Specific Role of Impaired Glucose Metabolism and Diabetes Mellitus in Endothelial Progenitor Cell Characteristics and Function

Kai-Hang Yiu, Hung-Fat Tse

Abstract—The disease burden of diabetes mellitus (DM) and its associated cardiovascular complications represent a growing and major global health problem. Recent studies suggest that circulating exogenous endothelial progenitor cells (EPCs) play an important role in endothelial repair and neovascularization at sites of injury or ischemia. Both experimental and clinical studies have demonstrated that hyperglycemia related to DM can induce alterations to EPCs. The reduction and dysfunction of EPCs related to DM correlate with the occurrence and severity of microvascular and macrovascular complications, suggesting a close mechanistic link between EPC dysfunction and impaired vascular function/repar in DM. These alterations to EPCs, likely mediated by multiple pathophysiological mechanisms, including inflammation, oxidative stress, and alterations in Akt and the nitric oxide pathway, affect EPCs at multiple stages: differentiation and mobilization in the bone marrow, trafficking and survival in the circulation, and homing and neovascularization. Several different therapeutic approaches have consequently been proposed to reverse the reduction and dysfunction of EPCs in DM and may represent a novel therapeutic approach to prevent and treat DM-related cardiovascular complications. (Arterioscler Thromb Vasc Biol. 2014;34:00-00.)

Key Words: diabetes mellitus

Diabetes mellitus (DM) is a complex metabolic disorder characterized by impaired glucose metabolism with hyperglycemia. Patients with DM have a 2- to 4-fold increased risk of developing cardiovascular complications compared with nondiabetic controls. This has been attributed to the occurrence of endothelial dysfunction that leads to the initiation and progression of atherosclerotic vascular disease and impaired neovascularization after ischemia induced by hyperglycemia. In patients with impaired glucose metabolism and DM, the vascular endothelium is challenged by inflammation, reactive oxygen species, and deletion of endothelial nitric oxide synthase (eNOS) with resultant endothelial dysfunction. The depletion and dysfunction of the circulating endothelial progenitor cells (EPCs) are thought to underlie the endothelial dysfunction in DM. In this review, the possible mechanisms, functional consequences, and the potential therapeutic approach for impaired EPCs in DM are discussed. A systematic literature search for full-text papers in the English language was performed using MEDLINE, Embase, and the Cochrane library through to February 2014. In the search phrases used, the following terms were combined with the phrase AND Diabetes; endothelial progenitor cells, cardiovascular disease, myocardial infarction, and stroke.

Alterations of EPCs in DM

EPCs were initially described as a pool of circulating bone marrow (BM)–derived CD34 progenitor cells that display vasculogenic potential. In response to stimuli such as exercise, tissue ischemia, and cytokines, EPCs can be mobilized from the BM into the peripheral circulation and contribute to endothelial repair and neovascularization at sites of injury/ischemia (Figure 1A). Circulating EPCs are mainly defined and enumerated using flow cytometry or colony-forming unit assay by their expression of a panel of surface markers such as CD34, CD133, and KDR. Nevertheless, there is significant overlapping in the results of these assays for EPCs and hematopoietic progenitor cells. Furthermore, 2 major subtypes of EPCs, early and late EPCs, can be isolated from circulating mononuclear cells using different culture durations and protocols. Both early and late EPCs are derived from circulating mononuclear cells and may have their distinct role in endothelial repair and vasculogenesis. In brief, early EPCs that emerge after 4 to 7 days of culture
Nonstandard Abbreviations and Acronyms

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BM</td>
<td>bone marrow</td>
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<tr>
<td>DDP-4</td>
<td>dipeptidyl peptidase 4</td>
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<td>DM</td>
<td>diabetes mellitus</td>
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<tr>
<td>eNOS</td>
<td>endothelial nitric oxide synthase</td>
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<tr>
<td>EPC</td>
<td>endothelial progenitor cell</td>
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<tr>
<td>SDF-1α</td>
<td>stromal-derived factor 1α</td>
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<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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originates from the hematopoietic lineage with a limited proliferative potential although they contribute to in vivo neovascularization via secretion of proangiogenic cytokines. Late EPCs appear after 1 to 3 weeks in culture and express CD34 as well as other endothelial markers, such as KDR and CD146. They have a potent proliferative capacity to form mature endothelial cells in vitro. In contrast to early EPCs, late EPC cultures are more difficult to establish and sometimes impossible in some patients with DM and cardiovascular disease.

Many clinical studies have demonstrated that number of circulating EPCs is reduced and their function impaired in terms of decreased proliferation, adhesion, and vasculogenesis in patients with type 1 and type 2 DM (Table I in the online-only Data Supplement). Similar alterations in EPCs are observed in patients with type 1 and type 2 DM, suggesting a close mechanistic link between hyperglycemia and the reduction and functional impairment of EPCs. Indeed, the reduction in circulating EPCs seems to correlate with glycemic control or severity of impaired glucose tolerance in subjects with or without established DM, respectively. Similarly, the deleterious effects of hyperglycemia on the number and function of late EPCs have also been reported. Late EPCs were found in an activated state in patients with DM. In vitro hyperglycemia or a diabetic intrauterine environment diminished late EPC colony formation, self-renewal capacity, and capillary-like tube formation. Moreover, decreased secretion of NO and vascular endothelial growth factor (VEGF), reduced activity of superoxide dismutase, and impaired function, such as migration and tube formation, are observed in activated late EPCs after exposure to a hyperglycemic condition or advanced glycation end products.

