The impact of insulin resistance on the kidney and vasculature

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Abstract | Insulin resistance is a systemic disorder that affects many organs and insulin-regulated pathways. The disorder is characterized by a reduced action of insulin despite increased insulin concentrations (hyperinsulinaemia). The effects of insulin on the kidney and vasculature differ in part from the effects on classical insulin target organs. Insulin causes vasodilation by enhancing endothelial nitric oxide production through activation of the phosphatidylinositol 3-kinase pathway. In insulin-resistant states, this pathway is impaired and the mitogen-activated protein kinase pathway stimulates vasoconstriction. The action of insulin on perivascular fat tissue and the subsequent effects on the vascular wall are not fully understood, but the hepatokine fetuin-A, which is released by fatty liver, might promote the proinflammatory effects of 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 insulin receptor is expressed on renal tubular cells and podocytes and insulin signalling has important roles in podocyte viability and tubular function. Renal sodium transport is preserved in insulin resistance and contributes to the salt-sensitivity of blood pressure in hyperinsulinaemia. Therapeutically, renal and vascular insulin resistance can be improved by an integrated holistic approach aimed at restoring overall insulin sensitivity and improving insulin signalling.

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In the 1960s, the development of validated assays to determine plasma insulin concentrations led to the identification of disproportionate hyperinsulinaemia — thereafter termed insulin resistance — in apparently healthy individuals with obesity. The discovery of the insulin receptor in the early 1970s[1] enabled the molecular mechanisms of reduced insulin action to be studied in 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 dyslipidaemia with high plasma concentrations of triglyceride.

Non-metabolic effects of insulin have been described in non-classical insulin-responsive organs and include increased pancreatic β-cell survival, endothelium-dependent vasodilation and renal sodium transport[6]. Unexpectedly, insulin has also been shown to influence brain function to regulate glucose metabolism and food-seeking behaviour[7]. Here, we describe the metabolic and non-metabolic effects of insulin on the vasculature and kidneys of otherwise healthy obese individuals with normal kidney function, including the molecular mechanisms of insulin-signal transduction and its impairment in insulin-resistant states.

Insulin signalling

Insulin is the central hormone involved in the control of glucose and lipid metabolism. The biological effects of insulin are mediated by binding to the insulin receptor, which consists of two extracellular insulin-binding α-subunits and two intracellular tyrosine kinase β-subunits. Alternate splicing of the insulin receptor gene results in two insulin receptor isoforms: insulin receptor A and insulin receptor B[8,9]. Insulin can also bind to the highly homologous insulin-like growth factor 1 receptor (IGF-1R) or to insulin receptor–IGF-1R heterodimers, albeit with reduced affinity compared to insulin-receptor binding[10]. Insulin-mediated receptor activation leads to activation and autophosphorylation of the receptor tyrosine kinase[11] and initiates various cascades of phosphorylation events.

The two major pathways of insulin receptor signal transduction are the insulin receptor substrate (IRS)–phosphatidylinositol 3-kinase (PI3K)–Akt (also known

Key points

- In addition to classical insulin target tissues (liver, skeletal muscle and white adipose tissue) insulin acts on most human organs and cell types, including the arterial vasculature and the kidney
- In insulin-resistant states such as obesity or type 2 diabetes mellitus, not only are the classical insulin effects impaired, but also the effects of insulin on the vasculature and the kidney
- Insulin stimulates its own delivery to target cells by actions on the vasculature involving increased capillary recruitment and endothelial transcytosis; these effects are impaired in insulin-resistant states
- Insulin resistance affects many aspects of kidney function, including renal haemodynamics, podocyte viability and tubular function
- The action of insulin on renal sodium handling is preserved in insulin resistance and contributes to sodium retention and arterial hypertension
- Renal and vascular insulin resistance can be improved through an integrated approach including lifestyle interventions and pharmacological agents

as PKB) pathway and the growth factor receptor-bound protein 2 (Grb2)–son of sevenless homologue 1 (SOS)–Ras–mitogen-activated protein kinase (MAPK, also known as ERK) pathway (FIG. 1A). IRS isoforms are intracellular adaptor proteins that are recruited to the activated insulin receptor. Phosphorylation of these isoforms on tyrosine residues by the insulin receptor initiates the recruitment and activation of PI3K[12,13]. Activated PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3), a membrane-anchored lipid second messenger that activates 3-phosphoinositide-dependent protein kinase 1 (PDK1) and recruits the serine/threonine kinase Akt. Importantly, Akt acts as a central node in the regulation of the biological effects of insulin. It phosphorylates and activates essential downstream kinases such as glycogen synthase kinase 3 (GSK3), mTOR complex 1 (mTORC1) and ribosomal protein S6 kinase (S6K) as well as transcriptional regulators such as forkhead box O family members (FoxO), sterol regulatory element binding protein (SREBP), peroxisome proliferator-activated receptor γ co-activator 1 (PGC1α), and the GTPase-activating protein Akt substrate 160 kDa (AS160)[14]. Subsequent to this activation, modulation of a network of metabolic regulators, controlled at the level of enzymatic activity or gene expression, leads to enhanced glucose uptake, glucose storage, lipid synthesis, protein synthesis and suppression of gluconeogenesis and lipolysis. PDK1 also activates the atypical protein kinases PKCζ and PKCλ/ι, which contribute to insulin-stimulated glucose uptake and lipid synthesis[15]. The insulin-induced Grb2–SOS–Ras pathway leads to activation of MAPK and is a major control mechanism of cell proliferation and differentiation. Other non-metabolic effects of insulin that are mediated via the PI3K–Akt–PKB pathway include inhibition of apoptosis and promotion of cell survival[16].

Insulin signal transduction must be tightly controlled to avoid severe metabolic and proliferative perturbations. Negative regulators of this signal transduction are often activated by insulin; this feedback mechanism inhibits the signalling pathway at the critical nodes, either insulin receptor–IRS or Akt. These negative regulators include phosphotyrosine and phosphoserine/threonine protein phosphatases (PTP1B, PP2A, PTP2B and PTB2C)[17,18], lipid phosphatases that control PIP3 levels (phosphatase and tensin homologue (PTEN) and SH2 domain-containing inositol phosphatase (SHIP))[19,20], and adaptor proteins of the insulin receptor and IRS (Grb and SOCS)[21,22]. Dysregulation of negative regulators of insulin signal transduction by chronic hyperactivation contributes to the development of insulin resistance (FIG. 1B). Another well-studied inhibitory mechanism of the insulin signalling pathway is serine/threonine phosphorylation of the insulin receptor and IRS through the insulin-mediated activation of serine/threonine kinases, predominantly c-Jun N-terminal kinase (JNK), IkB kinase (IKK), PKC, S6K1 and MAPK[23–27].

In the insulin-resistant state, the cellular response to insulin is reduced; therefore, activation of the insulin signalling pathway requires increased concentrations of insulin. Important contributors to insulin resistance are hyperinsulinaemia, hyperglycaemia, inflammation, lipid excess, mitochondrial dysfunction and endoplasmic reticulum (ER) stress[28]. Serine/threonine kinases are activated by elevated plasma levels of insulin, glucose or free fatty acids; by lipids or intermediates of lipid metabolism (such as ceramides and diacylglycerol); or by increased concentrations of cytokines, ER stress and increased levels of reactive oxygen species. Hyperactivation of protein phosphatases[29], increased expression of adaptor proteins, and enhanced O-*N*-acetylglucosamine-modification of insulin signalling molecules[30] are also related to the development of insulin resistance. As a consequence, the modified proteins show an impaired interaction with their signalling partners and altered affinities or enhanced protein degradation.

Regulation of the insulin signalling cascade and dysregulation of this cascade leading to insulin resistance are well described in classical insulin target tissues, which have important roles in the regulation of glucose and lipid metabolism. The insulin receptor is, however, ubiquitously expressed, and functional insulin signalling is found in other peripheral, non-classical insulin target tissues, including the vasculature[31] and the kidney[32]. In the kidney, insulin binds to its receptor in glomerular cells, podocytes[33], mesangial cells[34], endothelial cells, epithelial cells[35] and tubule cells[36]. This binding has diverse functions ranging from glucose uptake to regulation of glomerular function, gluconeogenesis and tubular transport.

Organ crosstalk

Insulin resistance is a hallmark of obesity, the metabolic syndrome and type 2 diabetes mellitus (T2DM). In the setting of insulin resistance, target cells show an attenuated response to insulin, which is compensated for by increased insulin secretion. Insulin resistance affects many organs and cellular insulin-regulated pathways; however, the extent of insulin resistance can vary considerably. Moreover, altered metabolic or hormonal signals as a result of insulin resistance affect organ crosstalk and promote organ dysfunction and disease (FIG. 2). In the kidney, obesity and insulin resistance

Impaired glucose tolerance
Defined as a plasma glucose concentration of 140–200 mg/dl (7.77–11.1 mmol/l) measured 2 h after an oral glucose load of 75 g.

