Effects of insulin resistance on endothelial progenitor cells and vascular repair

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ABSTRACT
Insulin resistance, a key feature of obesity, the metabolic syndrome and Type 2 diabetes mellitus, results in an array of metabolic and vascular phenomena which ultimately promote the development of atherosclerosis. Endothelial dysfunction is intricately related to insulin resistance through the parallel stimulatory effects of insulin on glucose disposal in metabolic tissues and NO production in the endothelium. Perturbations characteristic of insulin resistance, including dyslipidaemia, inflammation and oxidative stress, may jeopardize the structural or functional integrity of the endothelium. Recent evidence suggests that endothelial damage is mitigated by endogenous reparative processes which mediate endothelial regeneration. EPCs (endothelial progenitor cells) are circulating cells which have been identified as mediators of endothelial repair. Several of the abnormalities associated with insulin resistance, including reduced NO bioavailability, increased production of ROS (reactive oxygen species) and down-regulation of intracellular signalling pathways, have the potential to disrupt EPC function. Improvement in the number and function of EPCs may contribute to the protective actions of evidence-based therapies to reduce cardiometabolic risk. In the present article, we review the putative effects of insulin resistance on EPCs, discuss the underlying mechanisms and highlight potential therapeutic manoeuvres which could improve vascular repair in individuals with insulin resistance.

INTRODUCTION
Insulin resistance denotes the metabolic events which result in the disordered glucose homoeostasis encountered in several conditions, including obesity, the metabolic syndrome, pre-diabetes and Type 2 diabetes mellitus. Insulin resistance is associated with impaired downstream signal transduction when insulin binds to its receptor, so reducing glucose uptake in metabolic tissues. However, the metabolic effects of these molecular abnormalities have wider implications than disordered blood glucose regulation alone: dyslipidaemia, inflammation and a pro-thrombotic tendency are also hallmarks of insulin-resistant states [1]. The combined effect of these factors in insulin-resistant subjects results in a significantly increased risk of cardiovascular events [2–4]. Although many questions remain regarding the natural history of atherosclerosis, it is commonly accepted that

Key words: cardiometabolic risk, endothelial progenitor cell (EPC), insulin resistance, obesity, metabolic syndrome, Type 2 diabetes.
Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ADMA, asymmetric \(\omega^N,-N^G\)-dimethylarginine; Apoe, gene encoding apolipoprotein E; ARB, angiotensin II type 1 receptor blocker; BH4, tetrahydrobiopterin; CXCR4, CXC chemokine receptor 4; EPC, endothelial progenitor cell; G-CSF, granulocyte colony-stimulating factor; GH, growth hormone; HOMA-IR, homoeostasis model assessment of insulin resistance; IGF, insulin-like growth factor; MAPK, mitogen-activated-protein kinase; MMP, matrix metalloproteinase; NEFA, non-esterified fatty acid; NOS, NO synthase; eNOS, endothelial NOS; PDK, pyruvate dehydrogenase kinase; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome-proliferator-activated receptor; RAS, renin–angiotensin system; ROS, reactive oxygen species; SDF, stromal-cell-derived factor; TNF-\(\alpha\), tumour necrosis factor-\(\alpha\); VEGF, vascular endothelial growth factor.
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dysfunction of the vascular endothelium represents the earliest manifestation of the disease [5]. A closely coupled relationship between insulin resistance and endothelial dysfunction is now supported by a wealth of observational and mechanistic studies (for a review, see [6]). In health, the damaging effects incurred through exposure to risk factors are mitigated by endogenous processes which regenerate damaged endothelium and preserve the structural and functional integrity of the endothelial monolayer [7]. EPCs (endothelial progenitor cells), a rare population of circulating mononuclear cells, have been the subject of intense scrutiny as mediators of endothelial repair. In the present review, we highlight the components of the insulin-resistance phenotype, which may influence EPC-mediated endothelial repair, and summarize biochemical perturbations which potentially link insulin resistance and EPC dysfunction. This association is supported by indirect evidence at present, and we discuss further studies which are required to substantiate insulin resistance as a determinant of EPC function in vivo. We also review treatment options which may improve endothelial repair and highlight the remaining questions which require further research.

**INSULIN RESISTANCE AND CARdiovascular RISK**

Although genetic factors are likely to contribute to insulin resistance, nutritional excess and an increasingly sedentary lifestyle have contributed to a global rise in insulin-resistant disorders [8]. Increasing adiposity, particularly when concentrated within visceral deposits, promotes a pro-inflammatory state through the release of detrimental cytokines (or adipokines) [9]. These, along with adipose-tissue-derived NEFAs (non-esterified fatty acids; ‘free’ fatty acids), interfere with insulin signalling in the canonical insulin-responsive tissues [6]. As whole-body insulin sensitivity declines, pancreatic insulin secretion initially increases to maintain normoglycaemia. During this asymptomatic phase, hypertension, dyslipidaemia, inflammation and a prothrombotic state develop. Hence the vasculature may be exposed to an atherothrombotic biochemical milieu for many years [10], and only when pancreatic β-cells fail to secrete sufficient insulin to compensate for progressive insulin resistance will the individual be likely to develop symptoms related to hyperglycaemia. Globally, around 300 million people are anticipated to develop Type 2 diabetes by 2025 [8]; this represents a doubling of prevalence over 30 years, and is of major concern because of the 2–3-fold increase in cardiovascular risk associated with the condition [10,11]. The factors by which Type 2 diabetes promotes atherosclerosis are complex, and hyperglycaemia almost certainly contributes to vascular damage at this extreme of the spectrum of insulin-resistant disorders. However, cardiovascular risk correlates with insulin resistance in its milder forms: obesity, pre-diabetes and the metabolic syndrome are also associated with increased cardiovascular risk [12,13]. As around a quarter of ‘Western’ adult populations meet criteria for the metabolic syndrome, the public health implications of insulin resistance are of particular concern [14].