Reduced number and function of EPCs are also more prevalent in patients with type 1 and type 2 DM with diabetic nephropathy and macrovascular complications and correlate with the degree of atherosclerosis. These observations suggest that the burden of DM-related cardiovascular complications correlates with the number and function of EPCs; alterations in EPCs may contribute to the development and progression of atherosclerosis in patients with DM. This has been attributed to the decreased number of EPCs as well as impairment of their function and recruitment to injured vasculature, causing defective endothelial repair and neovascularization (Figure 1A). This has been confirmed by experimental studies (Table II in the online-only Data Supplement) wherein EPCs from patients with DM exhibit impaired re-endothelialization of an injured artery and limited ability to enhance neovascularization in ischemic tissue.

In contrast, a biphasic pattern in the change of EPCs has also been observed: a decreased number of EPCs seen in nonproliferative retinopathy but an increased number and clonogenic potential in patients with DM with proliferative retinopathy. In patients with type 1 DM and early nonproliferative diabetic retinopathy, only dysregulation of late EPCs with increased clonogenic potential but impairment of homing mechanism is observed. Similarly, functional impairments of EPCs are observed in patients with type 1 and type 2 DM with proliferative retinopathy despite an increase in their number and clonogenic potential. It is likely that the clonogenic potential and thus the number of late EPCs increased with the progression from nonproliferative to proliferative retinopathy. Furthermore, the proangiogenic activities of increasing number of dysfunctional EPCs may contribute to the pathological retinal neovascularization in late-stage diabetic retinopathy.

In addition to neovascularization, EPCs are involved in thrombus recanalization. It has been demonstrated that the presence of late EPCs has both anticoagulant and antifibrinolytic properties. Indeed, hyperglycemia may reduce plasminogen activator inhibitor-1 secretion and, therefore, diminish the antifibrinolytic property of EPCs. Such an impairment of EPC properties may further explain the occurrence of macrovascular complications in patients with DM.

**Mechanisms of Alteration of EPCs in DM**

Several major mechanisms have been proposed to explain the reduced number and dysfunction of EPCs associated with impaired glucose metabolism and DM. The underlying mechanisms by which hyperglycemia and insulin resistance can induce inflammation, oxidative stress, and deletion of NO have been extensively summarized in other reviews. Moreover, primary or secondary dyslipidemia in patients with DM can also interact with hyperglycemia and insulin to induce EPC dysfunction. In the following section, the deleterious effects of these mechanisms that affect EPCs at multiple stages of their lifecycle are discussed (Figure 1B).

**Differentiation and Mobilization**

Both human and animal studies have demonstrated that DM is associated with pathological changes in the BM, including microangiopathy with microvascular rarefaction, autonomic neuropathy, alteration of the vascular/osteoblastic progenitor niche, and depletion of hematopoietic tissue and the stem cell pool. Although these abnormalities might not cause a major deficit in hematopoiesis, they are associated with reduced differentiation and release of EPCs. Experimental and human studies also demonstrate that DM impairs the mobilization of EPCs in response to tissue ischemia or cytokines, such as granulocyte colony-stimulating factor. Among the several different mechanisms, eNOS dysfunction and altered cytokine gradients, for example, stromal-derived factor 1α (SDF-1α) between the BM and ischemic tissues in DM, may play major roles in the impairment of EPC mobilization. SDF-1α is a cytokine that contributes to EPC mobilization by stimulating CXCR4 on the cell membrane.
of EPCs. In DM, the increased BM SDF-1α level resulting from enhanced CD26/dipeptidyl peptidase 4 (DPP-4) activity and the decreased production of SDF-1α in the ischemic tissue lead to a reduced gradient of this chemoattractant to mobilize EPCs from BM to the circulation. One of the mechanisms of action of granulocyte colony-stimulating factor is via the cleavage of SDF-1α through the release of protease CD26/DDP-4 to reduce the local level of chemoattractant and thus mobilize stem cells from the BM. As a result, the increased BM SDF-1α level in DM reduces the ability of granulocyte colony-stimulating factor to mobilize stem cells, including EPCs, from the BM.

**Figure 1.**

A. Potential functional role of endothelial progenitor cells (EPCs) in vascular repair in normal subjects (top) and in patients with diabetes mellitus (DM; bottom). In subjects with DM, the number of bone marrow (BM) progenitor cells and their mobilization are reduced because of the alteration in the osteoblastic and vascular niches and the cytokines profiles, respectively, and the survival of circulating EPCs is reduced. As a result, the numbers of circulating EPCs are decreased in subjects with DM. Furthermore, the migratory, recruitment, and angiogenic functions of EPCs at ischemic tissue in subjects with DM are impaired and thus contribute to defective vascular repair and angiogenesis.

B. Potential mechanisms of impairment of the number and function of EPCs in DM induced by hyperglycemia and insulin resistance. Multiple pathophysiological mechanisms, including changes in inflammatory signals (e.g., nuclear factor-κB [NFkB], interleukin 8), oxidative stress (e.g., reactive oxygen species [ROS], thrombospondin 2 [TSP-2], and advanced glycation end products [AGEs]), nitric oxide (e.g., endothelial nitric oxide synthase [eNOS]), and Akt signal pathway, affect EPCs at multiple stages during their lifetime: differentiation and mobilization in BM, trafficking and survival in the circulation, and homing and neovascularization. G-CSF indicates granulocyte colony-stimulating factor (CSF); SCF, stem cell factor; SDF-1, stromal-derived factor 1; and VEGF, vascular endothelial growth factor.

