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EXPERIMENTAL

Hyperglycemia Interacts with Ischemia in a Synergistic Way on Wound Repair and Myofibroblast Differentiation

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Background: Hyperglycemia is known to adversely affect the outcome of ischemic insults, but its interaction with ischemia has not been investigated in wound repair yet. In this study, we develop a new animal model allowing to investigate the interaction between hyperglycemia and ischemia during the wound repair process. We focus on myofibroblast differentiation, a key element of wound repair.

Methods: Ischemia was inflicted in Wistar rats by resection of the femoral to popliteal arteries on the left side, whereas arteries were dissected without resection on the right side. Full-thickness skin wounds (1 cm^2) were created on both feet. Hyperglycemia was induced by injection of streptozotocin. Normoglycemic animals served as control (n = 23/group). Blood flow, wound closure, and myofibroblast expression were measured.

Results: Wound closure was significantly delayed in ischemic compared with nonischemic wounds in all rats. This delay was almost 5-fold exacerbated in hyperglycemic rats compared with normoglycemic rats, while hyperglycemia alone showed only a slight effect on wound repair. Delayed wound repair was associated with impaired wound contraction and myofibroblast differentiation.

Conclusions: Our model allows to specifically quantify the effect of hyperglycemia and ischemia alone or in combination on wound repair. We show that hyperglycemia amplifies the inhibitory effect of ischemia on wound repair and myofibroblast expression. Our data reveal for the first time the synergic aspect of this interaction and therefore stress the importance of a strict glycemic control in the management of ischemic wounds. *(Plast Reconstr Surg Glob Open 2015;3:e471; doi: 10.1097/GOX.0000000000000443; Published online 24 July 2015.)*

Diabetes mellitus is the most important factor predisposing to chronic wounds.[1] Up to 25% of patients with diabetes develop a chronic ulcer during their lifetime,[2,3] which is associated with significantly reduced life quality[4] and greatly increased mortality.[5,6] Chronic ulcers often require long-term and expensive therapy imposing a staggering burden on the public health systems.[7,8]

Current theories about the physiopathology of diabetic foot ulcers emphasize the importance of the classical triad of ischemia due to angiopathy, neuropathy, and infection.[9,10] By contrast, the role of hyperglycemia per se is not well elucidated and remains controversial.[3,11,12] Clinical studies reported that hyperglycemia may interfere with ischemia and adversely affect the outcome of ischemic insults,[13–17]

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but this interaction has not been investigated in wound repair yet.

During wound repair, local dermal fibroblasts and precursor cells from other sources differentiate into myofibroblasts by neoexpressing α-smooth muscle actin (α-SMA), conferring to them high contractile activity[18,19] and promoting wound contraction.[20] We previously demonstrated that ischemic wounds exhibit decreased levels of myofibroblast appearance and α-SMA expression with consequently a prolonged repair time.[21,22]

To date, there is no appropriate experimental model to specifically study the interaction between hyperglycemia and ischemia in the setting of wound repair. To address this question, we have developed an animal wound model designed to investigate ischemic wound repair in both normoglycemic and streptozotocin-induced hyperglycemic rats. Because ischemia was previously shown to impede myofibroblast differentiation,[22] we particularly focused on the behavior of these cells. In this study, we aimed to investigate the role of hyperglycemia on wound healing and whether hyperglycemia per se amplifies the negative effects of ischemia.

RESEARCH DESIGN AND METHODS

Animals

Forty-six male Wistar rats aged 90 days with a weight of 300–350 g (Charles River Laboratories, L'Arbresle Cedex, France) were fed a standard diet and given water ad libitum. For all surgical procedures, animals were anesthetized by inhalation of isoflurane 5%. The local ethics committee and veterinary authority approved all procedures according to Swiss guidelines.

Wound Model

As described previously,[17] through a longitudinal incision in the inguinal region that has been shaved, the femoral artery was dissected down to popliteal artery and resected in the left limb (ischemic limb) while conserved in the control right limb (nonischemic limb). After the arterial lesion, wounds were created on both legs on the dorsal aspect of the both feet in all animals by removing a full-thickness skin area of 1.2×0.8 cm (Fig. 1). All surgical procedures were performed under an operating microscope (Superflux 300, Carl Zeiss Vanospot, Zeiss, Germany).