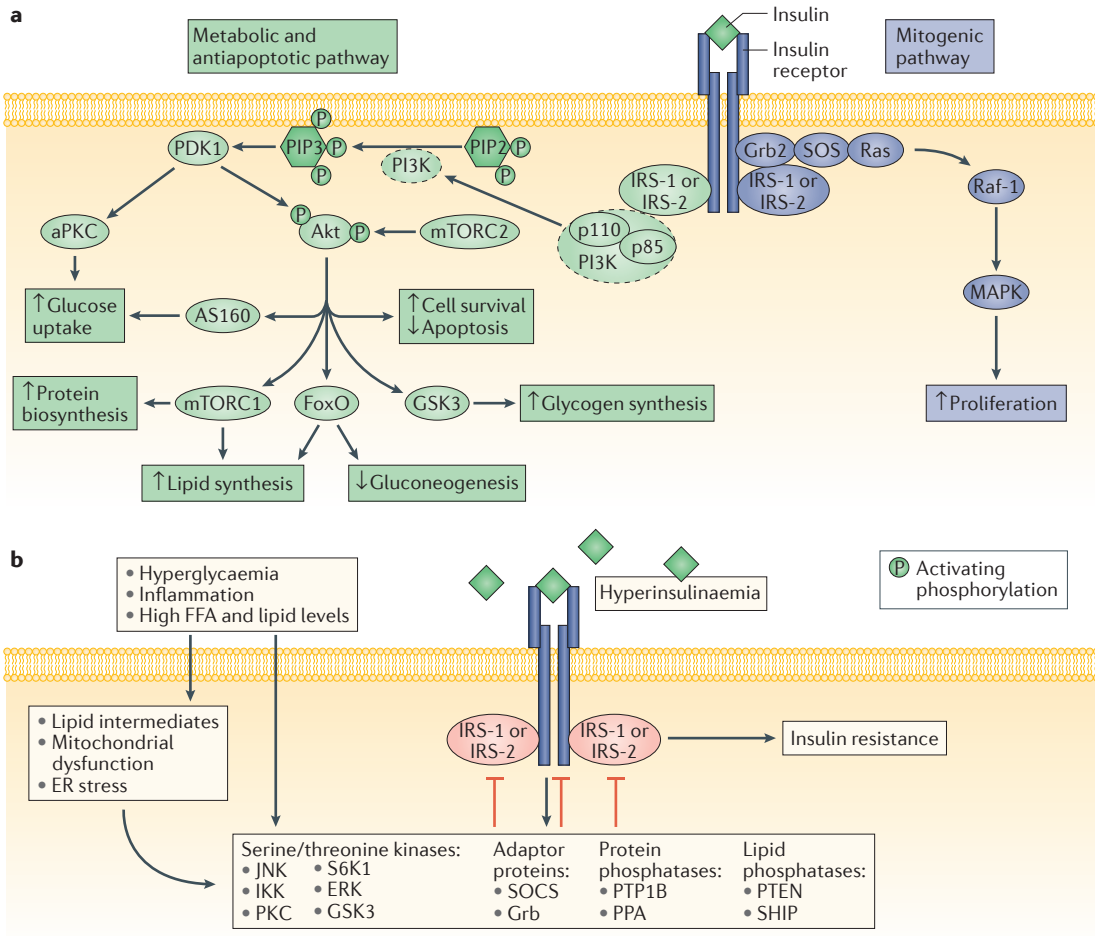

Figure 1 | **The insulin signalling pathway. a** | Activation of insulin signalling pathways. Insulin binding activates the insulin receptor and leads to the recruitment of insulin receptor substrate (IRS) isoforms and subsequent activation of the phosphatidylinositol 3-kinase (PI3K)–Akt pathway, which regulates glucose and lipid metabolism, protein biosynthesis, cell survival and apoptosis. Insulin-induced activation of the Ras–mitogen-activated protein kinase (MAPK) pathway increases cell proliferation. **b** | Negative regulation of insulin signalling pathways. Hyperglycaemia, hyperinsulinaemia, high plasma free fatty acid (FFA) levels and inflammation activate serine/threonine kinases, adaptor proteins and phosphatases either directly or via lipid intermediates, mitochondrial dysfunction or the induction of endoplasmic reticulum (ER) stress. Activation of these negative regulators results in a chronically reduced cellular response to insulin. aPKC, atypical protein kinase C; AS160, Akt substrate 160 kDa; FoxO, forkhead box O; Grb, growth factor receptor-bound protein; GSK3, glycogen synthase kinase 3; IKK, IκB kinase; JNK, c-Jun N-terminal kinase; mTORC, mTOR complex; PDK1, phosphatidylinositide-dependent protein kinase 1; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PKC, protein kinase C; PPA, protein phosphatase A; PTEN, phosphatase and tensin homologue; PTP1B, protein-tyrosine phosphatase 1B; p85, PI3K 85 kDa regulatory subunit α; p110, PI3K 110 kDa catalytic subunit α; Raf-1, RAF proto-oncogene serine/threonine-protein kinase; SHIP, SH2 domain-containing inositol phosphatase; SOCS, suppressor of cytokine signalling; SOS, son of sevenless homologue 1; S6K1, ribosomal protein S6 kinase β1.

are important risk factors for a decline in glomerular filtration rate (GFR) as well as for the onset and progression of chronic kidney disease (CKD)[37–41]. Visceral obesity is a stronger predictor of end-stage renal disease (ESRD) than is elevated body mass index[42]. Although visceral obesity might cause CKD by promoting metabolic diseases, studies indicate that even in the absence of well-known risk factors such as hypertension or diabetes, obesity *per se* might harm the kidney by causing hyperfiltration[43–45]. 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 levels[46]. Thus, insulin resistance itself and/or factors that promote insulin resistance might have a pathogenic role early in the development of CKD[47].

Some major organs and pathophysiological mechanisms are involved in both the development of insulin resistance and impaired kidney function. Defects in skeletal muscle, white adipose tissue and liver that result in impaired insulin signalling can induce hyperglycaemia, predominantly by impairing glucose disposal and increasing hepatic glucose production[48]. Evidence suggests that impaired brain insulin signalling[7] and an altered gut microbiome[49] are involved in this process. As the onset and progression of CKD may be independent

Visceral obesity
Increased waist circumference as a result of an accumulation of fat in the intra-abdominal compartments, such as the omentum majus.

Hepatokines
Factors that are secreted from the liver and act on other tissues.

of glycaemia, other factors such as humoral signals from metabolic tissues might have a role in the early stages of impaired renal function. The expansion of visceral adipose tissue, which is infiltrated by immune cells, is involved in this process[50]. Proinflammatory cytokine signalling not only induces insulin resistance, but also impairs kidney function[51]. In expanded visceral adipose tissue, infiltration by immune cells and proinflammatory cytokine signalling decrease the secretion of the anti-inflammatory adipokine adiponectin. This protein is considered to be important for sustaining whole-body insulin sensitivity and is thought to have beneficial effects on the kidney[52]. Furthermore, the expansion of adipose tissue results in increased production of leptin. This adipokine is thought to contribute to the development of glomerulosclerosis by stimulating increased production of transforming growth factor β1, which leads to the induction of proteinuria and renal expression of type IV collagen[53,54]. In addition, increased visceral obesity might be linked to the onset and progression of CKD through hyperinsulinaemia, inappropriate activation of the renin–angiotensin system and oxidative stress in the kidney. The resulting pathology includes an impaired blood pressure–natriuresis relationship, increased salt sensitivity of blood pressure, aldosterone excess, glomerular hypertension, endothelial dysfunction and vasoconstriction as well as matrix expansion[41,55].

When lipids accumulate in the liver, not only does hepatic glucose production increase and dyslipidaemia develop, but hepatokines are differently regulated[56–58]. Fetuin-A (also known as α-2-HS-glycoprotein) is the best studied of these hepatokines. Expression of fetuin-A is increased in non-alcoholic fatty liver disease[59–62] and inhibits insulin signalling[63]. We showed that fetuin-A induces cytokine expression in monocytes and adipose tissue[64], and we and others found that fetuin-A levels are associated with the risk of incident diabetes[65,66] and cardiovascular disease (CVD)[67,68]. In animals and *in vitro*, fetuin-A serves as an adaptor protein for saturated fatty acids, enabling them to activate Toll-like receptor 4. Thereby, fetuin-A induces inflammatory signalling and insulin resistance[69], important factors that drive the development of T2DM and CVD. In humans, we have found an association between plasma fetuin-A levels, plasma fatty acid levels and insulin resistance[70,71].

Fetuin-A might also be involved in the pathogenesis of CKD. In advanced CKD and in ESRD, fetuin-A might inhibit ectopic calcification, thus protecting the kidney[72]. However, in the early stages of CKD, when ectopic calcification is not present, the proinflammatory effects of fetuin-A may prevail to a large extent. Of note, an association between elevated fetuin-A levels and albuminuria has been reported in women with normal glucose tolerance; this relationship was independent of well-known risk factors for albuminuria[73]. Thus, elevated plasma fetuin-A levels might promote CKD through the induction of proinflammatory signalling in the kidney.