**Trafficcking and Survival**

As discussed above, the migration of EPCs to sites of injury/ischemia is mediated by the gradient of SDF-1α and other chemoattractants, such as VEGF and erythropoietin. In addition to the altered cytokine gradient, decreased NO, increased reactive oxygen species, and advanced glycation end products in DM impair this migration. These pathological processes induced by DM also increase apoptosis and decrease proliferation of EPCs. Both in vitro and in vivo studies have shown that hyperglycemia and its associated lipotoxicity with elevated oxidized low-density lipoprotein increases the senescence or apoptosis of EPCs mediated through multiple
different molecular pathways, including interleukin 8, Akt, protein kinase C, and p38 mitogen-activated protein kinase (Figure 1B).47,48,50,58,59

Homing and Neovascularization
It has been postulated that homing circulating EPCs to sites of ischemia can contribute to vascular repair by direct transdifferentiation into vascular endothelial cells and indirectly via the secretion of proangiogenic cytokines.8–11 As discussed above, the local release and thus the gradient of cytokines, especially SDF-1α, play a pivotal role in EPC homing. The blockade of either SDF-1α or its receptor CXCR4 prevents the recruitment of EPCs to injured sites.57 In patients with DM, the high glucose environment reduces the level of VEGF and SDF-1 secretion from endothelial cells via the hypoxia-inducible factor/hypoxia-responsive element pathway and DDP-4 activity.50 The reduced level of these cytokines may then impair the regulation of growth, migration, and survival of EPCs. Alternatively, exogenous administration of SDF-1α reversed the DM-induced EPC dysfunctional homing (Figure 2) and improved neovascularization and wound healing in animal models.56 In vitro culture of EPCs from patients with DM55,60 or from healthy subjects with a high glucose level61 as well as EPCs from animal models of DM56,59,62,63 exhibited impaired proliferation and angiogenesis. Similarly, multiple different molecular mechanisms related to hyperglycemia as discussed above have been proposed to explain the functional impairment and reduced survival of EPCs recruited to the sites of ischemia (Figure 1B).47,48,50 More recently, altered expression of microRNA, such as microRNA 126 and microRNA 130a, has been implicated in EPC dysfunction mediated through extracellular signal–regulated kinase, VEGF, and the phosphoinositide 3-kinase/Akt/eNOS signal pathway.64,65

Therapeutic Avenues to Restore EPC Alterations in DM
As summarized in Figure 2, several different approaches have been investigated to restore the dysregulation and dysfunction of EPCs mediated by DM.

Antidiabetic Agents
Because impaired glucose metabolism with hyperglycemia is the primary initiating event that induces EPC alteration in DM, and EPC dysfunction is closely related to the degree of hyperglycemia, improved hyperglycemic control should be the initial therapeutic target. Indeed, the severity of hyperglycemia seems to be negatively correlated with EPC number and function in patients with DM.14–20 In an animal model of type 1 DM, successful restoration of normoglycemia by islet transplantation increased the number of EPCs and improved their angiogenic function.66 Currently, there are only limited data on the optimal antidiabetic therapy to reverse EPC dysfunction in DM. Insulin therapy improves the clonogenic potential of EPCs in vitro67 and increases the number of EPCs in patients with type 2 DM.68 Metformin improved glycemic control and increased the number of EPCs in patients with type 2 DM.69 Nonetheless, add-on sulfonylureas (gliclazide) to metformin70 or thiazolidinediones71 were more effective than metformin alone in increasing circulating EPCs in patients with type 2 DM despite similar glycemic control. In addition to the hypoglycemic effect, thiazolidinediones have been shown to increase EPC production, reduce EPC apoptosis, and enhance their migratory capacity and reendothelization of injured artery via the activation of the Akt/eNOS pathway, as well as anti-inflammatory and antioxidative actions.28,35,59,72

Glucagon-like peptide-1 agonist and DPP-4 inhibitors are a newer class of antidiabetic agents that act by increasing the...
Acute hyperglycemia induces hypersecretion of glucagon, which can promote pancreatic tumor angiogenesis and growth in vivo. In contrast, there is potential concern of SDF-1 agonist (exenatide) can induce marked β-cell hyperplasia and increase the prevalence of preneoplastic lesions. The mobilization of BM EPCs by these incretin-based therapies may promote pancreatic tumor angiogenesis and growth in vivo.

**Approaches to Restore BM Mobilization**

Other than DDP-4 inhibitor, increased mobilization of EPCs from the BM in DM can be achieved by the administration of granulocyte colony-stimulating factor or erythropoietin, as well as the blockade of CXCR4 receptor in the BM using AMD3100 (Mozobil). This decreases the binding of SDF-1α to reduce the retention of EPCs. Previous clinical studies suggest that daily thiamine intake is associated with the level of circulating EPCs in patients with type 2 DM; thus, thiamine deficiency may be linked to impaired EPC mobilization. Experimental studies have demonstrated that treatment with benfotiamine, a liposoluble vitamin B1 with much higher bioavailability compared with thiamine, can reduce oxidative stress and activate the Akt/eNOS pathway to restore EPC number and their mobilization in BM.

γ-Tocotrienol, a vitamin E isoform, has also been shown to enhance mobilization of EPCs via increased expression of VEGF. Whether these vitamin supplements can restore the EPC number and function and thus improve vascular function in patients with DM requires future study.