Induction of Hyperglycemia

Hyperglycemia was induced by intraperitoneal injection of streptozotocin (50 mg/kg in 0.1 M citrate buffer, pH 4.5; Sigma-Aldrich, St. Louis, Mo.) 3 weeks before surgery. Glycemia was measured before surgery from tail venous blood by blood glucose test strips and just before euthanasia from carotid blood using glucose oxidase method (Glu; Roche Diagnostics, Rotkreuz, Switzerland). Rats with glucose levels >11.1 mM were included in the hyperglycemic group.

Experimental Groups

We compared streptozotocin-induced hyperglycemic animals with normoglycemic animals (n = 23 per group). By infliction of unilateral ischemia in both animal groups, we created the following 4 wound conditions: (1) hyperglycemic, nonischemic; (2) hyperglycemic, ischemic; (3) normoglycemic, nonischemic; and (4) normoglycemic, ischemic.

Percutaneous Laser Doppler Measurements

Laser Doppler flowmetry was performed to measure blood flow in the skin using a percutane-

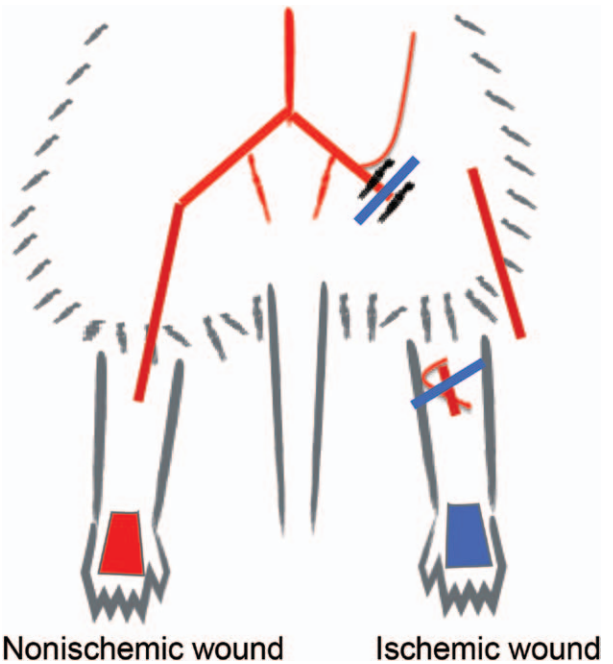

Fig. 1. A model with the en bloc resection of the arterial axis at the level of the femoral artery to the saphenous artery on the left limb and a sham operation (dissection of arterial bed without arterial lesion) on the right limb. Two rectangular wounds are then created on the dorsal aspect of each foot.

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ous laser Doppler perfusion monitor (PIM II Laser Doppler Perfusion Imager, LDPIwin 2.0.6 software, Lisca AB Berzelius Science Park, Linköping, Sweden). Measurements were carried out on the dorsal surface of the foot, immediately before creation of the ischemic injury (baseline) and during the observation period after the wounding. Results were expressed in arbitrary perfusion units.

Wound Repair Assessment

To avoid imprecisions due to irregular wound surface, wounds were traced on transparent sheets molding the surface every 2 days during the first week (d0 immediately after wounding, d1, d3, d5, d7) and then twice a week until complete wound repair. Transparent sheets were photographed, and wound surfaces were measured on photographs using a computer-assisted image analysis system (Image J, imagej.nih.gov/ij/).

At complete wound closure (ie, full epithelialization of the wound and absence of crust), the surface of hairless skin of the scar was measured and considered to correspond to the area of the wound healed by epithelialization. The surface of the wound healed by contraction was then estimated by subtraction of the epithelialized surface from the wound surface measured at day 0 (Fig. 2).