**INSULIN RESISTANCE AND ENDOTHELIAL DYSFUNCTION**

The monolayer of endothelial cells lining blood vessels subserves both mechanical and functional roles. A range of molecules produced by endothelial cells modulates a variety of homoeostatic processes, including vascular tone, platelet activation and thrombosis [5]. NO, produced from L-arginine by eNOS [endothelial NOS (NO synthase)], is perhaps the most important of these compounds, possessing vasodilatory, anti-inflammatory, antiplatelet and antioxidant properties [15–18]. NO is rapidly inactivated in the vessel wall by ROS (reactive oxygen species). The relative production by endothelial cells of NO and ROS is, therefore, of critical importance in vascular homoeostasis [19]. Other endothelium-derived molecules, such as endothelin, exert opposing effects [20]. Exposure to cardiovascular risk factors modifies this balance: the resulting endothelial dysfunction leads to vasoconstriction, platelet activation and inflammation, and ultimately promotes atherosclerosis.

Endothelial dysfunction is a consistent finding across the spectrum of insulin-resistant states. Individuals with Type 2 diabetes, obesity and the metabolic syndrome, and insulin-resistant men of South Asian origin all have endothelial dysfunction characterized by reduced NO bioavailability [21–26]. Co-existing vascular risk factors and many of the biochemical changes associated with insulin resistance, including inflammation, ROS, altered secretion of cytokines by adipocytes and increased NEFAs, may contribute to endothelial dysfunction indirectly [27–35]. Animal models allow the specific effects of insulin resistance to be studied in the absence of many of these confounding factors [36]. In mice with haploinsufficiency of the insulin receptor, which are glucose-competent, a modest decline in insulin signalling results in substantially impaired endothelial NO production and increased endothelial ROS production, highlighting the potential impact of insulin resistance on the vasculature in ‘pre-diabetic’ states [30,37]. Given that insulin promotes NO production by endothelial cells, such studies also suggest that ‘insulin resistance’ in endothelial cells may contribute to vascular pathology [38].

Insulin stimulates NO production in endothelial cells by a similar signalling pathway to which it promotes glucose uptake in liver, adipose tissue and skeletal muscle
Effect of insulin resistance on EPCs and vascular repair

Cumulative exposure to risk factors, such as insulin resistance, results in structural or functional damage to the endothelium by inducing biochemical cellular toxicity or promoting endothelial cell loss by apoptosis [49,50]. Endogenous reparative processes are now thought to mitigate this damage by regenerating injured endothelium. Mature endothelial cells have a finite proliferative capacity and have limited potential for repair. In 1999, however, Asahara et al. [51] discovered that a rare population of circulating cells, which express the haemopoietic progenitor marker CD34, could develop an endothelial phenotype in vitro when exposed to angiogenic growth factors. These cells, subsequently described as EPCs, were shown to promote angiogenesis in vivo when re-transplanted after hindlimb ischaemia. These findings challenged the long-held dogma that new vessel formation and endothelial regeneration in adults result solely from proliferation of mature endothelial cells, a process termed angiogenesis. Instead, potential for the embryonic process of vasculogenesis, in which blood vessels are formed de novo from angiogenic progenitor cells, appeared to be retained in adulthood. This hinted at persistence in adult animals of haemangioblasts, a putative common embryonic precursor of endothelial and haematological cells. Although a common precursor has not, to date, been detected in adult humans, the discovery of EPCs has stimulated intense interest in the role of these cells both in terms of new vessel formation and in the regeneration of damaged endothelium.

The expression of haemopoietic progenitor markers by EPCs suggests that they originate from bone marrow. This has been confirmed in animals and humans following bone marrow transplantation [51,52], although more recent work has also suggested the existence of tissue resident EPCs [53]. Mobilization of EPCs from the bone marrow into the circulation occurs in response to growth factors and cytokines, including VEGF (vascular endothelial growth factor), SDF-1α (stromal-cell-derived factor-1α), erythropoietin and oestrogens [54]. Vascular injury, particularly when associated with tissue ischaemia, is a potent mobilization stimulus, acting through the release of cytokines and chemokines in response to hypoxia [55–57]. Both VEGF and SDF-1α up-regulate bone marrow MMP-9 (matrix metalloproteinase-9) activity, which cleaves the progenitor cell membrane-bound kit ligand, allowing mobilization of progenitors into the bone marrow vascular zone [54]. Nitrosylation of MMP-9 by NO, released from bone marrow stromal cells, is required for VEGF-stimulated EPC mobilization [54].

After mobilization into the circulation, EPCs home or migrate toward regions of endothelial injury, where they adhere and proliferate before facilitating vascular repair. Chemokine signalling plays a major role in directing circulating progenitor cells to sites of injury. Up-regulation
Table 1  Summary of animal studies investigating EPCs in disorders of glucose regulation

<table>
<thead>
<tr>
<th>Study type</th>
<th>Principal findings</th>
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<tbody>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
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<tr>
<td>db/db genetic model of Type 2 diabetes/obesity</td>
<td>‘Late’ EPCs from db/db mice had reduced capacity to survive hypoxia and oxidant stress, and had impaired potential to promote vascularization of skin wounds. Diabetes was associated with impaired EPC migration and adhesion, reduced expression of VEGF and eNOS in EPCs, and reduced re-endothelialization after vascular injury. Up-regulation of thrombospondin-1 expression in EPCs may have accounted for some of these observations. Diabetes was associated with reduced numbers of bone-marrow-derived EPCs, which had increased ROS and impaired capacity to differentiate to form endothelial cells. These parameters were improved by systemic antioxidant treatment with SOD.</td>
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<tr>
<td>STZ-induced diabetes</td>
<td>Diabetes reduced the release of SDF in wounds, reduced circulating EPCs and impaired EPC mobilization due to reduced bone marrow eNOS phosphorylation. Hyperoxia and SDF administration acted synergistically to abrogate these defects. Decreasing intracellular superoxide by transgenic overexpression of MnSOD prevented the reduced eNOS and CXCR4 expression usually seen in diabetic EPCs exposed to hypoxia or hyperglycaemia.</td>
</tr>
<tr>
<td><strong>Rat</strong></td>
<td></td>
</tr>
<tr>
<td>STZ-induced diabetes</td>
<td>Diabetic rats failed to mobilize EPCs after hindlimb ischaemia/reperfusion injury. This was associated with a failure to up-regulate muscular HIF, and altered plasma SDF/VEGF response. Insulin, SCF and G-CSF pre-treatment partially corrected these defects.</td>
</tr>
<tr>
<td>Diet-induced insulin resistance</td>
<td>Olmesartan and pravastatin increased EPCs and reduced wire-injury-induced neointimal hyperplasia in insulin-resistant rats.</td>
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of SDF-1α in ischaemic tissues is driven largely by tissue hypoxia [58]. Interaction between SDF-1α and CXCR4 (CXC chemokine receptor 4) then facilitates homing of EPCs to sites of injury. EPCs contribute to endothelial repair by two principal mechanisms: (i) by proliferating to form new endothelial cells, or (ii) by releasing an array of pro-angiogenic cytokines and growth factors which stimulate the proliferation of other EPCs or local mature endothelial cells [59,60].

