



# Heal Thyself: SGLT2 Inhibition Limits Regenerative Cell Exhaustion and Heals Damaged Vessels

David A. Hess,<sup>1,2,3</sup> Daniella C. Terenzi,<sup>4,5</sup> and Subodh Verma<sup>1,4,5,6</sup>

*Diabetes* 2021;70:1620–1622 | <https://doi.org/10.2337/dbi21-0025>

Chronic metabolic dysfunction as a result of type 2 diabetes (T2D) increases susceptibility to the development of ischemic cardiovascular disease (CVD) (1). The search for therapies to improve cardiovascular health in individuals afflicted with T2D has led to the discovery and utilization of a novel class of glucose-lowering drugs called sodium–glucose cotransporter 2 inhibitors (SGLT2i). Located in the proximal tubule of the kidney, the SGLT2 receptor directs the reuptake of glucose and sodium into the circulation (2,3). Inhibition of this pathway results in glucosuria/natriuresis and represents a novel metabolic mechanism to reduce hyperglycemia during T2D.

Several randomized clinical trials in people with and without diabetes have now established a critical role for SGLT2i in the reduction of cardiovascular and cardiorenal events (4,5). While direct myocardial and indirect metabolic effects have been proposed to explain these benefits, recent data suggest that SGLT2 inhibition stimulates vascular repair via increased trafficking of bone marrow–derived provascular progenitor cells essential for vessel regeneration (6–9).

In this issue of *Diabetes*, Albiero et al. (10) definitively demonstrate that despite low levels of expression of SGLT2 on hematopoietic cells, SGLT2 inhibition with dapagliflozin improved endothelial repair through enhanced recruitment of hematopoietic stem/progenitor cell (HSPC) progeny from the bone marrow (BM) to the site of vessel injury. In concept, circulating HSPC represent critical intermediates in blood vessel homeostasis via the coordination of angiogenic, arteriogenic, and vasculogenic processes (11). Seminal contributions by the Fadini group and others have previously established a significant depletion in circulating provascular HSPC during T2D, which contributes to vascular dysfunction and increased risk of CVD (12–14). To assess

the role of HSPC following SGLT2i treatment, streptozotocin (STZ)-treated mice were administered dapagliflozin or vehicle for 2 weeks at a dose that decreased hyperglycemia by 20% and did not alter LKS (Lin<sup>−</sup>/c-kit<sup>+</sup>/Sca-1<sup>+</sup>) progenitor cell frequency in the BM (10).

Albiero et al. first revealed that dapagliflozin administration reduced the frequency of SIGLEC-1–expressing (CD169<sup>+</sup>) macrophages in the BM of STZ-treated mice by 70% (10). Although the role of CD169<sup>+</sup> macrophages in the M1 versus M2 polarization spectrum is unclear, elevated CD169<sup>+</sup> macrophage content in the BM of patients with diabetes acts to retain HSPC in the BM niche and prevents HSPC mobilization (15). Indeed, dapagliflozin treatment in STZ-treated mice normalized BM CD169<sup>+</sup> macrophage content and partially rescued the HSPC mobilization defect in response to granulocyte colony-stimulating factor (10).

Next, the authors examined the effect of improved HSPC mobilization on the induction of carotid artery wound healing. Remarkably, re-endothelialization after denudation injury was decreased 10-fold in hyperglycemic mice, and endothelial healing was significantly improved after dapagliflozin treatment (10). Elegant experiments, involving carotid artery denudation in chimeric mice reconstituted with green fluorescent protein–positive (GFP<sup>+</sup>) BM cells, effectively traced GFP<sup>+</sup> HSPC progeny localized to the site of carotid injury. Reduced recruitment of GFP<sup>+</sup> cells to the site of carotid injury in STZ-treated diabetic chimeric mice was reversed by dapagliflozin treatment (10). What was the phenotype of these regenerative GFP<sup>+</sup> cells? Multiparametric flow cytometry analyses demonstrated GFP<sup>+</sup>/LKS cells were rarely detected (<0.1) at the site of injury, whereas mature monocyte and neutrophil