Immunohistochemistry

Rats were killed at days 7, 14, 21, and 28 for tissue harvesting (n = 4 per time point, per group). The tissue was fixed in 4% buffered formaldehyde, followed by decalcification with 14% HCl. Immunostaining was performed on paraffin-embedded transverse sections using anti-α-SMA primary antibodies (a kind gift of Giulio Gabbiani, University of Geneva, Geneva, Switzerland). Before using the first antibody, antigen retrieval was performed by applying microwaves for 5 minutes in citrate buffer (10 mM; pH 6.0), followed by incubation with a goat anti-mouse-biotinylated IgG (Jackson Immunoresearch, West Grove, Pa.) and treatment with streptavidin-biotin-peroxidase (Dako, Glostrup, Denmark). The peroxidase activity was detected with diaminobenzidine (Serva, Heidelberg, Germany). Slides were counterstained with hemalun and mounted in Aquatex (Dako).

Sections were assessed with a Zeiss Axioskop 2 (Carl Zeiss) using a 3200 Kelvin tungsten light and a plan-Neofluar 40/0.75 lens. Images were acquired with a 3-chip CCD high-sensitivity Photonic Science Coolview camera (Carl Zeiss) using Image Access software (Imagic, Zurich, Switzerland). Three fields per section within the lesions were randomly selected. Images were subsequently analyzed using KS400 software (Kontron System, Zeiss Vision, Jena, Germany). To evaluate α-SMA expression, vessels were manually excluded from the image to retain only the area of interest. Results were given as α-SMA pixel/mm^2.

Statistical Methods

All values were expressed as the mean ± standard error of the mean. Data were analyzed with the use of Stata software (StataCorp, College Station, Tex.), version 11.0. Statistical analysis consisted in a comparison of data from hyperglycemic versus normoglycemic wounds, in both ischemic and nonischemic condition, using the nonparametric Kruskal-Wallis test followed by the measures to correct the α-error according to Bonferroni probabilities. Differences were considered significant at $P < 0.05$.

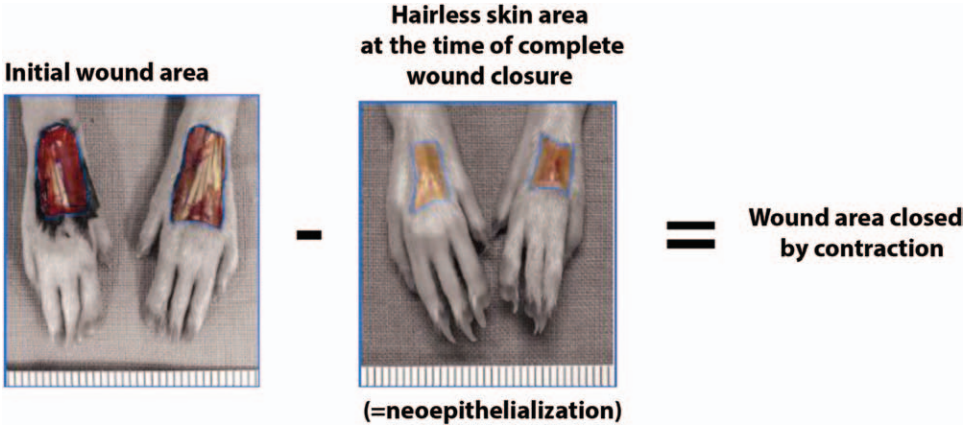

Fig. 2. The area of the wound is measured directly after surgery (d0). At the day of complete wound closure, the area of hairless skin is measured and considered to correspond to the area of the wound that was closed by re-epithelialization. Subtracting the area of re-epithelialization from the initial wound area provides the area closed by contraction.

RESULTS

Blood Flow Measurement

In nonischemic limbs, blood flow increased during the first days following wound creation, remained stable for 3 weeks, and returned to baseline. No difference in blood flow was observed between the normoglycemic and hyperglycemic groups in nonischemic conditions.

In ischemic limbs, we observed a substantial decrease in initial blood flow after resection of the arteries, which was similar in both normoglycemic and hyperglycemic groups (70% and 65% of initial value, respectively, for normoglycemic and hyperglycemic animals; not significant). Blood flow remained low in hyperglycemic animals over several weeks, whereas it progressively increased in normoglycemic animals reaching significant difference between both groups after day 14 (127% and 86%, respectively, of initial value; $P < 0.05$) (Fig. 3).