Vascular insulin resistance

In individuals with normal insulin sensitivity, insulin acts on the vasculature and causes endothelium-dependent vasodilation. This effect was first suggested through observations of hypotensive episodes following subcutaneous or intravenous administration of insulin[74] although distinguishing this effect from hypoglycaemia-induced counter regulation was difficult. In pioneering experiments in the early 1990s, Baron *et al.* reported an insulin-mediated increase in blood flow into

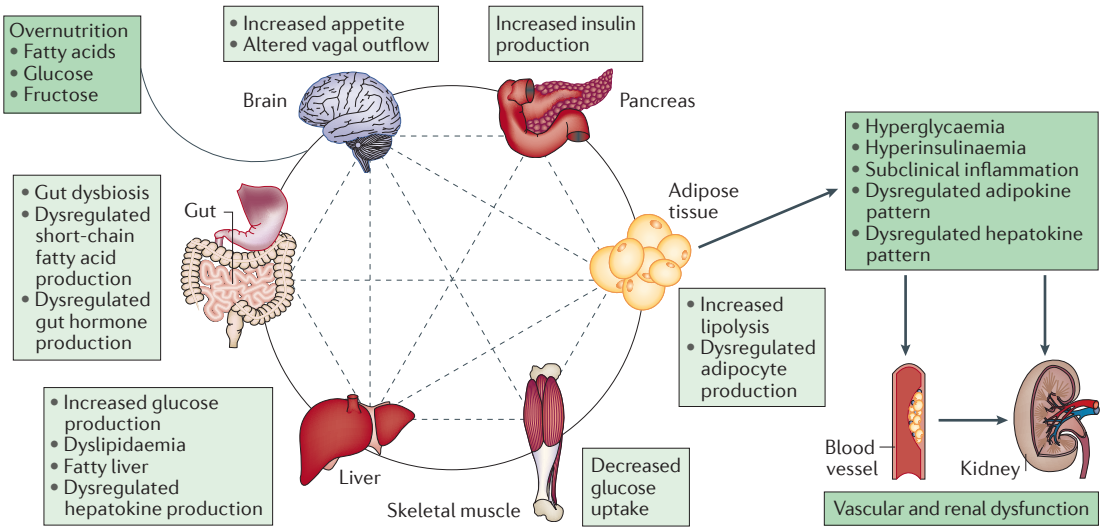

Figure 2 | **Organ crosstalk in the insulin-resistant state.** Insulin has metabolic effects on the liver, adipose tissue and skeletal muscle and non-classical effects on other organs, including the brain, gut, pancreas, vasculature and kidney. These effects are impaired in the insulin-resistant state, resulting in hyperglycaemia and hyperlipidaemia, which trigger several other pathophysiologic conditions in these organs. Furthermore, hyperglycaemia and hyperlipidaemia induce dysregulated adipokine and hepatokine production, hyperinsulinaemia and subclinical inflammation. These effects together with gut dysbiosis lead to a pathophysiological organ crosstalk that has an adverse impact on cardiovascular and renal systems.

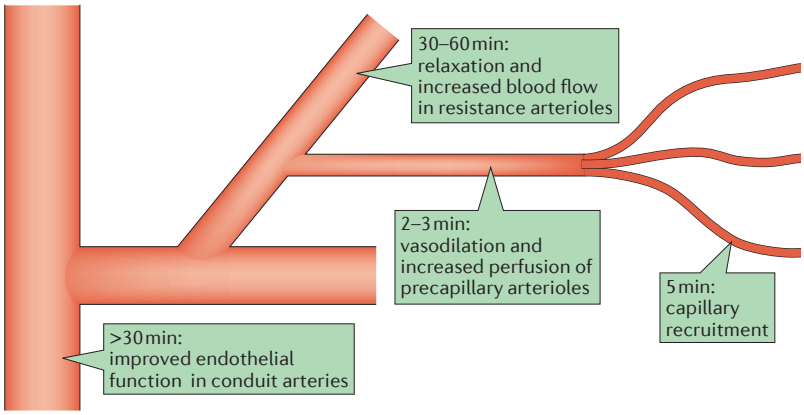

Figure 3 | **The effects of insulin on the arterial vasculature.** Insulin acts at all levels of the vascular tree. A few minutes after insulin stimulation, insulin induces capillary recruitment in skeletal muscle by vasodilation of the precapillary arterioles, followed by relaxation of the larger resistance arterioles, leading to enhanced blood flow. Accordingly, insulin improves endothelial function in conduit arteries. These actions of insulin are reduced in obese individuals and in patients with type 2 diabetes mellitus.

the skeletal muscle of the leg during a hyperinsulinaemic–euglycaemic clamp[75]. Compared to lean individuals, the dose–response curve of insulin was gradually shifted to the right and flattened in insulin-resistant obese individuals and in patients with T2DM[76,77]. As both the dose-response characteristic and the time course for glucose disposal paralleled the insulin-mediated vasodilatory effects, the researchers speculated that the effects of insulin on blood flow might contribute to, or even limit, the effects of insulin on glucose uptake in the skeletal muscle.

Detailed analysis revealed that insulin-mediated vasodilation in skeletal muscle occurs in two phases[78]. First, a few minutes after insulin stimulation, dilation of the precapillary arterioles results in an increase in the number of perfused capillaries without a change in blood flow (FIG. 3). Within 30 min, insulin induces the relaxation of larger resistance arterioles, thus increasing the overall blood flow to the limb to a maximum level after about 2 h. Thus the *in vivo* response to insulin is an integration of increased capillary recruitment and increased total blood flow, and both of these actions of insulin are reduced and retarded in insulin-resistant states such as obesity and T2DM[79].

Administration of insulin during a euglycaemic–hyperinsulinaemic clamp improved endothelial function at each of the three levels of the arterial tree (arteries, arterioles and capillaries) in healthy controls, but not in obese insulin-resistant patients, demonstrating the close link between metabolic and vascular insulin resistance[79]. Previous studies examining the mechanism behind the haemodynamic effects of insulin showed that application of the nitric oxide (NO) synthase inhibitor L-*N*-monomethyl-L-arginine abrogated insulin-induced vasodilation in skeletal muscle, suggesting a major role of insulin-stimulated NO generation in this process[80]. The early finding that the insulin receptor is expressed on vascular endothelium at all levels of the arterial tree is consistent with the existence of insulin-stimulated vasodilation[81]. Independent of the endothelium, insulin might also cause vasodilation by acting directly on vascular smooth muscle cells (VSMCs)[82].

Hyperinsulinaemic–euglycaemic clamp
Test used to quantify insulin resistance on a whole-body level. Continuous insulin infusion is used to maintain plasma insulin levels, whilst variable glucose infusion is used to maintain plasma glucose concentration at basal levels. When a stable plasma glucose concentration is achieved, the rate of glucose infusion is equal to the rate of glucose uptake by all of the body tissues.

Endothelial transcytosis of insulin

Insulin promotes its own delivery to the skeletal muscle microvasculature by inducing vasodilation, but must cross the tight endothelial barrier to access the interstitium and act on target cells. A specific, saturable mechanism transports insulin across endothelial cells[83]. Key experiments in animals have shown that the metabolic actions of insulin correlate more closely with interstitial concentrations than with plasma levels[84], suggesting that transendothelial transport is the rate-limiting step that determines insulin availability in target tissues.

In microvascular capillaries, insulin is transported through endothelial cells via clathrin-dependent entry and vesicle-mediated exocytosis, thus bypassing the lysosomal degradation pathway (FIG. 4)[85]. This transendothelial insulin transport is promoted by insulin-induced endothelial NO production, indicating that insulin enhances its own transport[86]. These findings explain why whole body insulin-induced glucose uptake is maximal after more than 1 h, whereas insulin-induced glucose uptake in isolated adipocytes or skeletal muscle cells occurs within minutes. They also explain reduced and retarded insulin-stimulated glucose uptake in insulin-resistant individuals, in whom the endothelial effects of insulin are impaired. In summary, insulin stimulates its own delivery to the target tissue through capillary recruitment, by increasing blood flow and through transcytosis. All of these processes are impaired in insulin-resistant states.

Endothelial NO signalling

Haemodynamic effects of insulin have been identified in various organs, indicating a ubiquitous NO-mediated vasodilatory action. After binding to the endothelial insulin receptor, insulin activates PI3K, PDK1 and Akt, leading to phosphorylation of endothelial NO synthase (eNOS) at Ser1177, enhanced eNOS activity and increased NO production[87] (FIG. 5A). Insulin also stimulates the MAPK pathway, which in endothelial cells induces the expression and secretion of endothelin-1, which stimulates vasoconstriction and proliferation of VSMCs[78]. In the insulin-resistant state, the PI3K pathway is impaired and subsequent NO production is reduced, whereas the MAPK pathway is either not affected or is enhanced, resulting in an imbalance that promotes vasoconstriction, VSMC proliferation and eventual hypertension[88] (FIG. 5B).

Adaptor protein containing PH domain, PTB domain and leucine zipper motif 1 (APPL1) has an important role in the regulation of vascular tone in endothelial cells[89]. This scaffold protein is involved in the signal transduction of adiponectin, which is an insulin sensitizer in classical insulin-target tissues[90]. In mice, transgenic overexpression of APPL1 prevented age-induced and obesity-induced impairment of insulin-mediated vasodilation and also reversed obesity-induced augmentation of insulin-evoked endothelin-1-dependent vasoconstriction[90].

By contrast, deletion of APPL1 caused selective impairment of the PI3K–Akt–eNOS pathway and augmented the MAPK1/3 signalling cascade in vascular endothelium, leading to decreased NO availability and enhanced endothelin-1 production, respectively. Thus the balance between vasodilatory and vasoconstrictory actions of insulin was shifted towards endothelial dysfunction (FIG. 5B). The MAPK pathway induces the expression of vascular cell adhesion proteins such as vascular cell adhesion molecule 1 and E-selectin in endothelial cells[78]. These proteins mediate the adhesion of leukocytes to the vascular endothelium, which might have a role in the development of atherosclerosis. In addition to its role in signal transduction of adiponectin, APPL1 blocks binding of the Akt inhibitor tribbles homologue 3 (TRB3) to Akt[91], and thus supports NO production at two intervention points of the PI3K pathway (FIG. 5A). Activated Akt phosphorylates Raf-1 at the inhibitory Ser259, thereby reducing the MAPK cascade. Hence, adiponectin controls insulin-mediated vasoreactivity through activation of APPL1 and AMP-activated kinase (AMPK)[92].

