

## Letter

### TO THE EDITOR

## Canagliflozin Improves the Recovery of Blood Flow in an Experimental Model of Severe Limb Ischemia

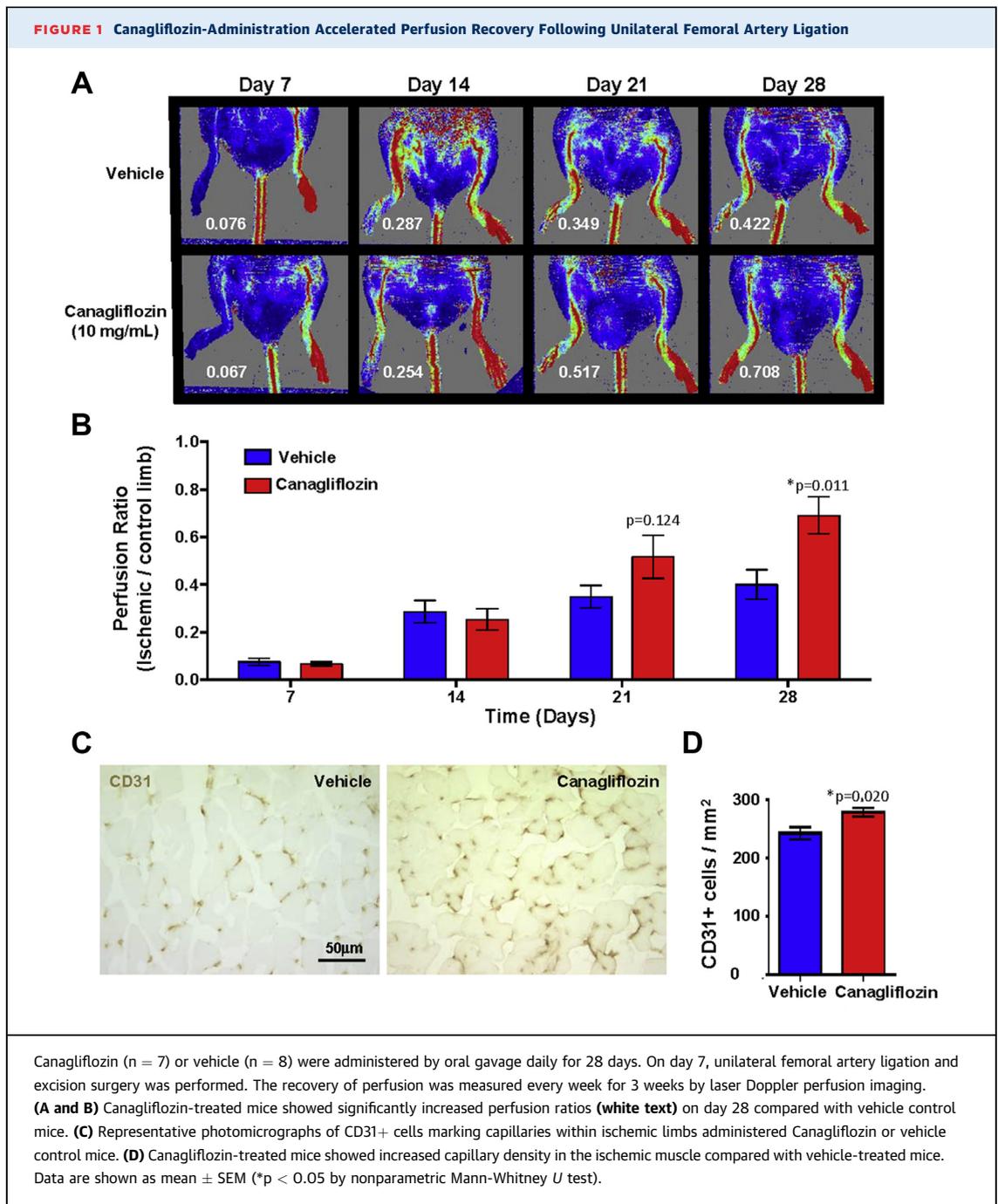


Canagliflozin, a sodium-glucose cotransporter 2 inhibitor (SGLT2i), was shown to reduce major adverse cardiovascular events in the recent CANVAS (CANagliflozin cardioVascular Assessment Study) program (1). Canagliflozin treatment was, however, also associated with a significant increase in the risk of lower limb amputations (hazard ratio [HR]: 1.97; 95% confidence interval [CI]: 1.41 to 2.75), with the majority occurring at the toe or transmetatarsal level. The strongest determinants of amputation risk were a history of prior amputation (HR: 20.9; 95% CI: 14.2 to 30.8) and the presence of peripheral artery disease at baseline (HR: 3.1; 95% CI: 2.2 to 4.5). The U.S. Food and Drug Administration subsequently issued a label warning for amputations that is specific to canagliflozin. Notably, although a recently published analysis using data from the U.S. Department of Defense Military Health System reported a signal for a higher risk of below-knee lower-extremity amputations with SGLT2i (2), another large-scale cardiovascular outcome trial that evaluated the SGLT2i empagliflozin observed no signal for amputations, even among participants with a history of peripheral artery disease (3). Although it has been widely speculated that the increased risk of amputation with canagliflozin may be attributed to changes in limb blood flow that are exaggerated in the setting of peripheral artery disease, there are no mechanistic and/or preclinical insights in this regard. Accordingly, we evaluated the impact of canagliflozin on blood flow recovery in a murine surrogate model of severe limb ischemia induced by unilateral femoral artery ligation and excision (FAL).

To assess the capacity for vascular regeneration during hindlimb ischemia, canagliflozin (10 mg/kg/day;

$n = 7$ ) or vehicle control ( $n = 8$ ) was administered to nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice via oral gavage daily for 28 days. On day 7, ligation and excision of a 3- to 5-mm segment of the femoral artery just proximal to the superficial caudal epigastric artery branch was performed as previously described (4,5), and laser Doppler perfusion imaging was performed weekly from days 7 through 28. Studies were approved by the Animal Care Committee at Western University, London, Ontario, Canada. Although this model does not assess chronic limb ischemia as a result of progressive and pervasive atherosclerosis, FAL surgery is a widely accepted model for the induction of acute and severe limb ischemia (4). Surprisingly, canagliflozin-treated mice demonstrated accelerated perfusion recovery compared with vehicle-treated mice (Figures 1A and 1B). Indeed, perfusion ratios in canagliflozin-treated mice were consistently increased at day 28 ( $p = 0.011$ ) compared with the vehicle-treated