**Approaches to Improve EPC Function and Survival**

Several other therapeutic approaches or agents that are commonly used in patients with DM to prevent or treat cardiovascular disease can also improve EPC number and function via different mechanisms. In addition to their lipid-lowering effect, statins have many pleiotropic effects that may explain their cardiovascular benefit. Both experimental and clinical studies demonstrate that statins increase the number and function of circulating EPCs by increasing the bioavailability of NO and reducing oxidative stress and apoptosis of EPCs. Blockade of the renin–angiotensin system with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers has also been shown to increase EPC number in patients with DM, possibly mediated by their anti-inflammatory and antioxidative actions through the suppression of angiotensin II. In addition, a combination of statin and angiotensin receptor blocker seems to have a synergistic effect to increase the number and function of EPCs in DM. Lifestyle modifications, including exercise and weight reduction, have also been shown to increase EPC number and function in patients with cardiovascular disease or DM.

**Biological Therapies**

Several different biological therapies that target the pathophysiological mechanisms of DM-mediated EPC dysfunction have been proposed. Several strategies by priming EPCs have been proposed to improve the function of EPCs in diabetic condition. The use of placenta growth factor has been shown to enhance EPC differentiation and improve postschismic neovascularization in diabetic mice. Pretreatment of EPCs with proangiogenic growth factors, including VEGF, basic fibroblast growth factor, and platelet-derived growth factor, improves incisional wound healing (as assessed by angiogenesis assay, Matrigel assay, and vessel densities) in diabetic mice. Furthermore, in vitro treatment with ephrin-B2/Fc improves the adhesion and migration of peripheral blood mononuclear cells, raises the number of circulating vascular progenitor cells, and enhances their proangiogenic potential in the diabetic mouse model. Moreover, folic acid treatment also shows to normalize the majority of the altered gene expression profiles of EPCs from patients with type 1 DM compared with healthy subjects.

In the diabetic mouse model, the overexpression of eNOS in EPCs improves their proangiogenic and antiatherogenic properties. Interestingly, recent studies have suggested that hydroxysulfide has proangiogenic effects, which improve wound healing via the restoration of EPC functions in diabetic mice. Similarly, direct administration of cytokines or a gene vector encoding those cytokines, such as SDF-1α and VEGF, or a cocktail of these cytokines derived from cultured EPCs of healthy pluripotent cell lines, such as human embryonic stem cells, can reverse EPC dysfunction in patients with DM. Although the number of EPCs in patients with DM did not correlate with the plasma level of adiponectin, treatment of EPCs with adiponectin prevented their senescence induced by hyperglycemia. Because downregulation of microRNA can contribute to EPC dysfunction in DM, microRNA-based treatment to restore the expression of microRNA 126 and microRNA 130a may be a potential novel therapeutic approach.

Finally, a direct delivery of EPCs isolated from BM to the injured/ischemia tissue may reverse the trafficking and homing defects induced by DM. Nevertheless, the proliferative and angiogenic capacity of exogenous EPCs is impaired in DM. An exogenous source of EPCs such as those derived from pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem cells, can avoid this issue. Nonetheless, other issues related to the use of such cell sources, such as risk of immune rejection and tumor formation, need to be addressed.

**Future Perspectives**

Although an association between endothelial dysfunction and vascular complications in DM is well established, emerging data from experimental and clinical studies suggest that alterations of EPCs may play a pivotal role in the development of microvascular and macrovascular complications. Current therapies that aim to control hyperglycemia, dyslipidemia, and hypertension have been shown to improve EPC number and function in patients with DM. It is unclear whether these improvements are simply a result of the control of these conventional risk factors or independent effects. More importantly, whether the additional pleiotropic effect of these agents on EPCs can further prevent microvascular...
and macrovascular complications in DM is also unknown. For example, thiazolidinediones seem to provide better improvement in EPCs compared with metformin, yet they fail to demonstrate any benefit on clinical outcomes in patients with DM. It is possible that existing methods used to determine EPCs are misleading. Indeed, EPCs are a heterogeneous population of progenitor cells with different stages of differentiation, and their surface marker profiles change throughout their lifespan. Furthermore, EPCs possess different phenotypes including a proinflammatory (harmful) or angiogenic (protective) capacity, depending on their surrounding environment. Therefore, the balance between the proportions of EPCs with these different phenotypes may play an important role in the pathogenesis of vascular diseases.

For the assessment of EPCs, the enumeration of EPCs based on their phenotypes, that is, early versus late EPCs as well as their in vitro and in vivo functional capacity, should be a preferable surrogate marker compared with simple measurement of EPCs as defined by the expression of surface markers. However, further standardization of cell isolation methods and culture protocols is urgently needed to allow future comparison between different studies. Moreover, future studies are needed to compare the uses of different agents and approaches, alone and in combination, as optimal therapeutic approaches to improve EPC function and thus clinical outcome in patients with DM.

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Disclosures
None.

References
Diabetes Mellitus and Endothelial Progenitor Cells


cardiovascular complications via the restoration of endothelial progenitor cell number and function in diabetes mellitus.

86. 85. 84. 83. 82. 76. 75. 74. 73. 72. 71. 70. 69. 68. 67. 66. 65. 64. 63. 62. 61. 60. 59. 58. 57. 56. 55. 54. 53. 52. 51. 50. 49. 48. 47. 46. 45. 44. 43. 42. 41. 40. 39. 38. 37. 36. 35. 34. 33. 32. 31. 30. 29. 28. 27. 26. 25. 24. 23. 22. 21. 20. 19. 18. 17. 16. 15. 14. 13. 12. 11. 10. 9. 8. 7. 6. 5. 4. 3. 2. 1. 0.