In addition to AMPK activators, glucagon-like peptide 1 (GLP-1) induces protein kinase A (PKA)-mediated NO production, which in turn stimulates muscle microvascular recruitment[93]. Accordingly, clinical trials in patients with T2DM have consistently shown that treatment with a GLP-1 receptor agonist reduces diastolic and systolic blood pressure by up to 5 mmHg[94].

Lessons from mouse models

To prove the causal involvement of insulin signal transduction in insulin-mediated vasodilation, an endothelial cell-specific, insulin-receptor-knockout mouse was generated[95]. Unexpectedly, arterial blood pressure was not increased in these mice, but tended to be lower than in wild-type controls. The knockout mice also had normal glucose homeostasis. As expression of both eNOS and endothelin-1 mRNA was reduced by approximately 50% in the knockout mice, the resulting reductions in both the pro-vasodilatory and pro-vasoconstrictive effects of insulin might have balanced each other out, leading to no net increase in blood pressure. Notably, mice that

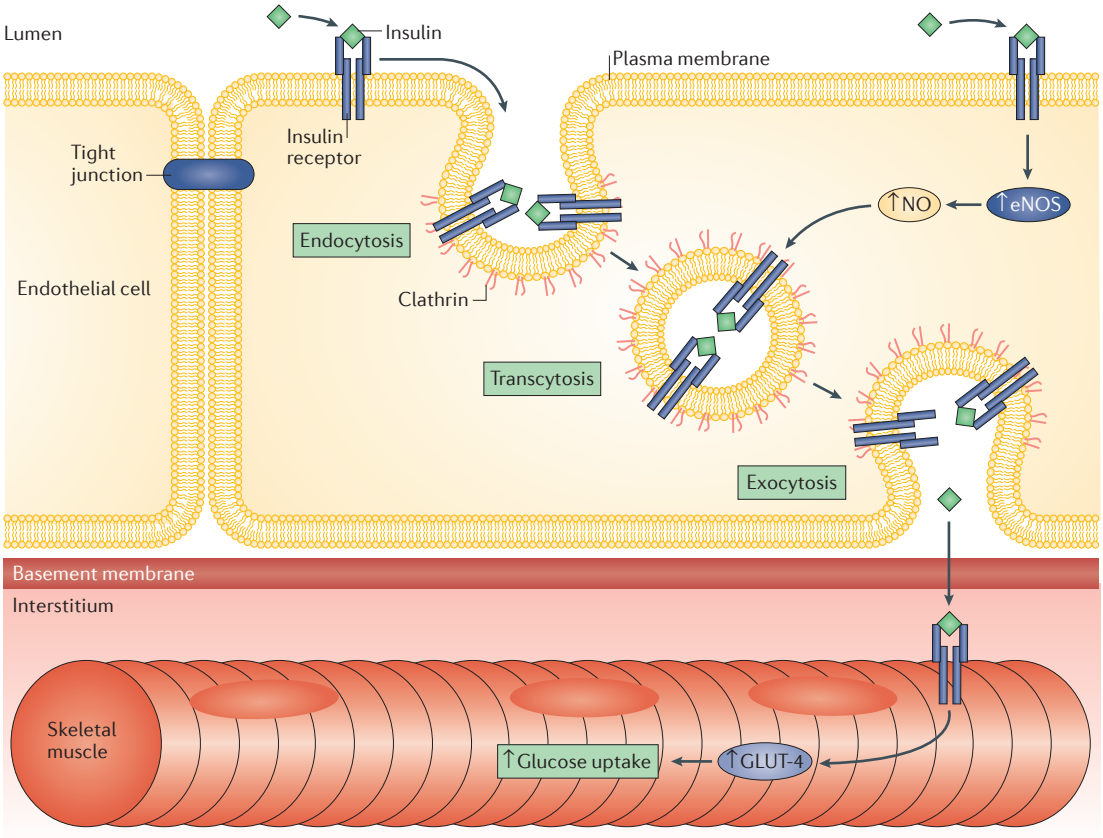

Figure 4 | **Transendothelial transport of insulin in a skeletal muscle capillary.** Insulin stimulates its own transendothelial transport by clathrin-dependent nitric oxide (NO)-mediated endocytosis and transcytosis. In skeletal muscle and in adipose tissue, the non-fenestrated microvascular endothelial monolayer forms a tight barrier that restricts free access of plasma constituents to the subendothelial interstitium and, if not activated, prevents interaction of blood cells with the endothelium. Insulin binds to the insulin receptor on the endothelial plasma membrane and the resulting complex is engulfed by clathrin-mediated endocytosis, transported through the endothelial cell and released by exocytosis. After passing through the basement membrane, insulin binds to its target cells and stimulates glucose uptake via facilitated glucose transporter member 4 (GLUT-4). Transcytosis of insulin is stimulated by NO, which is generated by insulin-stimulated activation of endothelial NO-synthase (eNOS). These insulin actions are impaired in patients with insulin resistance.

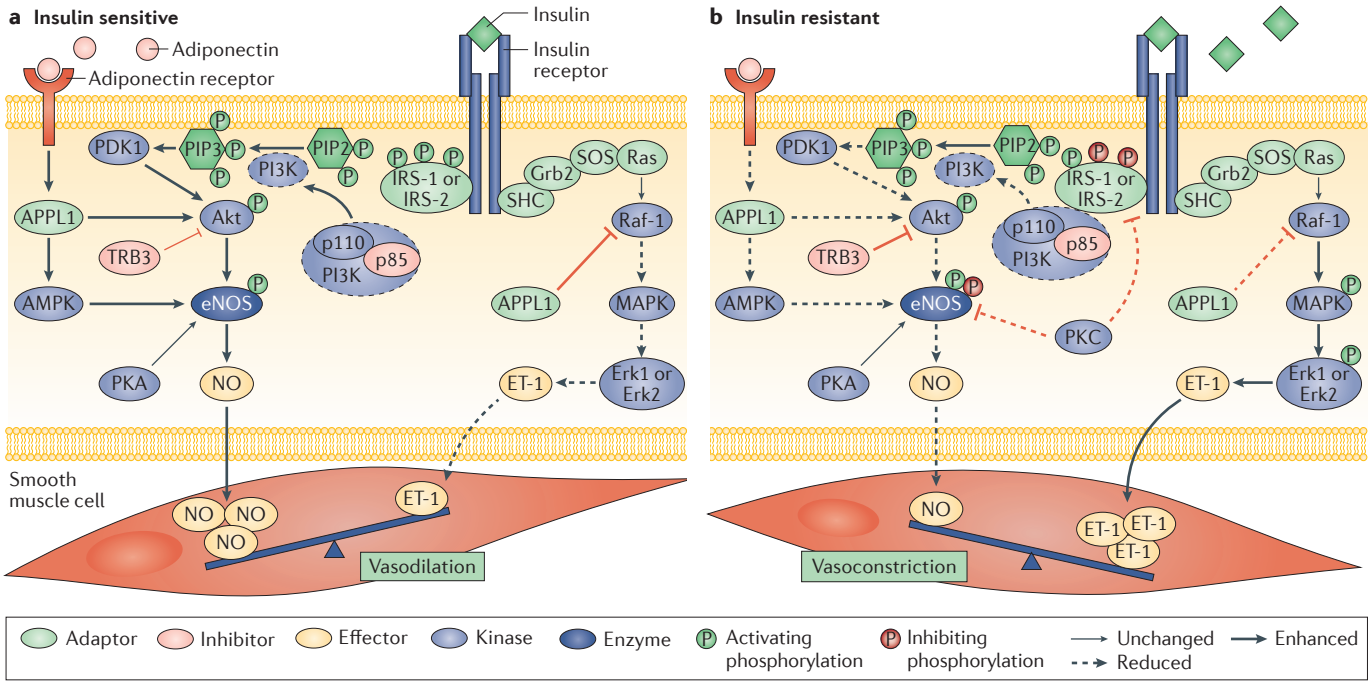

Figure 5 | **Insulin signal transduction in the endothelial cells of resistance arterioles.** **a** | Insulin has an essential role in maintaining vascular function by regulating the endothelial production of nitric oxide (NO) and endothelin-1 (ET-1), which act on vascular smooth muscle cells to stimulate vasodilation and vasoconstriction, respectively. Binding of insulin to its cognate receptor stimulates both the phosphatidylinositol 3-kinase (PI3K)–Akt axis and the mitogen-activated protein kinase (MAPK) axis of the insulin signalling cascade. Phosphorylation of endothelial NO-synthase (eNOS) on Ser1177 by activated Akt, AMP-activated kinase (AMPK) or protein kinase A (PKA), leads to increased production of NO. The insulin-sensitizing hormone adiponectin activates AMPK via the adaptor protein phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1), which competes with the Akt inhibitor tribble homologue 3 (TRB3), so further stimulates eNOS activity and subsequent NO production. APPL1 also inhibits RAF proto-oncogene serine/threonine-protein kinase (Raf-1), resulting in a reduction in ET-1 production. The action of NO, therefore, outweighs that of ET-1, resulting in vasodilation. **b** | In the setting of insulin resistance, various metabolic and inflammatory factors inhibit the actions of insulin receptor substrate 1 (IRS-1) and IRS-2, leading to reduced activation of the PI3K–Akt axis and a resulting reduction in NO production. As levels of adiponectin are reduced in patients with insulin resistance and/or obesity, APPL phosphorylation and activity is reduced, further shifting the insulin signal from the PI3K axis to the MAPK axis. Activation of protein kinase C (PKC) in insulin-resistant individuals results in phosphorylated of eNOS at the inhibitory Ser495, further reducing NO production. The action of ET-1, therefore, outweighs that of NO, resulting in vasoconstriction. As the expression of adhesion molecules is controlled by the MAPK axis, insulin resistance also results in overexpression of vascular cell adhesion molecule-1 and E-selectin. Expression of these adhesion molecules on the endothelial cell surface leads to leukocyte adhesion and further activation of the endothelium, which might be an early event in the development of atherosclerosis. Erk, extracellular-signal regulated kinase; Grb2, growth factor receptor-bound protein 2; PDK1, phosphatidylinositide-dependent protein kinase 1; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; p85, PI3K 85 kDa regulatory subunit α; p110, PI3K 110 kDa catalytic subunit α; SHC, Src homology 2 domain containing protein; SOS, son of sevenless homologue 1.

lack eNOS have hypertension, insulin resistance, fasting hyperinsulinaemia, hyperlipidaemia, and a 40% reduction in insulin-stimulated glucose uptake compared with controls[96].