<sup>1</sup>Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada

<sup>2</sup>Division of Vascular Surgery, St. Michael's Hospital, Toronto, Ontario, Canada

<sup>3</sup>Department of Physiology & Pharmacology, Western University, London, Ontario, Canada

<sup>4</sup>Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada

<sup>5</sup>Division of Cardiac Surgery, St. Michael's Hospital, Toronto, Ontario, Canada

<sup>6</sup>Department of Surgery, University of Toronto, Toronto, Ontario, Canada

Corresponding authors: David A. Hess, [dhess@robarts.ca](mailto:dhess@robarts.ca), and Subodh Verma, [subodh.verma@unityhealth.to](mailto:subodh.verma@unityhealth.to)

© 2021 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at <https://www.diabetesjournals.org/content/license>.

See accompanying article, p. 1767.

recruitment was abundant, with F4/80<sup>+</sup> macrophage and CD49d<sup>+</sup> granulocyte recruitment significantly increased after dapagliflozin treatment (Fig. 1). Previously documented as a provascular population by this group (16), CD49d<sup>+</sup> granulocyte mobilization, recruitment, and adhesion at the site of injury promoted re-endothelialization after dapagliflozin administration, identifying CD49d<sup>+</sup> granulocytes as novel therapeutic targets to promote endothelial repair during T2D (10).

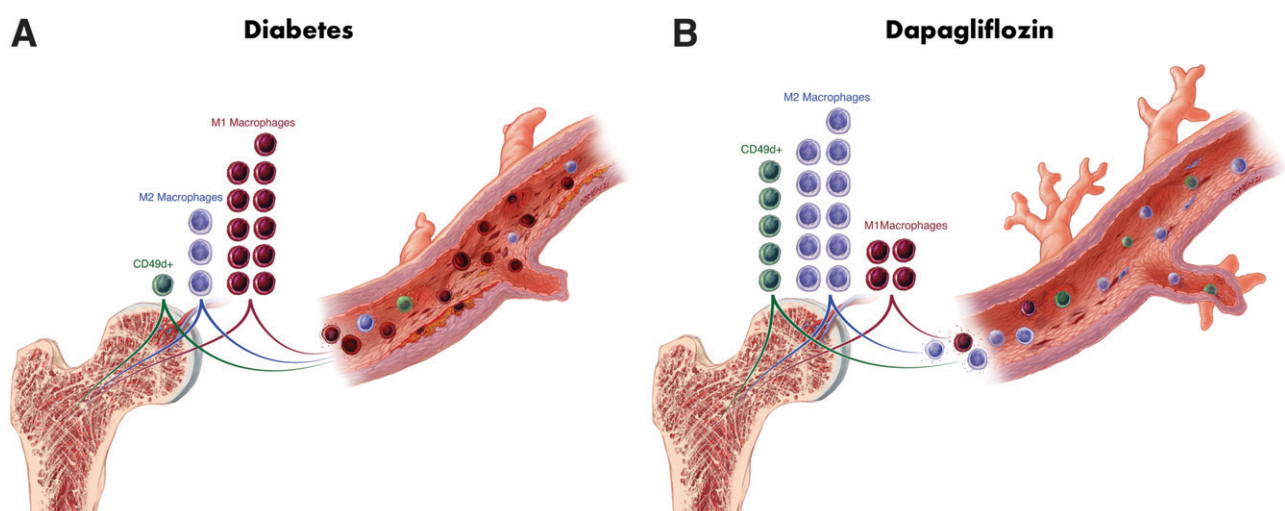
Is it simply glucose lowering that should be targeted, or are there unique benefits of SGLT2i beyond glucose lowering on BM cell regenerative flux? To address this question, the authors evaluated the influence of glucose lowering on endothelial wound healing through exogenous insulin administration or use of a ketogenic diet. Notably, insulin reduced systemic blood glucose (25%) and improved carotid endothelial healing—results similar to those seen with dapagliflozin therapy (10). In contrast, administration of a ketone drink did not reduce hyperglycemia but equally preserved endothelial healing (10). Therefore, endogenous vascular repair was stimulated, in part, through distinct alterations in systemic metabolism, including the improvement of glucose control and the induction of ketogenesis.