Circulatory system and macrovascular complications, as well as the potential novel therapeutic approaches to prevent and treat diabetes mellitus–related cardiovascular complications via the restoration of endothelial progenitor cell number and function in diabetes mellitus.


**Significance**

Diabetes mellitus and its associated cardiovascular complications represent major health threats. This review summarizes the recent understandings on the pathophysiological role of circulating exogenous endothelial progenitor cells on the occurrence and severity of microvascular and macrovascular complications, as well as the potential novel therapeutic approaches to prevent and treat diabetes mellitus–related cardiovascular complications via the restoration of endothelial progenitor cell number and function in diabetes mellitus.
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### Supplemental Table I: Clinical studies reporting endothelial progenitor cells (EPCs) alteration in patients with diabetes mellitus

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<td>Loomans et al (2004)¹</td>
<td>T1DM n=20, Control: n=20</td>
<td>Reduced cultured early EPCs (characterized by uptake of Dil-labeled adLDL, the binding of lectin UEA-1 and CD31+) in T1DM patients and inversely related with HbA1c.</td>
<td>Patients with T1DM had reduced EPCs and correlated closely with diabetic control.</td>
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<tr>
<td>Asnaghi V, et al. (2006)²</td>
<td>T1DM with (n=11) or without (n=12) DR Control n=11</td>
<td>Cultured EPCs as assessed by colony forming units was higher in T1DM patients with proliferative DR.</td>
<td>Increased clonogenic potential of EPCs in T1DM patients with diabetic retinopathy.</td>
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<tr>
<td>Sibal et al. (2009)³</td>
<td>T1DM n=74 Control n=80</td>
<td>CD34/VE-cadherin+, CD133/VE-cadherin+ and CD133/VEGFR-2+ EPC counts were significantly lower in T1DM patients, and significantly correlated with brachial artery FMD.</td>
<td>Low EPC counts confirm risk of macrovascular complications and may account for impaired endothelial function in T1DM patients</td>
</tr>
<tr>
<td>Brunner S, et al. (2009).⁴</td>
<td>T1DM with (n=60) or without (n=30) DR</td>
<td>CD34/CD133/CD309 EPCs was reduced in nonproliferative retinopathy but unchanged in proliferative DR compared with those without DR. However, CD34/CD133/CD309/CD31 mature EPCs was increased in proliferative DR as compared with those without DR.</td>
<td>In T1DM with DR, EPCs undergo stage-related regulation. In non-proliferative retinopathy, a reduction of EPCs was observed, and in proliferative DR, a dramatic increase of mature EPCs was observed</td>
</tr>
<tr>
<td>Dessapt et al. (2010)⁵</td>
<td>T1DM with (n=22) or without (n=22) microalbuminuria</td>
<td>Patients with microalbuminuria had lower circulating CD34+ and CD34/CD133+ EPCs and numbers of colony-forming unit, and impaired vascular endothelial growth factor mediated tube formation.</td>
<td>Circulating EPC number is reduced and function is impaired in T1DM patients with microvascular injury as reflected by the presence microalbuminuria.</td>
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<td>Study</td>
<td>T1DM n=16, Control n=26</td>
<td>CD34/KDR+ EPC was lower in T1DM patients than control and independently associated with carotid intima media thickness.</td>
<td>Young subjects with T1DM had lower EPCs that may contribute to subclinical carotid atherosclerosis.</td>
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<td>Palombo et al. (2011)&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>Reinhard H, et al (2011)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>T1DM with (n=37) or without (n=35) diabetic nephropathy</td>
<td>Cultured EPCs numbers were similar between asymptomatic CVD T1DM patients with or without diabetic nephropathy.</td>
<td>Asymptomatic patients with diabetic nephropathy had EPC numbers similar to normoalbuminuric patients.</td>
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<tr>
<td>*Hörtenhuber et al. (2013)&lt;sup&gt;8&lt;/sup&gt;</td>
<td>T1DM n=190, Control n=34</td>
<td>CD34/CD133/KDR+ EPCs was reduced in T1DM patients and correlated with HbA1c. EPC count was associated with glycemic control at 1 year of follow-up.</td>
<td>Optimization of glycemic control in T1DM patients may reduce cardiovascular burden by increasing circulating EPCs.</td>
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<td>Glowińska-Olszewska et al (2013).&lt;sup&gt;9&lt;/sup&gt;</td>
<td>T1DM n=52, Control n=36</td>
<td>CD34/VE-cadherin+ and CD34/VEGFR+ EPCs were higher in children with T1DM that inversely correlated with FMD.</td>
<td>Children with T1DM had higher circulating EPCs that negatively correlated with endothelial function.</td>
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**Type 2 Diabetes Mellitus**