The role of the insulin signalling cascade in the vasculature was further investigated using mice with deletion of IRS-1 (REF. 97). These mice had elevated plasma triglyceride levels and increased blood pressure, indicating a role for insulin signalling in regulating vascular tone. A role for IRS-1 in the regulation of human blood pressure has also been demonstrated; carriers of the IRS-1 Gly972Arg mutation had higher blood pressure and lower plasma nitrate and nitrite levels than non-carriers[98]. In addition, endothelial cells from donors with this mutation had lower eNOS expression and activity than those from noncarriers, indicating an important role for endothelial IRS-1 in the regulation of vascular tone in humans.

To ultimately answer the question of whether insulin-mediated capillary recruitment and transendothelial transport of insulin are related, or function independently, knockout mice were generated that lacked endothelial IRS-2, which is the major IRS isoform in endothelial cells[84]. In these mice, insulin signalling was impaired, as demonstrated by decreased Akt phosphorylation compared with controls after 60 min of insulin infusion during a hyperinsulinaemic–euglycaemic clamp, and a lack of Ser1177 phosphorylation of eNOS.

Quantitative analysis of the data indicated that, similar to humans, about 50% of the glucose uptake by skeletal muscle in mice was mediated by the endothelium via insulin-mediated vasodilation and insulin transport[84]. Insulin-mediated vasodilation and insulin delivery was not, however, observed in the liver of the endothelial IRS-2-knockout mouse. This difference can be explained by the different capillary structures of these tissues. Capillaries in skeletal muscle have occluded conjugations between non-fenestrated endothelial cells, whereas the sinusoid endothelium of the liver capillaries essentially enables free access of insulin and, thus, direct, fast action on hepatocytes. Considerations that are valid for the skeletal muscle and adipose tissue, therefore, do not apply to organs and organelles that do not have a tight endothelium, such as renal glomeruli with fenestrated endothelium.

The question of whether the effects of insulin on the vasculature are limited to skeletal muscle was addressed in a study that investigated whether insulin effects on vascular endothelium are present in the pancreatic islets[99]. This study, which used knockout mice, showed that the absence of IRS-2 in endothelial cells impairs islet blood flow, to a similar extent to that seen in skeletal muscle[99]. Pharmacological stimulation of islet blood flow almost completely restored insulin secretion in these animals, suggesting that insulin-induced and endothelium-mediated increases in blood flow might regulate insulin distribution in concert with other classical metabolic and hormonal stimuli. As hyperinsulinaemia leads to the downregulation of IRS-2 in endothelial cells, impaired insulin action might be one of the mechanisms that underlies the decrease and/or delay in insulin secretion in patients with obesity and/or T2DM.

Impact on renal haemodynamics

In rats, insulin induces NO-mediated vasorelaxation in interlobular arteries as well as in afferent and efferent arterioles[100]. In healthy individuals, insulin increased renal blood flow as analysed by clearance of para-aminohippuric acid during a hyperinsulinaemic–euglycaemic clamp[101]. This effect was abrogated by application of L-*N*-monomethyl-L-arginine, indicating that insulin stimulates NO generation in the renal vasculature[101]. In the insulin-resistant state, NO signalling is expected to be reduced in the renal vasculature, similar to classical insulin target organs. Indeed, renal vessels from insulin-resistant Zucker rats failed to dilate in response to insulin and acetylcholine, indicating endothelial dysfunction[102]. In addition, myogenic vasoconstriction in response to pressure was blunted, suggesting alterations in VSMCs of the media. Endothelial dysfunction of renal vessels leads to increased renovascular resistance and ultimately reduced renal blood flow. In a study that investigated the relationship between metabolic syndrome and vascular impairment, renal resistive index increased across the subgroups from healthy individuals to patients with metabolic syndrome, and was highest in those with both metabolic syndrome and T2DM[103]. Plasma concentration of adiponectin was an independent negative predictor of renal resistive index.

Renal resistive index
A measure of intrarenal vascular resistance.

Increased renovascular resistance owing to reduced insulin-stimulated NO production would be expected to reduce GFR. However, GFR is often increased in obese patients with metabolic syndrome or overt diabetes. Mediators other than insulin likely account for this glomerular hyperfiltration, which is also found in patients with type 1 diabetes mellitus (T1DM) and defines early diabetic nephropathy. Reduced tubuloglomerular feedback and dilation of afferent arterioles owing to increased reabsorption of sodium along with glucose, are thought to be the principal mechanisms leading to glomerular hyperfiltration[104].

Role of perivascular and renal sinus fat

Arteries of various sizes, including small arterioles but excluding the cerebral vasculature, are coated by adipose tissue. For a long time arteries were thought to be embedded in this perivascular fat tissue (PVAT) to protect and physically support the blood vessels. PVAT has, however, now been identified as an endocrine compartment that releases adipokines and other factors with vasodilatory activity. These factors can act directly on the vessel wall because, particularly in small arteries and microvessels, no anatomical barrier exists between the adventitia and PVAT[105,106]. The distribution of PVAT along vascular beds is not uniform, and PVAT differs in function and phenotype within different regions of a given blood vessel and also between different PVAT compartments, for example epicardial, periaortal, peritibial and renal sinus[107]. PVAT also differs from other fat compartments such as subcutaneous and visceral fat[108]. The PVAT cells express and secrete proinflammatory cytokines such as IL-6, IL-8, tumour necrosis factor and monocyte chemoattractant protein-1; the anti-inflammatory and insulin-sensitizing adipokine adiponectin; and the regenerative factor hepatocyte growth factor (FIG. 6). Extensive *ex vivo* studies of human PVAT have shown that fetuin-A stimulates the expression and secretion of proinflammatory cytokines[105,109]. The adipocyte-derived relaxing factor (ADRF), which has anti-contractile effects on the vascular wall[107], might be very important functionally, but has not yet been fully characterized. ADRF also has relaxing effects on VSMCs in the absence of endothelium, indicating an independent regulation of vascular tone by PVAT[110]. Several studies that aimed to identify the chemical nature of ADRF found evidence of low molecular weight compounds such as hydrogen sulphide and palmitate methyl ester[111].

Microvascular dysfunction occurs very early in the progression of vascular disease, even before clinical manifestations of vascular lesions. PVAT has been proposed as the cause of this dysfunction[112,113]. The quantity of PVAT surrounding the human brachial artery inversely associates with insulin sensitivity, but does not correlate with local endothelial dysfunction, as determined by flow-mediated dilation[114].

Few studies have investigated the role of perirenal sinus adipose tissue in metabolic health and renal function[115]. Among a subcohort of participants enrolled in the Framingham Heart Study, PVAT quantity around

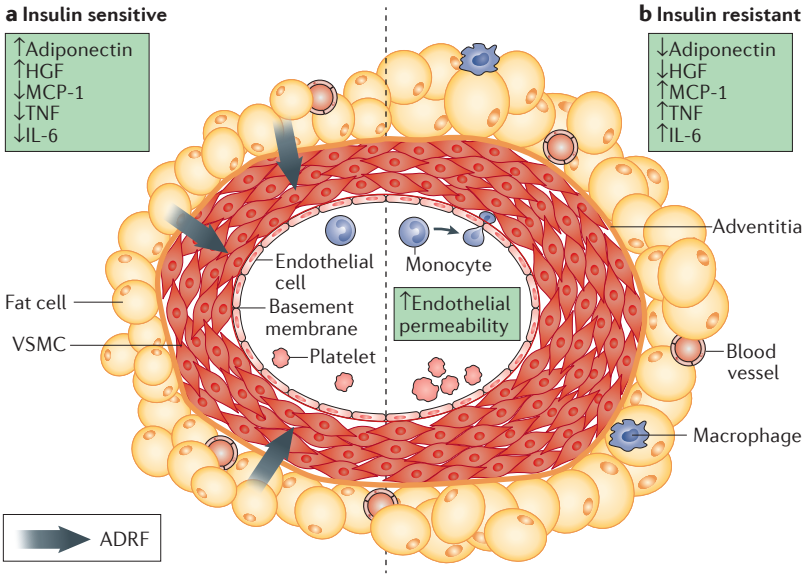

Figure 6 | **Perivascular adipose tissue influences vascular function.** Large and small arteries are surrounded by adipose tissue. **a** | In healthy, insulin-sensitive individuals, adiponectin levels are increased and perivascular fat cells secrete vasodilatory factors such as adipocyte-derived relaxing factor (ADRF). **b** | In the insulin-resistant state, perivascular fat cells increase in size and number, and secrete reduced levels of regenerative factors such as hepatocyte growth factor (HGF), and increased levels of proinflammatory factors such as IL-6, tumour necrosis factor (TNF) and monocyte chemoattractive factor 1 (MCP-1). These proinflammatory factors inhibit the phosphatidylinositol 3-kinase–Akt axis of insulin signal transduction, so increase endothelial permeability and enhance insulin resistance. MCP-1 promotes infiltration of monocytes and macrophages into the vascular wall and the surrounding adipose tissue, which might in turn further increase the proinflammatory state. The activated endothelium expresses adhesion factors that attract leukocytes and activate platelets, leading to enhanced permeability of the endothelium and potentially the development of atherosclerosis. VSMC, vascular smooth muscle cell.

the renal sinus was associated with increased risks of hypertension and CKD[116]. Another study found that pararenal and perirenal fat thickness were independent predictors of CKD, increased renal resistive index and hyperuricaemia in patients with T2DM[117]. Renal sinus fat mass was also associated with increased albumin excretion rate during exercise in healthy individuals at increased risk of T2DM[118]. In insulin-resistant individuals with fatty liver, GFR was found to correlate inversely with increased renal sinus fat[105].