Although the contribution of EPCs to new vessel formation has been the main focus of research for many investigators, several lines of evidence support a homoeostatic role of EPCs in maintaining endothelial integrity in adult animals and humans. Infusion of EPCs into Apoe<sup>−/−</sup> mice (mice lacking apolipoprotein E), which are prone to the development of atherosclerosis, improves endothelial function [61]. EPC infusion in these mice has also been shown to reduce atherosclerosis [62], although the evidence is conflicting and, in another study, EPC transfusion increased lesion size and impaired plaque stability [63]. On balance, however, these findings suggest that EPCs participate in background endothelial repair, a concept supported by analysis of gene expression profiles during the development of atherosclerosis in Apoe<sup>−/−</sup> mice [64]. In humans, indices of EPC number correlate with endothelial dysfunction [65], and reduced numbers of EPCs predict future cardiovascular events [66]. EPC infusions in rodents have been shown to augment endothelialization at sites of mechanical endothelial damage within large conduit vessels, such as after experimental wire injury [67]. Other recent work in Apoe<sup>−/−</sup> mice supports the concept of EPC-mediated endothelial repair, demonstrating that at sites of rapid endothelial turnover, such as vessel bifurcations, 3–4 % of endothelial cells co-express haemopoietic progenitor markers [68].

**EPCs AND INSULIN RESISTANCE**

Disorders of glucose regulation are associated with abnormalities in EPC biology, including reduced circulating numbers of EPCs, defective mobilization from bone marrow and impaired functional properties of EPCs implicit to their capacity to mediate endothelial repair. Tables 1 and 2 summarize studies carried out in animal models and humans respectively, which have investigated the effects of disorders of blood glucose regulation on EPCs.

Flow cytometric and cell culture analyses demonstrate consistently fewer circulating EPCs across the spectrum of insulin-resistant states. Individuals with Type 2 diabetes have reduced levels of circulating EPCs, which are correlated with disease severity [57,69–71]. Hyperglycaemia may partially explain this association, as Fadini et al. [72] demonstrated reduced numbers of EPCs in individuals with impaired glucose tolerance compared with those with normal glucose regulation. EPCs were...
negatively correlated with glucose levels after a glucose challenge, although a potential effect of insulin resistance on EPCs could not be assessed in that study as plasma insulin levels were not measured. EPCs were, however, negatively correlated with components of the metabolic syndrome and with HOMA-IR (homeostasis model assessment of insulin resistance), a score of insulin resistance, in a separate study of subjects across a wide range of cardiovascular risk [73]. EPCs were also found to be lower in another study of obese men with the metabolic syndrome compared with non-obese healthy controls [74]. In that study, EPCs were associated with BMI (body mass index) and correlated inversely with components of the metabolic syndrome, but insulin resistance was not studied separately. In a recent study of healthy men of South Asian descent, EPCs were significantly reduced compared with white European controls [24]. South Asian men were more insulin-resistant than white European participants, raising the possibility that insulin resistance may contribute to the effect of ethnicity on EPCs, although the HOMA-IR score was not found to be an independent predictor of EPC numbers in multiple regression analysis.

Reduced circulating EPCs may be attributable to a number of factors, including defective mobilization, decreased proliferation and shortened survival in the circulation [75–77]. As discussed in further detail below, insulin resistance is closely associated with abnormalities in NO bioavailability and PI3K/Akt signalling, both of which play a crucial role in EPC mobilization from the bone marrow [75,77–81].

After mobilization from bone marrow, effective homing to sites of endothelial injury, adhesion and integration into the endothelial layer, proliferation and