This report provides key mechanistic insights into cardiovascular protection following SGLT2i administration. Metabolic stabilization in the BM niche altered CD169<sup>+</sup> macrophage content to augment the differentiation and mobilization of circulating HSPC progeny that recruit to sites of endothelial damage to mediate healing. It is well known that chronic metabolic diseases like T2D increase glucotoxicity and oxidative stress in the BM (17,18) and contribute to a chronic

phenotype, termed provascular regenerative cell exhaustion, which alters the balance of HSPC differentiation to favor production of inflammatory versus regenerative cells (18). While several groups have established the potential of SGLT2i to restore provascular progenitor cell content in peripheral blood, Albiero et al. revealed the ability of SGLT2i to modify the BM niche through the production and release of mature provascular macrophage and CD49d<sup>+</sup> granulocyte subsets sensitive to chronic metabolic abnormalities. Thus, low-dose SGLT2 inhibition may reverse regenerative cell exhaustion and delay the onset or inhibit the progression of vascular dysfunction in patients with early T2D (HbA<sub>1c</sub> 6.0–6.5%) (19).

Arguably, the most transformative finding in this study is the introduction of CD49d<sup>+</sup> granulocytes with vascular-reparative function. Indeed, dapagliflozin treatment enhanced provascular CD49d<sup>+</sup> granulocyte recruitment to the site of injury and directly improved vascular repair within 3 days of damage (10). Further work on the characterization of these provascular cells in the peripheral circulation and the examination of CD49d<sup>+</sup> cell function in human patients with T2D administered SGLT2i or placebo is now warranted. This novel vascular regenerative mechanism may also have implications in nondiabetic models of atherosclerosis, such as in ApoE<sup>-/-</sup> mice with or without high-fat diet, and using other vascular injury models, such as femoral artery ligation-induced limb ischemia, as a model for lower extremity arterial disease (20).

Collectively, the study of Albiero et al. establishes novel mechanisms that clarify the critical role of BM-derived cellular subsets to directly enhance endothelial healing



**Figure 1**—Dapagliflozin improves the trafficking of provascular cells from the bone marrow and re-endothelialization after carotid artery injury. **A:** Diabetes is associated with significant depletion and dysfunction of circulating provascular regenerative cell subsets, leading to reduced blood vessel repair after ischemic injury. The bone marrow microenvironment demonstrated elevated CD169<sup>+</sup> macrophage content that propagates chronic inflammation and prevents the differentiation, mobilization, and recruitment of provascular monocytes and granulocytes to the site of endothelial injury. **B:** Dapagliflozin administration indirectly reduced proinflammatory CD169<sup>+</sup> macrophages in the bone marrow, enhanced the trafficking of provascular CD49d<sup>+</sup> granulocytes to the site of endothelial injury, and improved healing of the damaged endothelium.

during diabetes. These findings will usher in a new vascular regenerative paradigm for the translation of metabolism-altering approaches to reverse provascular regenerative cell depletion and dysfunction during chronic metabolic diseases associated with ischemic CVD.

**Duality of Interest.** S.V. reports receiving research grants and/or speaking honoraria from Amarin, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, EOCI Pharmacomm Ltd., HLS Therapeutics, Janssen, Merck, Novartis, Novo Nordisk, Pfizer, PhaseBio, Sanofi, Sun Pharmaceuticals, and the Toronto Knowledge Translation Working Group. No other potential conflicts of interest relevant to this article were reported.