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<th>Study</th>
<th>T2DM n=20, Control n=20</th>
<th>Cultured EPCs in T2DM patients had decreased proliferation and impaired tubule formation in Matrigel assay compared with controls.</th>
<th>EPCs in T2 DM patients exhibit alterations in functions important for blood vessel growth.</th>
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<td>Tepper et al (2002)&lt;sup&gt;10&lt;/sup&gt;</td>
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<td>Fadini et al (2005).&lt;sup&gt;11&lt;/sup&gt;</td>
<td>T2DM n=51, Control n=17</td>
<td>CD34/KDR+ EPCs is reduced in patients with T2DM. T2DM patients with PVD had reduced CD34+ EPCs.</td>
<td>Patients with T2DM, in particular to those with PVD, had reduced EPCs. The finding suggested that depletion of EPCs may contribute to the pathogenesis of PVD.</td>
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<tr>
<td>*Pistrosch F et al. (2005).&lt;sup&gt;12&lt;/sup&gt;</td>
<td>T2DM n=10, Control N=10</td>
<td>Number and migratory function of EPCs were reduced in T2DM patients which improved with after treatment with rosiglitazone.</td>
<td>PPARgamma-agonist rosiglitazone increases number and migratory activity of cultured EPCs in T2DM patients.</td>
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<td>Bahlmann FH, et al (2005).</td>
<td>T2DM n=38</td>
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<tr>
<td>Fadini et al (2006).</td>
<td>T2DM with (n=72) or without PVD (n=55)</td>
<td>CD34/KDR+, CD34+, CD133+ EPCs were reduced in T2DM patients with PVD compared with those without PVD, and EPC levels were negatively correlated with the severity of PVD. The clonogenic and adhesion capacity of cultured EPCs were also significantly lower in T2DM patients with PVD.</td>
<td>EPC decrease in T2DM patients was related to PVD severity and that EPC function was altered in those patients with PVD.</td>
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<td>Wang CH, et al. (2006).</td>
<td>T2DM n=36</td>
<td>CD34/KDR+ EPCs level, migratory response and the adhesive capacity increased after 8 weeks of pioglitazone</td>
<td>Pioglitazone increased the number and improved the functional properties of EPCs in T2DM patients</td>
</tr>
<tr>
<td>Fadini et al (2007).</td>
<td>Middle-aged individuals n=219</td>
<td>CD34+ and CD34+/KDR+ EPCs were reduced in those with T2DM and were negatively correlated with glucose tolerance.</td>
<td>EPCs were negatively associated with glucose tolerance and maybe a cause of high incidence of cardiovascular damage in patients with pre-diabetes.</td>
</tr>
<tr>
<td>*Sorrentino SA, et al. (2007)</td>
<td>T2DM n=30, Control n=10</td>
<td>Impaired in-vivo reendothelialization of wire-induced carotid artery injured by diabetic EPCs. EPCs from diabetic individuals had a substantially increased superoxide production and impaired NO bioavailability. Rosiglitazone therapy normalized NAD(P)H oxidase activity, restored NO bioavailability, and improved in vivo reendothelialization capacity of EPCs from diabetic patients</td>
<td>In vivo reendothelialization capacity of EPCs derived from T2DM patients was severely impaired partially related to increased NAD(P)H oxidase-dependent superoxide production and subsequently reduced NO bioavailability which can be reversed by rosiglitazone.</td>
</tr>
<tr>
<td>Egan et al (2008).</td>
<td>T2DM n=98, Control n=39</td>
<td>T2DM patients had reduced CD34/KDR+ and KDR+ EPCs levels. Putative KDR+ EPCs were further reduced in T2DM patients with cardiovascular and microvascular complications.</td>
<td>T2DM patients had reduced EPCs which was negatively associated with severity of DM related complications.</td>
</tr>
<tr>
<td>Author(s) and Year</td>
<td>Subset</td>
<td>Outcome(s)</td>
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<tr>
<td>*Makino J, et al. (2008).&lt;sup&gt;19&lt;/sup&gt;</td>
<td>T2DM n=34</td>
<td>CD34+EPC significantly increased after 24 weeks of pioglitazone</td>
<td>Pioglitazone therapy increased CD34+ EPCs in T2DM patients</td>
</tr>
<tr>
<td>Wong et al (2008).&lt;sup&gt;20&lt;/sup&gt;</td>
<td>T2DM n=88, Control n=91</td>
<td>T2DM patients had lower CD133/KDR+ and CD34/KDR+ EPCs levels, and FMD, which were positively correlated with daily thiamine intake.</td>
<td>Reduced EPCs and FMD in T2DM patients was related to a reduced daily intake of thiamine.</td>
</tr>
<tr>
<td>Chen et al (2009).&lt;sup&gt;21&lt;/sup&gt;</td>
<td>T2DM n=51, Non-diabetic n=23</td>
<td>The number of CD34+/KDR+ and CD133/KDR+ EPCs levels were similar between patients with T2DM and controls. The migratory function of cultured EPCs was impaired in T2DM patients with and without critical leg ischemia and non-diabetic patients with critical leg ischemia compared with control.</td>
<td>The migratory function of EPCs was impaired in T2DM patients even in those without critical leg ischemia, suggesting impaired migratory function of EPCs may contribute to impaired neovascularization and critical limb ischemia in T2DM patients.</td>
</tr>
<tr>
<td>Makino H, et al. (2009)&lt;sup&gt;22&lt;/sup&gt;</td>
<td>T2DM n=85</td>
<td>CD34+ EPCs negatively correlated with urinary albumin excretion rate and T2DM patients with lower EPCs had increased urinary albumin excretion after 12 months follow-up.</td>
<td>Decreased number of circulating EPCs may be involved in the progression of diabetic nephropathy</td>
</tr>
<tr>
<td>Tan K, et al. (2010)&lt;sup&gt;23&lt;/sup&gt;</td>
<td>T2DM n=23, Control n=22</td>
<td>CD34+/CD45-EPCs were increased in T2DM patients with proliferative DR, but their migratory function was impaired.</td>
<td>EPCs from T2DM patients with proliferative DR are mobilized into the circulation but may be unable to migrate and repair damaged capillary endothelium.</td>
</tr>
<tr>
<td>*Jaumdally RJ, et al. (2010)&lt;sup&gt;24&lt;/sup&gt;</td>
<td>CVD patients with (n=14) or without (n=10) T2DM</td>
<td>80mg atorvastatin increased CD34/CD133+ EPCs and angiopoietin-2, decreased VEGF in T2DM patients.</td>
<td>High-dose atorvastatin increased circulating EPCs, reduced VEGF and increased Ang-2 in T2DM patients with CVD</td>
</tr>
<tr>
<td>Fadini GP, et al (2010).&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Subjects with different degree of impaired</td>
<td>Biphasic distribution on the level of CD34+ cells with significantly reduced in impaired glucose tolerance and in newly</td>
<td>Reduction of circulating EPCs occurred after the onset of T2DM and progression of diseases</td>
</tr>
</tbody>
</table>
glucose tolerance n=425 diagnosed T2DM and then decreased further after 20 years of diabetes compared with control.