### Table 2: Summary of human studies investigating EPCs in disorders of glucose regulation

<table>
<thead>
<tr>
<th>Study type</th>
<th>Principal findings</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>In vivo study in patients with Type 2 diabetes</td>
<td>G-CSF-mobilized autologous PB-MNCs, administered by multiple intramuscular injections, augmented indices of limb perfusion in diabetic patients with peripheral arterial disease when compared with patients randomized to no therapy.</td>
<td>[157]</td>
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<tr>
<td>Cell culture from patients with Type 2 diabetes</td>
<td>Diabetes was associated with impaired EPC proliferation, adhesion to endothelial monolayers and incorporation into endothelial tubular capillary-like networks. Rosiglitazone increased EPC numbers and migratory function. Pre-treatment, diabetes was associated with similar EPC numbers, but with impaired migratory function. EPCs from patients with diabetes had blunted migration towards SDF and VEGF due to structural rigidity. These defects were improved using an NO donor. Oral hypoglycaemic agents or insulin increased EPC numbers in patients with newly diagnosed Type 2 diabetes. Prior to treatment, diabetes was associated with similar EPC numbers, but with impaired migratory function.</td>
<td>[57, 141, 87, 143, 71, 141, 115, 143, 110]</td>
</tr>
<tr>
<td>FACS enumeration of EPCs from patients with the metabolic syndrome</td>
<td>Components of the metabolic syndrome were associated with fewer EPCs and acted synergistically in reducing EPC count.</td>
<td>[73]</td>
</tr>
<tr>
<td>FACS enumeration of EPCs from obese men with the metabolic syndrome</td>
<td>Obese men with the metabolic syndrome had fewer EPCs than controls. Intensive lipid-lowering therapy increased EPCs to control levels.</td>
<td>[74]</td>
</tr>
<tr>
<td>FACS enumeration of EPCs from patients with Type 2 diabetes</td>
<td>Diabetes was associated with reduced circulating EPCs in patients with peripheral arterial disease. EPC numbers correlated inversely with plasma glucose. Abnormal glucose tolerance was associated with reduced numbers of circulating EPCs. Diabetes was associated with reduced circulating EPCs defined using multiple surface marker definitions. CXCR4 expression was also impaired.</td>
<td>[70, 72, 69]</td>
</tr>
<tr>
<td>Cell culture of cord blood from infants of mothers with gestational diabetes</td>
<td>Gestational diabetes was associated with a trend towards reduced cord blood EPCs. Post-hoc analysis revealed maternal insulin treatment to be associated with greater numbers of cord blood EPCs. In vitro hyperglycaemia or a diabetic intrauterine environment impaired late outgrowth EPC colony formation, self-renewal and capillary network formation. EPC proliferation was reduced and senescence increased.</td>
<td>[146, 177]</td>
</tr>
</tbody>
</table>
differentiation are essential pre-requisites for EPCs to contribute to vascular repair. EPCs from humans and animals with Type 2 diabetes have multiple functional defects in vitro, including impaired migration to chemotactic stimuli, reduced proliferative potential and diminished ability to form vascular-like structures, which are likely to limit their regenerative capacity [57,82]. These functional deficits appear to be biologically relevant in vivo, as Li et al. [82] have demonstrated that re-endothelialization following endothelium-denuding injury was impaired in mice with Type 2 diabetes [82]. Unfortunately, such studies do not allow the effects of insulin resistance to be considered separately from those of hyperglycaemia and other metabolic derangements characteristic of diabetes.

Homing of EPCs to sites of vascular injury is dependent on an interaction between locally produced chemokines and the CXCR4 receptor on EPCs. Diabetes is associated with a decreased expression of the chemokine SDF-1α in injured tissues [83] and reduced expression of CXCR4 in peripheral mononuclear cells [69], which may inhibit recruitment of EPCs from the circulation.

In the following sections, we highlight putative mechanisms by which insulin resistance may adversely affect the contribution of EPCs to endothelial regeneration (Figure 2).

**NO bioavailability**

Several of the steps required for endothelial regeneration, including EPC mobilization from bone marrow, homing to sites of injury and proliferation to mediate endothelial repair, are critically dependent on NO. Reduced NO bioavailability, which is a consistent finding in insulin-resistant humans and animals [21,30,37,84], impairs EPC mobilization and function in experimental models. In mice with genetic deletion of eNOS, studies have revealed that NO is essential for mobilization of progenitor cells from the bone marrow in response to distinct stimuli [21,30,37,75,84]. Physical exercise and statins also mobilize EPCs from the bone marrow via a partially NO-dependent mechanism, which is abolished by concomitant treatment with the NO synthase inhibitor L-NAME (N^G^-nitro-L-arginine methyl ester) [85,86]. NO also plays a vital role in the normal function of EPCs after mobilization; both in vitro and in vivo studies have demonstrated an essential requirement for NO in migration, homing and augmentation of neovascularization. In mice with deletion of eNOS, infusion of wild-type EPCs rescues the defective neovascularization, whereas infusion of eNOS<sup>−/−</sup> EPCs has no effect [75]. These findings strongly suggest that the ability of EPCs to synthesize NO is an essential prerequisite for their biological functions. Taken together, the available findings suggest that intact NO bioavailability in bone marrow cells, EPCs and the vascular wall may be required to facilitate effective repair.

In a mouse model of diabetes, reduced bone marrow NO bioavailability and decreased eNOS phosphorylation were associated with reduced EPC numbers and defective mobilization [56]. Activation of
Inflammation and adipokines

Alterations in the secretory profile of adipocytes have been implicated in mediating the association between insulin resistance and endothelial dysfunction, particularly in the setting of obesity. Adipocytes secrete a range of hormones, cytokines and other pro-inflammatory molecules which participate in complex cross-talk between adipose depots, metabolically active tissues and the vascular wall. insulin-resistant conditions, including obesity, the metabolic syndrome and Type 2 diabetes, share a common association with low-grade inflammation, characterized by increased circulating levels of CRP (C-reactive protein), IL-6 (interleukin-6) and TNF-α (tumour necrosis factor-α) [94]. Systemic inflammation is recognized to contribute to vascular disease by effects on mature endothelial cells such as the stimulation of pro-atherogenic adhesion molecules [95]. Inflammatory mediators also attenuate EPC survival, differentiation and function [96]. Conversely, inflammatory molecules released following vascular insults stimulate the production of growth factors and cytokines which are necessary to facilitate EPC release and homing to sites of injury [97]. It is likely that a transient inflammatory response associated with acute vascular injury is required for EPC mobilization, but that persistent low-level inflammation may have deleterious effects, resulting in decreased numbers of circulating EPCs [98].

In the setting of obesity, adipocytes contribute to a chronic inflammatory state by secreting the pro-inflammatory cytokine TNF-α [94]. TNF-α exposure reduces the proliferation of EPCs when studied in an ex vivo culture assay, an effect mediated through activation of the p38 MAPK pathway [93]. As discussed previously, in skeletal muscle cells of insulin-resistant individuals, signalling via this pathway is enhanced by the hyperinsulinaemia that accompanies chronic insulin resistance [45]. Whether P13K/Akt and MAPK signalling are differentially regulated in EPCs in insulin-resistant subjects is yet to be determined.