Effects of insulin on the kidney

As well as its putative actions on the renal vasculature, insulin can act on virtually all renal cell types, including mesangial cells, podocytes and tubular epithelial cells. Both isoforms of the insulin receptor are widely expressed in the kidney[32]. The kidney is unique in that insulin can access its target cells both from the lumen (in the case of podocytes) and from the basolateral side (in the case of tubular cells)[119]. Studies of insulin signalling in the kidney have focused on podocytes and tubular cells and have revealed a variety of effects, including glucose uptake, regulation of ion transport and prevention of apoptosis.

Glomerular endothelial cells

Glomerular endothelial cells express the insulin receptor and respond to insulin binding with increased NO generation, similar to the extrarenal vasculature[32]. Insulin signalling does not stimulate glucose uptake or remodelling of the actin cytoskeleton of glomerular endothelial cells. Owing to its fenestration, the glomerular endothelium is freely permeable to insulin, which can cross the basement membrane and enter the Bowman space and tubulus. Insulin-mediated endothelial NO signalling is, therefore, not a limiting factor for the transport of insulin to subendothelial cells, such as VSMCs (FIG. 4). This finding suggests that changes in insulin plasma concentrations readily affect renal mesangial cells and podocytes. Insulin-mediated NO generation could, therefore, contribute to the increase in GFR that occurs after a meal[120]. NO might also be an important player in the crosstalk between podocytes and mesangial cells, which are located adjacent to each other and separated only by the glomerular basement membrane.

Mesangial cells

In mesangial cells, insulin is a potent survival factor that confers protection from apoptotic stimuli through stimulation of the PI3K–Akt pathway[121] (FIG. 1A). Increased insulin-induced MAPK signalling has been shown to stimulate large conductance Ca^{2+}-activated K^+ (BKCa) channels, resulting in mesangial cell relaxation and possibly increased proliferation[122]. Impaired insulin signalling in mesangial cells might be associated with reduced GFR, as shown in a cross-sectional study of 670 individuals that investigated the effects of the Gly972Arg variant of IRS1 (REF. 123). Mesangial cells transfected with this variant exhibited attenuated insulin-stimulated phosphorylation of IRS-1 and Akt. Silencing of the insulin receptor in mesangial cells resulted in the formation of homodimeric IGF-1R and increased IGF-1 signalling[124], which could potentially lead to cell growth and proliferation. In mesangial cells from rats with streptozotocin-induced diabetes, insulin reversed the mitogenic action of IGF-1 by regulating signal transducer and activator of transcription 5A–suppressor of cytokine signalling 2 expression[125]. IGF1 signalling was increased in mesangial cells kept in insulin-deficient media, and resulted in increased cell proliferation and enhanced synthesis of fibronectin and collagen IV[126]. Furthermore, mesangial cells that overexpressed solute carrier family 2, facilitated glucose transporter member 1 (GLUT-1), produced fibronectin and collagen IV in the absence of insulin, indicating that elevated glucose utilization induces mesangial matrix production[127]. Together, these studies suggest that reduced insulin signalling in mesangial cells could contribute to mesangial cell hypertrophy, proliferation and matrix deposition, which are characteristic of early diabetic nephropathy.

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

Podocytes

Podocytes respond to insulin stimulation with an increase in PI3K and MAPK signalling, resulting in glucose uptake via GLUT-4, similar to skeletal muscle cells[33]. In addition to energy uptake, this response seems to underlie adaptive changes in 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 podocyte actin cytoskeleton[128]. Nephrin enables the insertion of vesicles that contain GLUT-1 and GLUT-4 into the plasma membrane by interacting with the vesicular SNARE protein vesicle-associated membrane protein 2. As nephrin expression is reduced with progression of diabetic nephropathy and albuminuria, insulin signalling is also expected to be reduced, and thus might negatively affect podocyte integrity.

The action of insulin on podocytes also includes upregulation of transient receptor potential cation channels and BKCa channels[129,130]. In podocytes isolated from insulin-resistant obese diabetic mice, failure of insulin to phosphorylate Akt was associated with reduced cell viability[131]. The strongest evidence for the *in vivo* relevance of insulin on podocyte function comes from mouse models with podocyte-specific deletion of the insulin receptor[132]. These knockout mice developed albuminuria at 8 weeks of age as a result of severe podocyte damage and loss of podocyte foot processes. At 13 weeks of age, light microscopy revealed podocyte loss, glomerulosclerosis and mesangial expansion, features that are characteristic of human diabetic nephropathy. Moreover, remodelling of the actin cytoskeleton was severely impaired, leading to loss of podocyte architecture and morphology. All of these changes occurred in the absence of hyperglycaemia, underscoring the role of normal insulin signalling and glucose uptake in podocyte health. A lack of insulin signalling in podocytes has also been found to impair PI3K-dependent translocation of the transcription factor X-box-binding protein 1 into the nucleus, resulting in maladaptive ER-stress signalling and podocyte dysfunction[133].

Tubular epithelial cells

The effects of insulin 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 loop of Henle in rabbits and dogs[134–136]. In addition, acute intravenously administered insulin stimulated reabsorption of phosphate, potassium and water[136,137]. By contrast, in rats chronic infusion of insulin over 5 days transiently reduced sodium excretion on the first day, but was associated with an increase in blood pressure[138]. Extensive studies in dogs under various conditions such as insulin resistance, chronic adrenaline infusion, reduced kidney mass, high-salt intake or chronic angiotensin II infusion did not demonstrate that chronic insulin administration causes sustained sodium retention or hypertension[139]. Much of the controversy as to whether insulin has antinatriuretic effects was resolved when it became clear that hyperglycaemia is required to induce the sodium-retaining effect of hyperinsulinaemia, indicating that the euglycaemic–hyperinsulinaemic clamp is not an appropriate model to study the pathogenesis of hypertension in hyperglycaemic patients with diabetes[139]. When sustained hyperglycaemia and hyperinsulinaemia were induced at the same time, formerly resistant dogs developed sodium retention during chronic insulin administration that was sufficient to antagonize the natriuresis caused by hyperglycaemia[140].

Distal tubule. In cells that express the epithelial sodium channel (ENaC), insulin stimulates Na^+ transport by upregulating the abundance of membrane ENaC, an effect that is dependent on PI3K activity[141]. Increased PI3K signalling by insulin leads to activation of mTORC2 and serum/glucocorticoid-regulated kinase 1 (SGK1), which are essential serine-threonine kinases involved in the regulation of ENaC in the distal tubule (FIG. 7). SGK1 is heavily upregulated by aldosterone on a transcriptional and activity level via activation of PI3K and PDK1. In turn, SGK1 increases ENaC membrane expression by inhibiting its ubiquitin ligase Nedd4-2-mediated internalization and degradation[142]. Elevated glucose concentration also increases *SGK1* gene expression; this increase might be one of the critical factors that underlies the stimulation of sodium retention in T2DM[143]. An increase in SGK1 expression in response to hyperglycaemia would also explain the development of sodium retention upon chronic insulin infusion in dogs[140].

Surprisingly, knockout mice with deletion of the insulin receptor in the distal tubule (using the Cre-loxP recombination system and a kidney-specific cadherin promoter) developed hypertension and impaired natriuresis following a sodium load[144]. The researchers suggested that a reduction in renal NO signalling as a result of absent insulin signalling could account for this finding. A subsequent study confirmed that reduced insulin signalling in the knockout mice conferred salt sensitivity of blood pressure by reducing renal NOS expression[145]. In a second mouse model with deletion of the insulin receptor in the collecting duct using an aquaporin-2 promoter, 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 insulin receptor deletion and abrogated by inhibition of PI3K and mTOR[147]. Together, the available data confirm that insulin acts as an antinatriuretic hormone in the distal tubule.