## References

- Mechanick JI, Farkouh ME, Newman JD, Garvey WT. Cardiometabolic-based chronic disease, adiposity and dysglycemia drivers: JACC state-of-the-art review. *J Am Coll Cardiol* 2020;75:525–538
- Zelniker TA, Braunwald E. Mechanisms of cardiorenal effects of sodium-glucose cotransporter 2 inhibitors: JACC state-of-the-art review. *J Am Coll Cardiol* 2020;75:422–434
- Verma S, McMurray JJV. SGLT2 inhibitors and mechanisms of cardiovascular benefit: a state-of-the-art review. *Diabetologia* 2018;61:2108–2117
- McGuire DK, Shih WJ, Cosentino F, et al. Association of SGLT2 inhibitors with cardiovascular and kidney outcomes in patients with type 2 diabetes: a meta-analysis. *JAMA Cardiol* 2021;6:148–158
- Connelly KA, Bhatt DL, Verma S. Can we DECLARE a victory against cardio-renal disease in diabetes? *Cell Metab* 2018;28:813–815
- Hess DA, Terenzi DC, Trac JZ, et al. SGLT2 inhibition with empagliflozin increases circulating provascular progenitor cells in people with type 2 diabetes mellitus. *Cell Metab* 2019;30:609–613
- Rahadian A, Fukuda D, Salim HM, et al. Canagliflozin prevents diabetes-induced vascular dysfunction in apoe-deficient mice. *J Atheroscler Thromb* 2020;27:1141–1151
- Steven S, Oelze M, Hanf A, et al. The SGLT2 inhibitor empagliflozin improves the primary diabetic complications in ZDF rats. *Redox Biol* 2017;13:370–385
- Vallon V, Verma S. Effects of SGLT2 inhibitors on kidney and cardiovascular function. *Annu Rev Physiol* 2021;83:503–528
- Albiero M, Tedesco S, Amendolagine FI, et al. Inhibition of SGLT2 rescues bone marrow cell traffic for vascular repair: role of glucose control and ketogenesis. *Diabetes* 2021;70:1767–1779
- Qadura M, Terenzi DC, Verma S, Al-Omran M, Hess DA. Concise review: cell therapy for critical limb ischemia: an integrated review of preclinical and clinical studies. *Stem Cells* 2018;36:161–171
- Terenzi DC, Al-Omran M, Quan A, Teoh H, Verma S, Hess DA. Circulating pro-vascular progenitor cell depletion during type 2 diabetes: translational insights into the prevention of ischemic complications in diabetes. *JACC Basic Transl Sci* 2018;4:98–112
- Zafar N, Krishnasamy SS, Shah J, et al. Circulating angiogenic stem cells in type 2 diabetes are associated with glycemic control and endothelial dysfunction. *PLoS One* 2018;13:e0205851
- Fadini GP, Sartore S, Albiero M, et al. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. *Arterioscler Thromb Vasc Biol* 2006;26:2140–2146
- Albiero M, Poncina N, Ciciliot S, et al. Bone marrow macrophages contribute to diabetic stem cell mobilopathy by producing oncostatin M. *Diabetes* 2015;64:2957–2968
- Cappellari R, D'Anna M, Menegazzo L, et al. Diabetes mellitus impairs circulating proangiogenic granulocytes. *Diabetologia* 2020;63:1872–1884
- Mangialardi G, Spinetti G, Reni C, Madeddu P. Reactive oxygen species adversely impacts bone marrow microenvironment in diabetes. *Antioxid Redox Signal* 2014;21:1620–1633
- Terenzi DC, Trac JZ, Teoh H, et al. Vascular regenerative cell exhaustion in diabetes: translational opportunities to mitigate cardiometabolic risk. *Trends Mol Med* 2019;25:640–655
- Albiero M, Menegazzo L, Boscaro E, Agostini C, Avogaro A, Fadini GP. Defective recruitment, survival and proliferation of bone marrow-derived progenitor cells at sites of delayed diabetic wound healing in mice. *Diabetologia* 2011;54:945–953
- Sherman SE, Bell GI, Teoh H, et al. Canagliflozin improves the recovery of blood flow in an experimental model of severe limb ischemia. *JACC Basic Transl Sci* 2018;3:327–329