In addition to pro-inflammatory molecules, adipocytes secrete a range of cytokines (adipokines) which have been implicated in the complex inter-relationship between metabolic and vascular homeostasis. For example, the adipokine leptin induces NO-mediated vasodilation via the P13K/Akt pathway in mature endothelial cells [99,100]. Insulin enhances leptin-induced eNOS activation and subsequent NO production in endothelial cells, suggesting an interaction between insulin and leptin signalling pathways [101]. Leptin receptors are present on human EPCs and, although it has been demonstrated that leptin can affect EPC function, the effects are complex [102]. At physiological concentrations, leptin increased tubule formation in cultured EPCs, but at higher concentrations this effect was reduced and EPC migration was inhibited [102]. In a more recent study, leptin increased the expression of integrins in EPCs and enhanced their capacity to adhere to mature endothelial cells or extracellular matrix [103]. These effects translated into reduced neointima formation and enhanced re-endothelialization when leptin-stimulated...
human EPCs were infused in a mouse model of vascular injury [103]. Typically, obese insulin-resistant humans have increased circulating leptin concentrations and have been postulated to exhibit ‘leptin resistance’ [104]. Whether EPCs from insulin-resistant individuals are resistant to the effects of leptin remains to be determined.

The adipokine adiponectin plays a protective role in insulin-resistant conditions [105]. Circulating adiponectin concentrations decrease during the development of diet-induced insulin resistance and are inversely correlated with endothelial NO bioavailability [105]. Adiponectin directly stimulates NO production in endothelial cells by phosphorylation of eNOS [106]. Recent findings have begun to establish an association between adiponectin and the number and function of EPCs. Mice with a deficiency in adiponectin, for example, fail to mobilize EPCs in response to ischaemia, whereas adenovirus-mediated delivery of adiponectin increases circulating EPCs in both adiponectin-null mice and control animals [107]. In addition to serving as a putative EPC-mobilizing agent, adiponectin stimulates the incorporation of EPCs into endothelial cell networks in vitro and acts as a chemoattractant in EPC migration assays [107]. The molecular mechanisms for these effects of adiponectin are not known. In patients with coronary artery disease, circulating adiponectin concentrations were correlated with circulating EPCs [108]. Although adiponectin concentrations are known to be reduced in obese insulin-resistant humans, and this may be expected to impact adversely on EPCs, the relationship between adiponectin levels and EPCs has not yet been studied in this group.

**ROS**

Increased production of ROS is a characteristic feature of cardiovascular disease states and is associated with atherosclerosis [19]. In endothelial cells, ROS exert direct cytotoxic effects, react with NO to diminish NO bioavailability and form peroxynitrite anions, which are powerful oxidants [19]. Although EPCs from healthy individuals are postulated to be relatively resistant to oxidant stress, increased ROS, resulting from increased production or diminished endogenous antioxidant defences, may lead to EPC dysfunction in those at risk of cardiovascular disease. In animals models, conditions characterized by increased ROS are associated with reduced numbers of circulating EPCs [109,110]. Incubation of EPCs in vitro with H$_2$O$_2$ profoundly reduces their number by inducing apoptosis [111]. ROS have also been implicated in the reduced migratory capacity of EPCs in rats treated with the NO donor isosorbide mononitrate [112]. GPX-1 (gluthathione peroxidase-1)-deficient mice, which are unable to detoxify increased cellular ROS, have an inability to mobilize EPCs in response to ischaemia or VEGF [113]. EPCs isolated from these mice also have reduced ability to neutralize oxidant stress in vitro and have an impaired capacity for migration towards VEGF [113]. EPCs are less able to tolerate oxidative stress in the setting of insulin resistance and obesity [114]. As increased production of ROS is a characteristic feature of insulin resistance and diabetes, this has important implications for the integrity of endothelial repair in these conditions. Sorrentino et al. [115] demonstrated that the in vivo re-endothelialization capacity of EPCs from humans with Type 2 diabetes is severely impaired, at least partly as a result of increased superoxide production by the enzyme NADPH oxidase and subsequent reduced NO bioavailability. Similar changes in NADPH-oxidase-mediated superoxide production and decreased NO bioavailability have been demonstrated in the setting of insulin resistance [30]. Neovascularization in response to limb ischaemia in mice with diabetes is improved by decreasing intracellular superoxide [116]. Recently, systemic antioxidant therapy was shown to increase the numbers and endothelial differentiation of EPCs in mice with diabetes [117].

Although at high concentrations ROS induce cytotoxic effects, at low levels ROS may serve as essential intracellular signalling molecules. In endothelial cells, ROS derived from NADPH oxidase stimulate redox-sensitive signalling pathways leading to angiogenic responses [118]. In keeping with this, it has recently been reported that ROS derived from Nos2-based NADPH oxidase play a critical role in mobilization, homing and angiogenic capacity of EPCs [119]. The potential effects of insulin resistance on NADPH-oxidase-derived ROS in EPCs are worthy of future study.

Under certain pathological conditions, eNOS itself can be a source of superoxide instead of NO [120]. This 'uncoupling' of eNOS, which is typically associated with reduced levels of the enzymatic co-factor BH$_4$ (tetrahydrobiopterin), contributes to vascular dysfunction in the setting of diabetes [120,121]. Uncoupled eNOS was recently shown to be an important source of superoxide in EPCs from humans with diabetes and in the bone marrow of rats with diabetes [110]. Manipulation of intracellular BH$_4$ levels may, therefore, be a potential target to improve EPC function in this setting [110,122].

**Direct effects of insulin and IGF-I on EPCs**

Thus far we have discussed how the spectrum of molecular, biochemical or signalling abnormalities associated with insulin resistance may impact adversely on the capacity for EPCs to participate in vascular homeostasis. The potential for insulin, and the related glucoregulatory peptide IGF-I, to impact directly on EPCs remains underexplored. Findings, however, suggest that these peptides may play a role in the mobilization and differentiation of EPCs. For example, in a small study of patients with poorly controlled Type 2 diabetes, insulin therapy led to an increase in circulating EPCs [123]. In that study, insulin-mediated EPC mobilization was significantly enhanced in subjects with the SDF-1
Putative mechanisms by which insulin resistance modulates EPC function and associated potential therapeutic strategies