Proximal tubule. Insulin signalling in the proximal tubule is thought to affect renal gluconeogenesis, which accounts for up to 25% of the glucose released into the circulation[148]. Reduced insulin signalling could, therefore, lead to an increase in gluconeogenesis, which would in turn contribute to hyperglycaemia. In mice with deletion of the insulin receptor in the proximal tubule (driven by the glutamyltransferase promoter), fasting glucose levels

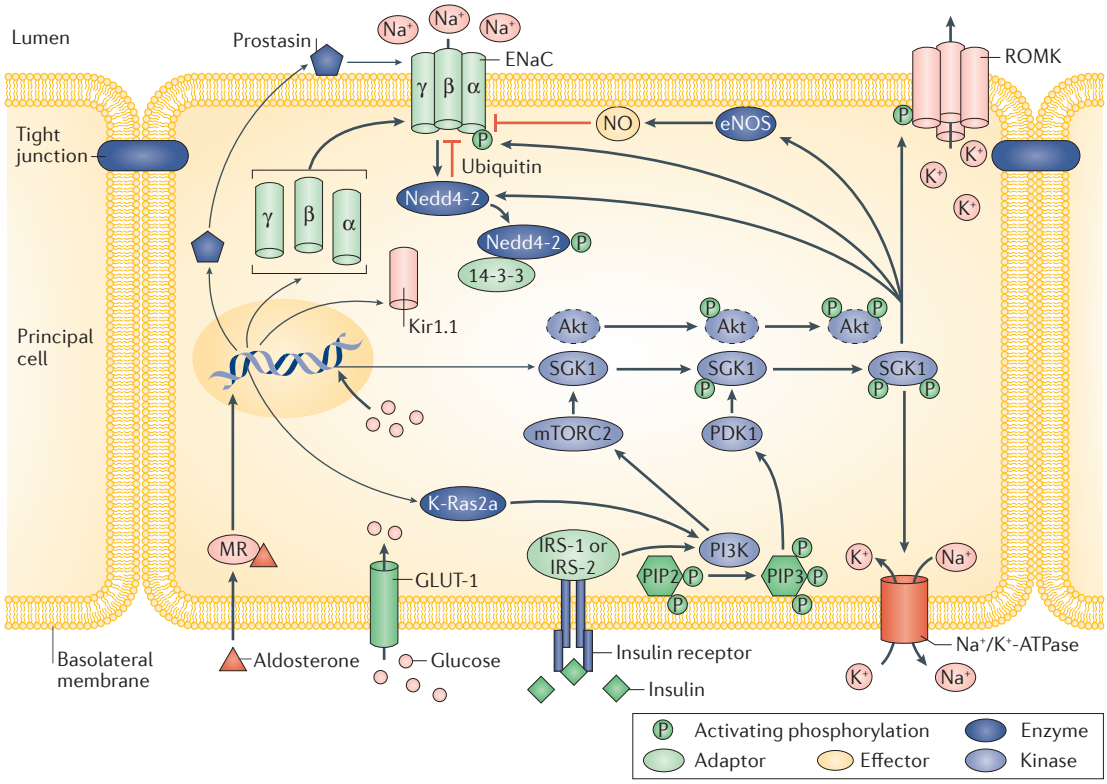

Figure 7 | **Insulin signalling in the principal cell of the aldosterone-sensitive distal nephron.** Serum-and glucocorticoid-inducible kinase 1 (SGK1) is the central effector of insulin and aldosterone in the distal tubule. SGK1 expression is rapidly induced on a transcriptional level by aldosterone–mineralocorticoid receptor (MR) signalling and by hyperglycaemia. Insulin signalling leads to activation of SGK1 via sequential phosphorylation at Ser422 by mTOR complex 2 (mTORC2)[164] and Thr256 by phosphatidylinositide-dependent protein kinase 1 (PDK1). Activated SGK1 increases epithelial sodium channel (ENaC) membrane abundance and currents via direct and indirect effects, including inhibition of the ubiquitin ligase Nedd4-2. SGK1 also stimulates the renal outer medullary potassium channel (ROMK) and the Na/K-ATPase, resulting in increased Na transport. Akt1 is of minor importance as an effector of insulin in the principal cell, although it is similarly phosphorylated and has the same downstream targets as SGK1. IRS, insulin receptor substrate; Kir1.1, inward rectifier potassium channel 1; GLUT-1 glucose transporter 1; K-Ras2a, KRAS proto-oncogene; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PI3K, phosphatidylinositol 3-kinase; 14-3-3, 14-3-3 protein.

were elevated and the activity of glucose-6-phosphatase (the enzyme that catalyses the final step of gluconeogenesis and is responsible for the 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 showed disinhibition of 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), which is accomplished by sodium/glucose cotransporter (SGLT) 1 and SGLT2; the latter cotransporter contributes to 97% of renal glucose reabsorption[104]. SGLT2-mediated transport is upregulated by insulin and involves phosphorylation of SGLT2 via insulin receptor signalling[151]. Upregulation of SGLT2 activity by insulin might serve to minimize postprandial glucose losses. The renal expression of SGLT2 in animal models of diabetes does not follow a clear pattern and is model dependent[152]. Glucose reabsorption is increased in patients with T2DM[153], however, suggesting that SGLT2 upregulation is not affected by insulin-resistance in this disease.

Effects of insulin receptor deletion

Mouse models with insulin receptor deletion are thought to represent the most extreme form of insulin resistance. However, the validity of the results obtained with these models with regard to understanding insulin resistance in humans is not always clear. Even under conditions of severe insulin resistance in humans, complete disruption of insulin receptor signalling does not occur. Moreover, the models are often equivocal and do not fully agree with the complex situation seen in patients; some findings are unexpected and conflicting. Mice with constitutive deletion of the insulin receptor are massively hyperglycaemic and die of diabetic ketoacidosis within a few days after birth, despite developing hyperinsulinemia[154,155]. By contrast, patients with genetic deficiency of the insulin receptor exhibit fasting hypoglycaemia, retarded growth and develop ketoacidosis

only after a meal[156]. Mice with specific deletion of the insulin receptor in skeletal muscle, which in humans is thought to be insulin resistant from the early stages of diabetes, were normoglycaemic and not hyperinsulinemic[157]. These mice had increased fat cell mass and obesity, however, suggesting a redirection of energy and fat storage similar to the situation seen in humans. Deletion of the hepatic insulin receptor resulted in severe fasting hyperglycaemia, disinhibition of gluconeogenesis and glucose intolerance[158]. These changes again resemble the human situation given the importance of the fatty liver to insulin resistance[61].

Data from mice with deletion of the insulin receptor in various renal cell types clearly have to be interpreted with great caution. Contrary to the expectation that reduced insulin signalling might cause natriuresis, the first published mouse model using an insulin-receptor deletion under the Ksp promoter showed reduced sodium excretion[144]. Subsequent studies with a more defined model involving deletion of ENaC in the distal tubule fitted better with the role of insulin in promoting sodium retention[146,147]. In contrast to patients with diabetic nephropathy, the deleterious effect of insulin-receptor deletion in podocytes has been shown to occur in the setting of 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, insulin-receptor-knockout models only highlight single distinct aspects of human insulin resistance, either within an organ, or in the whole organism, and represent a small piece of the complex mosaic underlying systemic insulin resistance.

Renal insulin resistance

Despite compelling evidence that the kidney is an insulin-responsive organ in which insulin regulates various functions, whether the kidney in general or in part is affected by insulin resistance similarly to the classical target organs is not clear. The human kidney abundantly expresses insulin receptor B, which is the isoform found in classically insulin-responsive organs[9], and glucose uptake is insulin-dependent in some renal cells (such as podocytes), but not in others (such as tubule and mesangial cells).

In rats with insulinopenic and insulin-resistant diabetes, insulin signalling, as represented by Akt phosphorylation, was reduced only in isolated glomeruli, whereas tubuli were not affected[159]. In glomeruli, the phosphorylation of other downstream effectors of insulin, such as eNOS and GSK3α, were similarly reduced. However, no change in MAPK phosphorylation was found, indicating resistance only of the IRS-1–PI3K pathway of insulin signalling (FIG. 1A). Moreover protein expression of IRS-1 was reduced but could be restored by inhibition of PKCβ. Together these data indicate that glomeruli, but not tubuli, can develop insulin resistance. The study did not determine whether glomerular insulin resistance is confined to endothelial cells or also involves podocytes and mesangial cells or whether insulin sensitivity was retained in the whole tubulus or only in parts.

Tubular sodium transport

If the tubular epithelium is not affected by insulin resistance, hyperinsulinaemia will lead to stimulation of renal sodium transport and promote sodium retention and salt-sensitive hypertension, features that are frequently encountered in obese patients with metabolic syndrome. Indeed, insulin infusion during a euglycaemic–hyperinsulinaemic clamp induced a similar reduction of sodium excretion in obese adolescents[160] and in patients with T2DM, compared to healthy individuals, whereas the effect on peripheral glucose uptake was reduced only in insulin-resistant obese and diabetic patients[161]. The site of sodium retention during hyperinsulinaemia is the distal tubule, and this retention involves stimulation of ENaC by PI3K–SGK1 (FIG. 7). Sodium retention can, however, also involve the proximal tubule, in which the stimulatory effect of insulin on sodium transport is preserved in rat models and in patients with T2DM[162,163]. Sodium retention in the proximal tubule was associated with preserved expression of IRS-2 and Akt phosphorylation, whereas IRS-1 expression was reduced in the renal cortex. The expression of transcription factors such as FoxO1 and SREBP-1, which are involved in the downregulation of IRS-2 in the fatty liver, was also preserved, indicating subtle differences in altered insulin signalling during insulin-resistance in the liver and the kidney.

Importantly, in addition to hyperinsulinaemia, hyperglycaemia stimulates sodium transport in the proximal and distal nephron by transcriptional upregulation of *SGK1*, which is activated by consecutive insulin-dependent and PI3K-dependent phosphorylation at two sites[142,143,164]. Hyperinsulinaemia and hyperglycaemia converge to enhance sodium transport in the distal tubule and to promote net sodium retention, which might override hyperglycaemia-induced, osmosis-driven diuresis in the proximal tubule[140].