<table>
<thead>
<tr>
<th>Study</th>
<th>T2DM n</th>
<th>Control n</th>
<th>EPCs count</th>
<th>EPC dysfunction in T2DM might be partly improved by strict glycemic control.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Churdchomjan W, et al. (2010)</td>
<td>n=36</td>
<td>n=20</td>
<td>CD34/VEGFR2+ EPCs count was decreased in T2DM patients compared with controls and there was an inverse correlation between the EPC numbers with plasma glucose and HbA1C. The number and function of EPCs in patients with good glycemic control were recovered compared with those with poor glycemic control.</td>
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<tr>
<td>*Reinhard H, et al (2010).</td>
<td>n=28</td>
<td></td>
<td>Culture EPCs count increased by 35% after 90 days of multifactorial (metformin, aspirin, statin and angiotensin II receptor blocker) treatment</td>
<td>Numbers of EPC derived from peripheral blood mononuclear cells increased significantly after multifactorial intervention in T2DM patients.</td>
</tr>
<tr>
<td>*Fadini GP, et al. (2010).</td>
<td>n=32</td>
<td></td>
<td>4 weeks treatment of sitagliptin significant increased EPCs and SDF-1alpha and decreased monocyte chemoattractant protein-1 in T2DM patients</td>
<td>Sitagliptin increases circulating EPCs in T2DM patients with concomitant upregulation of SDF-1alpha.</td>
</tr>
<tr>
<td>Brunner S, et al. (2011).</td>
<td>T2DM with (n=66) or without (n=60) CVD</td>
<td></td>
<td>CD34/CD133+, CD34/CD133/CD30+, and CD34/CD133/CD309/CD31+ EPCs were stepwise reduced in CVD T2DM patients with increased severity of DR.</td>
<td>T2DM patients with CVD showed a strong retinopathy-stage-dependent depletion of all angiopoietic EPCs.</td>
</tr>
<tr>
<td>Li M, et al, (2011)</td>
<td>T2DM n=95 Control n=95</td>
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<td>CD133+ and CD133/KDR+ EPCs were independently associated with the presence of T2DM, and the levels of CD34+ and CD34/KDR+ EPCs were independently associated with Hba1c.</td>
<td>Different subtype of circulating EPCs were associated with T2DM and hyperglycemia</td>
</tr>
<tr>
<td>Yue et al. (2011)</td>
<td>T2DM n=234, Control n=121</td>
<td></td>
<td>T2DM patients had reduced CD34/KDR+ and CD133/KDR+ EPCs.</td>
<td>EPCs and arterial stiffness were closely related to glycemic control in patients with</td>
</tr>
<tr>
<td>Authors</td>
<td>Study Design</td>
<td>Results</td>
<td>Conclusion</td>
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<tr>
<td>Yiu YF et al (2011)</td>
<td>T2DM n=280, Control n=73</td>
<td>Patients with T2DM had reduced CD133/KDR+ EPCs and correlated with vitamin D deficiency and impaired brachial FMD.</td>
<td>Vitamin D deficiency might contribute to depletion of immature EPCs and endothelial dysfunction in T2DM patients.</td>
<td></td>
</tr>
<tr>
<td>Van Ark J, et al (2012)</td>
<td>T2DM with (n=35) or without (n=16) CVD Non-T2DM with (n=36) or without (n=19) CVD</td>
<td>CD34+ and CD34/KDR+ EPCs were reduced in T2DM patients. Culture EPCs was reduced in T2DM patients with CVD compared with those without CVD. However, there was no difference in circulating smooth muscle progenitor cells.</td>
<td>Disturbed ratio between EPCs and smooth muscle progenitor cells might contribute to CVD in T2DM patients.</td>
<td></td>
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<tr>
<td>Moon JH, et al (2012)</td>
<td>T2DM without CVD n=73</td>
<td>CD34/CD133/CD309+ EPCs were lower in T2DM patients with carotid artery plaques, and lower EPC counts independently correlated with carotid artery plaque formation.</td>
<td>Reduced EPC count in T2DM patients was associated with carotid atherosclerotic plaque formation in T2DM patients.</td>
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<tr>
<td>Zhao CT, et al (2012)</td>
<td>T2DM n=87</td>
<td>T2DM patients (n=34) with impaired myocardial function as determined strain imaging had lower number of CD34+ EPCs than those with normal myocardial function (n=53).</td>
<td>Myocardial dysfunction in T2DM patients was related to depletion of EPCs.</td>
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</tbody>
</table>

**Abbreviations:** CVD=cardiovascular diseases; DR=diabetic retinopathy; EPC= endothelial progenitor cells; FMD=flow-mediated dilatation; NO= nitric oxide; PVD=peripheral vascular disease; T1DM= type 1 diabetes mellitus; T2DM= type 2 diabetes mellitus.