Clinical and pre-clinical studies have been used to derive the list. Not all have studied EPCs directly.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Potential therapeutic strategy</th>
</tr>
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<tbody>
<tr>
<td>Decreased NO bioavailability</td>
<td>Lifestyle modification, statins, ACEIs/ARBs, PPAR-γ agonists, metformin, and oestrogens</td>
</tr>
<tr>
<td>Oxidative stress (ROS)</td>
<td>Antioxidants, statins, and lifestyle modification</td>
</tr>
<tr>
<td>Disturbed PI3K/Akt signalling</td>
<td>PPAR-γ agonists, statins, ACEIs/ARBs, lifestyle modification, and oestrogens</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Statins, immunotherapy, lifestyle modification, and PPAR-γ agonists</td>
</tr>
<tr>
<td>Unfavourable adipokine profile</td>
<td>Lifestyle modification, PPAR-γ agonists, ACEIs/ARBs, statins, and metformin</td>
</tr>
<tr>
<td>Insulin/IGF receptor</td>
<td>Insulin, IGF-I, and GH</td>
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</table>

3′-A/G allele, a polymorphism known to be associated with increased EPC mobilization. [123,124]. Evidence is also emerging that insulin stimulates the clonogenic and angiogenic potential of EPCs. Humpert and co-workers [125] have demonstrated that insulin stimulates the outgrowth in vitro of EPCs from patients with Type 2 diabetes. Intriguingly, this effect was unaltered by blocking the insulin receptor in EPCs, but was completely abrogated by IGF-I-receptor blockade. The IGF-I-receptor-dependent effect of insulin on EPC growth was largely mediated by the MAPKs ERK1/2 (extracellular-signal-regulated kinase) and p38 [125].

IGF-I has complementary actions to insulin in glucose counter-regulation and contributes to systemic NO bioavailability by stimulating NO production in endothelial cells [126]. Low IGF-I levels are recognized as an independent risk factor for cardiovascular disease [127]. In middle-aged humans, GH (growth hormone) treatment, which increases circulating IGF-I levels, increased circulating EPCs, improved colony forming and migratory capacity, enhanced incorporation into tube-like structures and increased eNOS expression [79]. In vitro, IGF-I stimulates EPC differentiation, migratory capacity and the ability to incorporate into vascular networks, actions mediated via the IGF-I receptor [79]. IGF-I increases endothelial NO synthase expression, phosphorylation and activity in a PI3K/Akt-dependent manner in EPCs [79].

Taken together, these studies demonstrate important direct actions for insulin and IGF-I on EPCs which influence multiple facets of EPC biology. As we discuss later, however, it remains unknown whether EPCs from subjects with systemic insulin resistance are themselves ‘resistant’ to these effects of insulin and IGF-I.

**POTENTIAL THERAPEUTIC APPLICATIONS**

Given the critical balance between endothelial damage induced by exposure to risk factors and endothelial repair by circulating progenitor cells, modulation of endothelial regeneration presents an exciting therapeutic opportunity through which to prevent cardiovascular disease. Many lifestyle and pharmacological interventions with established cardiovascular benefits favourably affect endothelial function or the capacity for EPC-mediated vascular repair. In addition, cell-based therapies, in which endogenous progenitor cells are mobilized from the bone marrow or ex-vivo-expanded progenitor cells are transfused, are showing promise in the treatment of established vascular disease. Putative targets for therapeutic manipulation of EPCs in insulin-resistant individuals are summarized in Table 3.

**Lifestyle measures**

Regular physical activity improves endothelial function and decreases the incidence of cardiovascular events. In healthy volunteers, exercise mobilizes EPCs into the circulation [128] and, in patients with stable coronary artery disease, moderate exercise training for 28 days leads to a significant increase in circulating EPCs and reduced EPC apoptosis [129]. Exercise training improves insulin sensitivity in obesity [130], and weight loss improves endothelial function, insulin sensitivity and increases EPC numbers in obese individuals [26,131,132]. In mice, exercise training enhances EPC mobilization in an NO- and VEGF-dependent manner, diminishes neointima formation after vascular injury and enhances new blood vessel formation in response to ischaemia [86].

**RAS (renin–angiotensin system)**

The cardiovascular benefits of ACEIs (angiotensin-converting enzyme inhibitors) and ARBs (angiotensin II type 1 receptor blockers) are well established. Both types of drugs improve endothelial function, reduce inflammation and may improve insulin sensitivity [133]. Inhibition of the RAS mobilizes EPC in rodents [134]. Increased numbers and function of EPCs in patients with stable coronary artery disease following treatment with the ACEI ramipril may contribute to its cardioprotective effect [135]. Similarly, the ARBs olmesartan or irbesartan increase the numbers of EPCs in subjects with Type 2 diabetes, independent of their BP (blood pressure)-lowering actions [136].
Statins

Statins have a proven role in both primary and secondary prevention of cardiovascular disease. In addition to their fundamental effects on lipoprotein metabolism, statins may modulate vascular repair by up-regulating EPC numbers and inducing EPC differentiation through activation of the PI3K pathway and stimulation of NO production [78]. It is not clear whether these favourable effects of statins on EPCs remain intact in insulin-resistant humans in whom signalling via the PI3K/Akt pathway is defective and NO bioavailability is reduced. However, it has been shown that combination therapy consisting of olmesartan and pravastatin significantly suppressed neointimal formation and increased EPC numbers following arterial injury in insulin-resistant rats [137].

PPAR agonists

PPARs are a family of nuclear receptors with wide-ranging effects on gene transcription. PPAR-α, PPAR-δ and PPAR-γ agonists have emerged as potentially useful drugs to treat metabolic diseases. PPAR-γ agonists (thiazolidinediones) are in widespread clinical use as insulin-sensitizing drugs with favourable effects on classical cardiovascular risk factors. In pre-clinical studies, PPAR-γ agonists reduce atherosclerosis, limit restenosis following vascular injury and improve endothelial function through multiple mechanisms [138]. Clinical studies suggest that the PPAR-γ agonist pioglitazone reduces cardiovascular events in patients with Type 2 diabetes [139,140]. PPAR-γ agonists may favourably modulate EPC numbers and function. Rosiglitazone normalizes impaired EPC migratory activity and increases EPC numbers in culture [141], and reduces NADPH oxidase activity and improves re-endothelialization capacity of EPCs in patients with Type 2 diabetes [115]. Pioglitazone increases the number and function of EPCs and decreases EPC apoptosis [80,142,143]. It is unclear to what extent the in vivo effects of PPAR-γ agonists on EPCs are attributable to improvements in insulin sensitivity compared with their anti-inflammatory or lipid-modifying actions.