Renal gluconeogenesis

With the exception of periportal hepatocytes, only proximal tubular cells are capable of gluconeogenesis, which is suppressed by insulin[165]. In patients with T2DM, both renal and hepatic gluconeogenesis are increased and contribute to hyperglycaemia during the fasting state, termed impaired fasting glucose[166]. This finding suggests that the proximal tubule is resistant to the suppressive action of insulin on gluconeogenesis, as is the case in the liver. This hypothesis is consistent with findings in insulin-resistant rodents with specific deletion of the insulin receptor in the proximal tubule, which show disinhibition of gluconeogenesis[149,150]. By contrast, tubular sodium transport seems to be spared from insulin-resistance; this finding can be explained by differences in intracellular signalling, such as alterations in the expression of IRS-1 and IRS-2 (REF. 163) and/or the involvement of SGK1, which regulates a variety of sodium channels. Reduced IRS-1 expression might be responsible for

Kimmelstiel–Wilson lesions
The typical histopathological hallmark of diabetic nephropathy, which is characterized by nodular glomerulosclerosis.

Impaired fasting glucose
Defined as a plasma glucose concentration of 110–126 mg/dl (6.11–6.99 mmol/l) in the fasting state.

Oral glucose tolerance test
Test used to screen for disturbances in glucose metabolism and insulin resistance.

Liver steatosis
Accumulation of excess fat in the liver.

disinhibition of gluconeogenesis [162] and reduced NO production[159], whereas preserved IRS-2 expression seems to maintain stimulation of sodium transport in the proximal tubule. This evidence is supported by a study using IRS-1-knockout and IRS-2-knockout mice that showed attenuated stimulation of sodium transport exclusively in mice with deletion of IRS-2 (REF. 167).

Treatment of insulin resistance

Weight loss

The strong association between obesity and insulin resistance suggests that lifestyle changes that result in weight loss and increased muscle mass might restore whole-body insulin sensitivity. Indeed, lifestyle interventions (increased physical activity and decreased caloric intake) improved insulin sensitivity (estimated using an oral glucose tolerance test) and were associated with reductions in total, visceral and ectopic fat mass in individuals with impaired or normal glucose tolerance[168]. MRI studies showed that visceral adipose tissue and hepatic lipid levels can be significantly reduced by lifestyle interventions and that their baseline values are predictive of improvement in insulin sensitivity[169,170].

Similar to the improvement in whole-body insulin sensitivity, renal and vascular insulin resistance is expected to improve with lifestyle intervention. Studies focusing on the insulin sensitivity of renal and vascular function are rare, however, and cannot really discriminate between systemic and organ-specific effects. The available lifestyle intervention studies with renal end points showed a reduction in glomerular hyperfiltration and albuminuria, suggesting normalization of afferent vasodilatation, filtration pressure and possibly podocyte dysfunction[171]. These interventions are also thought to improve salt sensitivity and to reduce blood pressure[172], suggesting an effect on tubular sodium handling. With regard to vascular insulin resistance, lifestyle interventions improved both the macrocirculation and microcirculation, as indicated by increased flow-mediated vasodilation in the brachial artery and insulin-induced vasodilation in cutaneous vessels[173,174]. Weight loss after lifestyle intervention is not uniformly associated with improved insulin resistance, however, and a substantial proportion of patients do not respond to such intervention, possibly as a result of persistent visceral obesity and, more importantly, liver steatosis[170,175]. Bariatric surgery results in a large loss of body weight so is more effective than lifestyle intervention in improving insulin sensitivity, but this treatment can only be considered in patients with morbid obesity[176].

Pharmacological treatment

Metformin and PPARγ agonists. Insulin resistance can be improved by treatment with metformin through an as yet unclear mechanism, and with thiazolidinediones, which activate peroxisome proliferator-activated receptor γ (PPARγ). Metformin is increasingly being recommended in the very early stages of insulin resistance and pre-diabetes, whereas treatment with thiazolidinediones, such as pioglitazone, is indicated in patients with overt diabetes[177]. These agents lower blood glucose concentration predominantly by stimulating lipogenesis in the white adipose tissue, while reducing hyperinsulinaemia, suggesting that they are insulin-sensitizers. Metformin predominantly affects the liver, whereas pioglitazone acts on the vasculature and the kidney. Pioglitazone has direct effects on various cell types, including endothelial cells, VSMCs, podocytes and tubular cells. Treatment with this agent improves endothelial function and lowers blood pressure, probably by a direct vasodilatory effect[178].

In patients with diabetes, several studies have shown significant reductions in albuminuria with thiazolidinediones. A meta-analysis of these studies reported that such treatment reduced albuminuria by 25–65%, depending on whether spot urine samples or collected urine samples were analysed[179]. In the randomized controlled DREAM trial, which included 5,269 patients with pre-diabetes (defined as impaired fasting glucose and/or impaired glucose tolerance), rosiglitazone treatment significantly reduced the occurrence of renal outcomes over 3 years of follow-up (12.3% in the intervention group versus 15.0% in the placebo group; HR 0.82, 95% CI 0.69–0.98), predominantly by reducing the progression of albuminuria[180]. The incidence of estimated GFR loss ≥30% was also reduced with borderline significance (3.15% in the intervention group versus 4.0% in the placebo group; HR 0.77, 95% CI 0.58–1.04). However, rosiglitazone increased the risk of developing heart failure (0.53% in the intervention group versus 0.08% in the placebo group; HR 7.04, 95% CI 1.60–31.0), which is the most severe adverse effect of thiazolidinedione treatment and has limited its widespread use. This complication is caused by stimulation of sodium retention by the kidney, involving activation of ENaC and upregulation of SGK1 (REF. 181).

Another clinically important effect of thiazolidinediones 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. Pioglitazone was found to suppress mRNA and protein expression of fetuin-A in the Fao hepatoma cell line[182]. Interestingly, rosiglitazone, but not metformin, also inhibited fetuin-A expression in this study. In addition, the PPARγ inhibitor GW 9662, reversed pioglitazone-induced suppression of fetuin-A[182]. These data suggest that thiazolidinedione derivatives might have common characteristics with regard to fetuin-A suppression, possibly through PPARγ activation. In agreement with these findings, pioglitazone, but not metformin or exercise, decreased fetuin-A levels during a 6-month intervention period, although similar beneficial effects on insulin sensitivity were reported in the three groups[183].

Insulin. An important pathophysiological factor that causes insulin resistance is elevated plasma glucose levels. The association between increased blood glucose levels and insulin resistance is called glucotoxicity, or more accurately glucolipotoxicity[184,185]. As a result of chronic hyperglycaemia, insulin signalling is inhibited, which is also seen in patients with T1DM[186]. Glucolipotoxicity is not only associated with insulin resistance

Homeostasis model assessment of insulin resistance
A simple quantitative measure of insulin resistance calculated from the plasma fasting glucose level and insulin concentration.

but also with β-cell dysfunction. A therapeutic approach to hyperglycaemia in T2DM is insulin treatment to reverse glucotoxicity, which results in recovery of residual β-cell function and improvement of insulin resistance. The ORIGIN randomized controlled trial investigated early treatment with long-acting insulin in patients with dysglycaemia including pre-diabetes and T2DM[187]. The results showed that early insulin treatment can prevent the manifestation and exacerbation of T2DM[188] and significantly reduce the incidence of eye and kidney disease in patients with a baseline haemoglobin A_{1c} level >6.4%[189].

SGLT2 inhibitors. SGLT2 inhibitors might be a promising new pharmacological approach to overcome insulin resistance. These drugs inhibit proximal tubular glucose reabsorption in an insulin-independent manner and can induce glucose losses of 50–150 g in patients with diabetes[190], depending on the level of hyperglycaemia. Although SGLT2 inhibitors lack a systemic effect, studies in humans and animals suggest that these drugs improve insulin sensitivity. In a small study that included 12 patients with T2DM, treatment with the SGLT2 inhibitor dapagliflozin improved peripheral glucose uptake (analysed using a euglycaemic–hyperinsulinaemic clamp) and reduced plasma insulin concentrations[191]. The effects of dapagliflozin occurred within 2 weeks of treatment, well before the induction of substantial weight loss. These findings might be explained by immediate correction of hyperglycaemia by SGLT2 inhibition, leading to regression of glucotoxicity.

In a study that included 66 patients with T2DM, the effects of SGLT2 inhibition on insulin secretion and peripheral glucose uptake were detectable even after a single dose of empagliflozin[190]. This finding can also be explained by the beneficial effects of immediate lowering of plasma glucose concentration with a resulting reduction in glucotoxicity. 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 homeostasis model assessment of insulin resistance. In addition to possibly improving insulin sensitivity, SGLT2 inhibitors have beneficial effects on cardiovascular outcomes[192] and on renal end points[193] via hitherto poorly defined mechanisms. These drugs are emerging as a new therapeutic approach to treat patients with insulin resistance and T2DM.

Conclusions

Renal and vascular insulin resistance develops as part of the complex mosaic of systemic insulin resistance and affects organ-specific functions and organ crosstalk. Dysregulation of insulin-regulated pathways in the kidney and vasculature culminate in and sustain the pathophysiological alterations found in metabolic syndrome, such as reduced endothelial function, increased sodium retention and renal gluconeogenesis. These changes occur early in the course of the development of T2DM and determine subsequent renal and vascular damage. To prevent microangiopathic and macroangiopathic diseases, interventions in patients with insulin resistance are required to halt the progression of, or even reverse, pathophysiological alterations. Identification of molecular mechanisms that promote renal and vascular insulin resistance is warranted to better understand organ crosstalk involving classical and non-classical insulin-responsive organs.

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The authors declare no competing interests.