cohort (Figure 1B). Canagliflozin treatment was also associated with modest hemoglobin concentration compared with control treatment (hematocrit:  $44.3 \pm 2.2\%$  for vehicle [ $n = 4$ ],  $49.3 \pm 3.7\%$  for canagliflozin [ $n = 5$ ];  $p = 0.22$ ). Finally, capillary density quantification was performed on frozen adductor muscle sections using a rat antimouse CD31 antibody using peroxidase-labeled antirat secondary antibody and visualized with 3,3'-diaminobenzidine. CD31<sup>+</sup> cells/mm<sup>2</sup> were enumerated in a blinded fashion from 4 fields/section  $\times$  3 sections (Figure 1C). Importantly, CD31 is expressed primarily on endothelial cells and is used as a strong marker to label newly formed capillaries in the ischemic region, where increased CD31<sup>+</sup> cell capillary density correlates directly with the recovery of tissue perfusion after FAL surgery. Consistent with the recovery of perfusion by laser Doppler perfusion imaging, canagliflozin-treated mice showed significantly increased capillary density in the ischemic limb compared with vehicle control mice, which was potentially mediated by enhanced activation of both arteriogenic and angiogenic mechanisms (Figure 1D).

Collectively, and contrary to our hypothesis that canagliflozin treatment would delay the recovery of perfusion after FAL surgery, these data suggest that



canagliflozin administration did not impair the efficiency of endogenous vascular recovery. Therefore, in this preclinical model, the cellular and molecular machinery that mediate vascular remodeling via endogenous angiogenic processes were not impaired and were possibly heightened during canagliflozin treatment. Thus, the results described

herein suggest that the increased risk of amputation reported with canagliflozin in the clinical setting may not be related to changes in limb blood flow induced by hemoconcentration. Follow-up preclinical studies are warranted using alternate hindlimb ischemia models to fully assess the potential effect of canagliflozin on peripheral artery disease.

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

## REFERENCES

1. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med* 2017;377:644-57.
2. Udell JA, Yuan Z, Rush T, Sicignano NM, Galitz M, Rosenthal N. Cardiovascular outcomes and risks after initiation of a sodium glucose co-transporter 2 inhibitor: results from the EASEL population-based cohort study. *Circulation* 2018;137:1450-9.
3. Verma S, Mazer CD, Al-Omran M, et al. Cardiovascular outcomes and safety of empagliflozin in patients with type 2 diabetes mellitus and peripheral artery disease: a subanalysis of EMPA-REG OUTCOME. *Circulation* 2018;137:405-7.
4. Putman DM, Liu KY, Broughton HC, Bell GI, Hess DA. Umbilical cord blood-derived aldehyde dehydrogenase-expressing progenitor cells promote recovery from acute ischemic injury. *Stem Cells* 2012;30:2248-60.
5. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964-7.