Although not in clinical use, the PPAR-δ agonist GW501516 protects against nutritional obesity, and improves insulin sensitivity and glucose tolerance in animals [144]. In terms of vascular homeostasis, PPAR-δ activation increases proliferation of human EPCs, protects them from hypoxia-induced apoptosis and enhances EPC function [91]. These actions by PPAR-δ activation in EPCs are mediated by the PI3K/Akt pathway [91].

Insulin, IGF-I and GH

Insulin is increasingly used to achieve glycaemic control in subjects with Type 2 diabetes who are resistant to oral therapies. Although there is some evidence that short-term insulin treatment may improve cardiovascular outcomes following myocardial infarction [145], the effects of longer term insulin therapy on cardiovascular risk are controversial. As discussed previously, recent findings suggest that treatment with insulin and certain insulin analogues may mobilize EPCs and improve EPC parameters in vitro [125]. Further studies are required to assess the effects in vivo. Other results demonstrate that maternal insulin therapy increases EPCs in fetal circulation independently of glycaemic control [146]. As insulin does not pass the placental barrier, an indirect effect of insulin on the fetal environment must be postulated. Insulin treatment has also been shown to abrogate the down-regulation of tissue VEGF production associated with diabetes and improve EPC mobilization in a rat model of hindlimb ischaemia [147].

IGF-I has glucose-lowering and insulin-sensitizing properties, with recent studies supporting beneficial effects on EPCs [79]. Although IGF-I has been used in the treatment of Type 2 diabetes in humans, its clinical utility is limited by poor tolerability and adverse effects. Whether short-term treatment with IGF-I could find a role in enhancing endothelial repair following acute ischaemic insults such as myocardial infarction is not known. GH supplementation, which may have significant health benefits in elderly individuals, may provide an alternative method of increasing circulating IGF-I levels and favouring EPC-mediated vascular repair [79,148].

Oestrogens

Oestrogens mobilize EPCs from bone marrow and reduce neointimal formation following arterial injury in animal models [149]. These effects are dependent on NOS activity [150] and FGF-2 (fibroblast growth factor-2) [151]. In vitro, oestrogens induce EPC proliferation and migration by stimulating the PI3K pathway [152], and have been shown to reduce EPC senescence [153]. EPCs cultured from female donors have enhanced adhesion and clonogenicity, compared with those from males, with increased angiogenic potential in vivo [154]. In healthy fertile women, EPCs are mobilized cyclically in response to changing hormonal status, raising the possibility that the higher EPCs levels contribute to gender differences in cardiovascular risk [154,155]. The influence of hormonal status on EPCs in women with insulin resistance or diabetes, and the clinical potential of oestrogens as an EPC-enhancing therapy, have yet to be studied.

Cell-based therapies

Major obstacles to employing cell-based therapies to modulate endothelial repair in subjects at risk of cardiovascular disease are the depletion and impaired regenerative properties of EPCs characteristic of these individuals. Therapeutic intervention, therefore, requires augmentation of EPC numbers or improvement in their function. Few studies to date, however, have been carried out in individuals with insulin resistance or diabetes. G-CSF (granulocyte colony-stimulating factor) is a potent stimulus for EPC mobilization and accelerates
re-endothelialization following vascular injury in animal models [156]. Huang et al. [157] transplanted G-CSF-mobilized peripheral blood mononuclear cells, enriched for EPCs, into patients with Type 2 diabetes and critical limb ischaemia. Improvements in limb perfusion and reduced requirements for amputation were observed [157]. G-CSF-mobilized bone marrow cells proved to be less effective in the setting of stable coronary artery disease however [158]. That study also raised concerns that G-CSF mobilization increases the risk of serious adverse events, including myocardial infarction and death, perhaps by promoting a systemic inflammatory response [158]. In acute myocardial infarction, intracoronary infusion of G-CSF-mobilized bone marrow cells leads to improvements in myocardial perfusion and left ventricular systolic function [159,160]; however, significantly higher rates of in-stent restenosis were observed in patients undergoing concomitant coronary artery stenting in one such study [160]. Other potential mobilizing agents include erythropoietin [81], oestrogen [149] and VEGF [161]. Studies of intra-coronary infusion of bone-marrow-derived progenitors, harvested without the use of mobilizing agents, have yielded conflicting results in the setting of ischaemic heart disease [162–166]. The discrepancies may relate to differences in the cell fractions administered, along with small sample sizes and variations in outcome measures adopted.

Ex vivo expansion of cultured peripheral-blood-derived EPCs represents an alternative approach for cell-based therapy. Ex-vivo-expanded human EPCs retain their reparative potential in vivo in the setting of myocardial or limb ischaemia [167,168]. In support of a potential role for ex-vivo-expanded EPCs in augmenting endothelial repair, systemic transfusion of cultured EPCs accelerates re-endothelialization [67], improves endothelial function [169] and prevents atherosclerosis [62] in animal models. Cardiovascular risk factors may inhibit endothelial repair by affecting the microenvironment of the vascular wall or by directly affecting EPCs. Diabetes exerts a direct effect on EPCs, as transfusion of EPCs harvested from a donor animal with diabetes results in impaired endothelial regeneration following experimental injury regardless of the diabetes status of the recipient [82]. This implies that ex vivo manipulation of cultured EPCs to restore their reparative potential may be required for this approach to be successful in the setting of insulin resistance or diabetes. Consistent with this suggestion, ex vivo pre-treatment of EPCs with a novel eNOS transcription enhancer partially reversed the impaired functional activity of EPCs from patients with ischaemic cardiomyopathy [170]. Similarly, transfusion of human EPCs transfected with an adenovirus encoding VEGF significantly reduced distal ischaemia in a murine model of hindlimb ischemia [171]. Further work is required in order to determine whether ex vivo manipulation of EPCs can overcome the functional defects associated with insulin resistance and diabetes.

