus. *The Journal of clinical investigation* **102**, 619-624, doi:10.1172/jci2415 (1998).
167 Zheng, Y. *et al.* Roles of insulin receptor substrates in insulin-induced stimulation of renal proximal bicarbonate absorption. *Journal of the American Society of Nephrology : JASN* **16**, 2288-2295, doi:10.1681/asn.2005020193 (2005).
168 Schafer, S. *et al.* Lifestyle intervention in individuals with normal versus impaired glucose tolerance. *European journal of clinical investigation* **37**, 535-543, doi:10.1111/j.1365-2362.2007.01820.x (2007).
169 Machann, J. *et al.* Follow-up whole-body assessment of adipose tissue compartments during a lifestyle intervention in a large cohort at increased risk for type 2 diabetes. *Radiology* **257**, 353-363, doi:10.1148/radiol.10092284 (2010).
170 Stefan, N. *et al.* A high-risk phenotype associates with reduced improvement in glycaemia during a lifestyle intervention in prediabetes. *Diabetologia* **58**, 2877-2884, doi:10.1007/s00125-015-3760-z (2015).
171 Cohen, J. B. & Cohen, D. L. Cardiovascular and renal effects of weight reduction in obesity and the metabolic syndrome. *Current hypertension reports* **17**, 34, doi:10.1007/s11906-015-0544-2 (2015).
172 Rocchini, A. P. *et al.* The effect of weight loss on the sensitivity of blood pressure to sodium in obese adolescents. *The New England journal of medicine* **321**, 580-585, doi:10.1056/nejm198908313210905 (1989).
173 Lavrencic, A., Salobir, B. G. & Keber, I. Physical training improves flow-mediated dilation in patients with the polymetabolic syndrome. *Arteriosclerosis, thrombosis, and vascular biology* **20**, 551-555 (2000).
174 Vinet, A. *et al.* Impact of a lifestyle program on vascular insulin resistance in metabolic syndrome subjects: the RESOLVE study. *The Journal of clinical endocrinology and metabolism* **100**, 442-450, doi:10.1210/jc.2014-2704 (2015).
175 Thamer, C. *et al.* High visceral fat mass and high liver fat are associated with resistance to lifestyle intervention. *Obesity (Silver Spring, Md.)* **15**, 531-538, doi:10.1038/oby.2007.568 (2007).
176 Fenske, W. *et al.* Obesity-related cardiorenal disease: the benefits of bariatric surgery. *Nat Rev Nephrol* **9**, 539-551, doi:10.1038/nrneph.2013.145 (2013).
177 7. Approaches to Glycemic Treatment. *Diabetes care* **39 Suppl 1**, S52-59, doi:10.2337/dc16-S010 (2016).
178 Sarafidis, P. A. & Lasaridis, A. N. Actions of Peroxisome Proliferator–Activated Receptors–γ Agonists Explaining a Possible Blood Pressure–Lowering Effect. *American Journal of Hypertension* **19**, 646-653, doi:10.1016/j.amjhyper.2005.12.017 (2006).
179 Sarafidis, P. A., Stafylas, P. C., Georgianos, P. I., Saratzis, A. N. & Lasaridis, A. N. Effect of thiazolidinediones on albuminuria and proteinuria in diabetes: a meta-analysis. *American journal of kidney diseases : the official journal of the National Kidney Foundation* **55**, 835-847, doi:10.1053/j.ajkd.2009.11.013 (2010).
180 Dagenais, G. R. *et al.* Effects of ramipril and rosiglitazone on cardiovascular and renal outcomes in people with impaired glucose tolerance or impaired fasting glucose: results of

the Diabetes REduction Assessment with ramipril and rosiglitazone Medication (DREAM) trial. *Diabetes care* **31**, 1007-1014, doi:10.2337/dc07-1868 (2008).
181 Artunc, F. *et al.* Lack of the serum and glucocorticoid-inducible kinase SGK1 attenuates the volume retention after treatment with the PPARgamma agonist pioglitazone. *Pflugers Archiv : European journal of physiology* **456**, 425-436, doi:10.1007/s00424-007-0401-5 (2008).
182 Ochi, A. *et al.* Direct inhibitory effects of pioglitazone on hepatic fetuin-A expression. *PloS one* **9**, e88704, doi:10.1371/journal.pone.0088704 (2014).
183 Mori, K. *et al.* Effects of pioglitazone on serum fetuin-A levels in patients with type 2 diabetes mellitus. *Metabolism: clinical and experimental* **57**, 1248-1252, doi:10.1016/j.metabol.2008.04.019 (2008).
184 Poitout, V. & Robertson, R. P. Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocrine reviews* **29**, 351-366, doi:10.1210/er.2007-0023 (2008).
185 Bensellam, M., Laybutt, D. R. & Jonas, J. C. The molecular mechanisms of pancreatic beta-cell glucotoxicity: recent findings and future research directions. *Molecular and cellular endocrinology* **364**, 1-27, doi:10.1016/j.mce.2012.08.003 (2012).
186 Kaul, K., Apostolopoulou, M. & Roden, M. Insulin resistance in type 1 diabetes mellitus. *Metabolism: clinical and experimental* **64**, 1629-1639, doi:10.1016/j.metabol.2015.09.002 (2015).
187 Hanefeld, M., Monnier, L., Schnell, O. & Owens, D. Early Treatment with Basal Insulin Glargine in People with Type 2 Diabetes: Lessons from ORIGIN and Other Cardiovascular Trials. *Diabetes therapy : research, treatment and education of diabetes and related disorders*, doi:10.1007/s13300-016-0153-3 (2016).
188 Gerstein, H. C. *et al.* Basal insulin and cardiovascular and other outcomes in dysglycemia. *The New England journal of medicine* **367**, 319-328, doi:10.1056/NEJMoa1203858 (2012).
189 Gilbert, R. E. *et al.* Basal insulin glargine and microvascular outcomes in dysglycaemic individuals: results of the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial. *Diabetologia* **57**, 1325-1331, doi:10.1007/s00125-014-3238-4 (2014).
190 Ferrannini, E. *et al.* Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *The Journal of clinical investigation* **124**, 499-508, doi:10.1172/jci72227 (2014).
191 Merovci, A. *et al.* Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *The Journal of clinical investigation* **124**, 509-514, doi:10.1172/jci70704 (2014).
192 Zinman, B. *et al.* Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *The New England journal of medicine* **373**, 2117-2128, doi:10.1056/NEJMoa1504720 (2015).

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Author contributions

All authors contributed equally to researching the data for the article, discussing the article´s content, writing the article and review/editing of the manuscript before submission.

Competing interests statement

No conflict to declare.