* Studies on therapeutic agents on EPC
### Supplemental Table II. Studies reporting alteration and therapeutic effects of EPCs alteration in diabetic animal model.

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal model</th>
<th>Finding</th>
<th>Interpretation</th>
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</thead>
<tbody>
<tr>
<td>Awad O, et al (2006)(^{36})</td>
<td>Hindlimb ischemic model in STZ-induced diabetic mice</td>
<td>CD34+ EPCs or CD14+ monocytic progenitor cells derived from peripheral blood improved healing and vascular growth after limb ischemia.</td>
<td>CD14(^+) cells could provide an alternative therapeutic option for people with diabetes when their CD34(^+) EPC number and function are compromised.</td>
</tr>
<tr>
<td>Li m, et al. (2006)(^{37})</td>
<td>Wire-induced carotid denudation in db/db mice</td>
<td>Diabetic EPCs exhibited decreased migration and adhesion activities in vitro. Vascular endothelial growth factor and endothelial NO synthase expressions were reduced but thrombospondin-1 mRNA expression was significantly upregulated in diabetic EPCs. Reendothelialization of the injured artery was impaired by malfunctioning EPCs in diabetes.</td>
<td>Change in expression of thrombospondin-1 in diabetic EPCs might contribute to the impaired reendothelialization after arterial injury.</td>
</tr>
<tr>
<td>Fadini et al (2006).(^{38})</td>
<td>Hindlimb ischemia-reperfusion injury model in STZ induced diabetic rats</td>
<td>EPCs were unable to be mobilized in diabetic rats compared to control. Insulin, G-CSF and SDF-1(\alpha) partially restored the mobilization of EPCs in diabetic rats.</td>
<td>Administration of G-CSF and SDF-1, and blood glucose control with insulin might offer a therapeutic strategy for diabetic ischemic syndromes via improving mobilization of EPCs.</td>
</tr>
<tr>
<td>Ohshima M, et al. (2009)(^{39})</td>
<td>db/db mice</td>
<td>Diabetic mice had reduced Flk-1/CD34+ EPCs and higher intracellular ROS levels, with lower potency of endothelial differentiation compared with control. Superoxide dismutase-mimic decreased the intracellular ROS level and increased number and potency of differentiation of EPCs.</td>
<td>Antioxidant therapy attenuated the diabetes-related impairment of EPCs reducing oxidative stress</td>
</tr>
<tr>
<td>Kang L, et al</td>
<td>Hindlimb ischemic</td>
<td>Diabetic mice had reduced circulating</td>
<td>Impairment of ischemia-induced EPC</td>
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<tr>
<td>Reference</td>
<td>Model/Condition</td>
<td>Findings</td>
<td>Summary</td>
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<tr>
<td>(2009)</td>
<td>Model in STZ-induced diabetic mice</td>
<td>EPCs and increased plasma endothelial microparticles. Following hindlimb ischemia, diabetic mice exhibited suppressed EPC mobilization, a reduction in the expected increase in capillary density and suppressed restoration of transcutaneous oxygen pressure in the ischemic tissue.</td>
<td></td>
</tr>
<tr>
<td>Olkawa et al (2010).</td>
<td>STZ-induced diabetic mice</td>
<td>Alteration of the bone marrow structure with depletion of hematopoetic component and fatty degeneration in mice with T1DM. Cultured endothelial cells from T1DM mice showed impaired migratory function, network formation and adhesiveness to BM mononuclear cells.</td>
<td>The study suggested that microangiopathy was presence in the bone marrow of diabetic mice.</td>
</tr>
<tr>
<td>Albiero et al (2011).</td>
<td>Hindlimb skin wound in STZ-induced diabetic mice</td>
<td>BM derived EPCs were reduced, increased apoptosis and decreased proliferation in granulation tissue of diabetic mice compared with control mice.</td>
<td>Diabetes delayed wound healing in association with defective recruitment, survival and proliferation of BM derived EPCs.</td>
</tr>
<tr>
<td>Kuliszewski et al (2013).</td>
<td>Lean Zucker, obese Zucker (model of metabolic syndrome [MS]) and Zucker diabetic fatty rats</td>
<td>Circulating EPCs were reduced in both MS and DM rats. Cultured EPCs from mice MS model had reduced EPCs differentiation, greater apoptosis, reduced migratory response and matrigel tubule formation, similar in diabetic model. Both obese Zucker and Zucker diabetic fatty rats had reduced EPCs recruitment in the ischemic hindlimb.</td>
<td>EPCs were reduced with functional impairment in both diabetic and MS model.</td>
</tr>
</tbody>
</table>
Westerweel PE, et al (2013)\textsuperscript{44}  

| Model          | STZ-induced diabetic mice | In diabetic mice, bone marrow EPC levels were unaffected, but the circulating EPC levels in blood were lower at baseline and mobilization with G-CSF/SCF was attenuated. | Diabetes induces alterations in the progenitor cell supportive capacity of the bone marrow stroma, which could be partially responsible for the attenuated EPC mobilization and reduced EPC levels. |

Abbreviations as in Table I; G-CSF=granulocyte-colony stimulating factor; ROS=reactive oxidative species; SDF-1\textalpha =stromal cell-derived factor 1\textalpha; STZ=streptozotocin.
Supplemental References