**REMAINING QUESTIONS AND FUTURE RESEARCH**

We have thus far reviewed the many molecular and biochemical consequences of insulin resistance with may impact adversely on EPC-mediated vascular repair. Although the results linking insulin resistance with endothelial dysfunction are compelling, a similar causal relationship between insulin resistance and EPC dysfunction is speculative and requires further corroboration. This question will only satisfactorily be addressed by ongoing mechanistic studies in the laboratory to determine the role of insulin signalling pathways in EPCs and large-scale cross-sectional studies in insulin-resistant populations with sufficient statistical power to detect independent effects of insulin resistance on EPCs. The following areas merit particular attention.

**Characterization and definition of EPCs**

A major obstacle to delineating the contribution of any risk factor to EPCs and vascular homeostasis is a lack of clarity and consistency over the identification and characterization of EPCs. Limitations of the two common approaches employed to isolate and enumerate EPCs, namely in vivo adhesion and growth assays and selection by cell-surface phenotype using flow cytometry with fluorescently labelled antibodies, have been extensively reviewed elsewhere [172]. Flow cytometric studies are limited by the lack of a unique complement of cell-surface markers which prospectively identify cells that participate in endothelial repair in vivo. Cell culture assays of ‘early outgrowth’ cells, which were used to identify EPCs in the majority of studies reviewed in the present article, probably identify cells of macrophage/monocyte lineage which, although they may participate in endothelial regeneration and vascular homeostasis, do not themselves differentiate to form mature endothelial cells [173]. Possible effects of insulin resistance on ‘late outgrowth’ or ‘endothelial colony-forming cells’, which possess the potential for clonal expansion and formation of endothelial monolayers [174,175], have not been studied to date. Only when these findings are available will it be possible to accurately identify the specific effects of vascular risk factors, including insulin resistance, on cell-mediated endothelial repair.

**Insulin resistance compared with diabetes**

To date, the most convincing observational and experimental results to support an association between EPC dysfunction and insulin resistance derive from individuals with diabetes [57,69,82]. It is difficult in such studies to separate the effects of insulin resistance from other components of the diabetes phenotype. Certainly, long-term
exposure to high glucose concentrations in vitro inhibits colony-forming ability, proliferation activity and migration activities of EPCs [176]. Additionally, EPCs derived from human neonates exposed to a diabetic intrauterine environment have abnormal functional properties [177]. Therefore the EPC dysfunction observed in the setting of Type 2 diabetes may, at least in part, be attributable to the toxic effect of glucose. However, the evidence that intensively reducing glycaemia in adults reduces the risk of macrovascular disease is questionable. Several recent large-scale trials in Type 2 diabetes failed to demonstrate a benefit from intensive glucose lowering on macrovascular events [178–180], although long-term follow-up from UKPDS (UK Prospective Diabetes Study) revealed a reduced incidence of myocardial infarction with this approach [181]. In contrast, studies targeting insulin resistance with metformin or pioglitazone have reported a reduced incidence of macrovascular events [139,140,181,182]. In keeping with a specific detrimental effect of insulin resistance on EPC function, it has been shown that treatment with the insulin-sensitizing drug rosiglitazone improves EPC numbers and functional parameters independently of glycemic control [141]. Furthermore, preliminary work from our laboratory in mice with haploinsufficiency of insulin receptors suggests that insulin resistance, in the context of normal glucose regulation, is associated with reduced numbers of EPCs and delayed endothelial regeneration following vascular injury [183]. However, large-scale studies in humans are necessary to determine whether insulin resistance is an independent predictor of EPC numbers and function.

Systemic compared with cell-specific insulin resistance
The term ‘insulin resistance’ encompasses both a biochemical state associated with impaired glucose uptake in metabolically active tissues and diminished responsiveness to the direct effects of insulin on subcellular signalling pathways. EPC-related phenomena in the setting of insulin resistance may, therefore, be due to the systemic effects of insulin resistance (for example oxidative stress, inflammation and increased NEFAs) or resistance to biological effects of insulin in bone marrow cells, EPCs or the vascular wall. Murine studies in which EPCs were transfused following endothelial-denuding injury implicate defects of both EPCs and the vascular wall in the impaired endothelial repair in the setting of diabetes [82]. It is becoming increasingly apparent that resistance to the effects of insulin resistance may confer disparate effects in the various cell types contributing to atherosclerosis. Although it is recognized that cell-specific effects of insulin resistance may have opposing effects on atherosclerosis in endothelial cells [38] and macrophages [184,185], relatively little is known about the effects of insulin or insulin resistance in bone marrow cells or in EPCs themselves. Further studies are required to delineate the effects of insulin resistance on all cell types involved in EPC mobilization, homing and integration into the vascular wall. Although recent findings suggest that ‘early outgrowth’ EPCs in vitro respond to exogenous insulin through IGF-1-receptor signalling [125], the relative abundance of insulin, IGF-1 or hybrid receptors on the cell surface of these cells has not been studied. It is also not yet known whether progenitor cells express these receptors or respond to insulin in vivo. Further examination of insulin-mediated responses, including cross-talk with IGF-1-receptor signalling, is required in these cells and, arguably more importantly, in ‘late outgrowth’ EPCs.

CONCLUDING REMARKS
The last decade has witnessed a paradigm shift in vascular biology following the recognition that endogenous regenerative processes maintain endothelial integrity and contribute to vascular homoeostasis. An association between endothelial dysfunction and insulin resistance is well-established, but accumulating results now suggest that defective endothelial repair may contribute to vasculopathy in individuals at risk of cardiometabolic disease. The burden of cardiovascular disease currently attributable to diabetes may be eclipsed in future by an increasing recognition of the much larger group with insulin resistance associated with ‘pre-diabetes’, the metabolic syndrome or obesity [186]. Only by unravelling the complex interactions between insulin resistance, EPCs and endothelial repair will we be able to shift the balance to favour maintenance of vascular health in this growing population.

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