Wound Closure

Wounds of hyperglycemic animals healed significantly slower than those of normoglycemic animals; this delay was exacerbated in ischemic conditions (Fig. 4 and Table 1). In nonischemic conditions, hyperglycemic wounds were completely closed after 17.2 ± 0.5 days, slower compared with 15.1 ± 0.4 days for normoglycemic wounds ($P = 0.01$). In ischemic conditions, wound healing time was 36 ± 3.1 days for hyperglycemic wounds, hence twice as slow compared with 19.3 ± 0.8 days for normoglycemic wounds ($P = 0.002$). The delay related to ischemia was almost 5-fold longer in hyperglycemic rats compared with normoglycemic rats (~19 vs 4 delayed days), while hyperglycemia alone showed slight effect (Table 1).

At the time of complete wound closure, in both nonischemic and ischemic conditions, the surface of newly formed epithelium, that is, hairless skin, was significantly larger in hyperglycemic animals compared with normoglycemic animals. This difference indicates that the wound surface proportion closed by contraction was significantly smaller in hyperglycemic animals. In nonischemic condition, the proportion of wound closed by contraction was 70% ± 2% in hyperglycemic versus 84% ± 2% in normoglycemic wounds ($P = 0.001$), whereas in ischemic conditions, it was 49% ± 9% in hyperglycemic versus 74% ± 3% in normoglycemic wounds ($P = 0.005$). The impaired wound contraction related to ischemia was stronger in hyperglycemic rats compared with normoglycemic rats (Table 2 and Fig. 5).

Histology

Hyperglycemic wounds showed a substantial decrease in myofibroblast marker expression in both ischemic and nonischemic conditions (Fig. 6). Nonischemic/normoglycemic wounds showed a strong myofibroblast presence at 7 days post-wounding followed by a gradual decrease in α-SMA expression. By contrast, nonischemic/hyperglycemic wounds displayed a delayed and lower α-SMA expression with a peak at day 14 (0.02 ± 0.01 α-SMA pixel/mm^2), almost 5-fold lower compared with the peak expression in nonischemic/normoglycemic wounds (0.1 ± 0.04 α-SMA pixel/mm^2) ($P = 0.006$). Myofibroblast occurrence was also significantly lower in ischemic/normoglycemic wounds (0.02 ± 0.01 vs 0.1 ± 0.04 α-

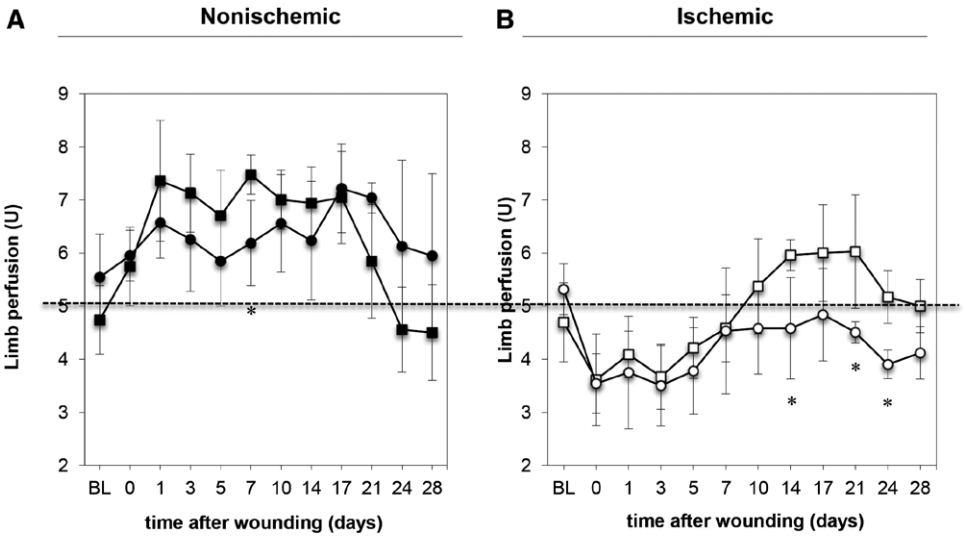

Fig. 3. Time course of wound perfusion of normoglycemic (squares) and hyperglycemic (circles) animals in nonischemic (A) and ischemic (B) conditions. Mean ± SEM; *$P < 0.05$ vs normoglycemic animals; $n = 7$/group. BL indicates baseline.

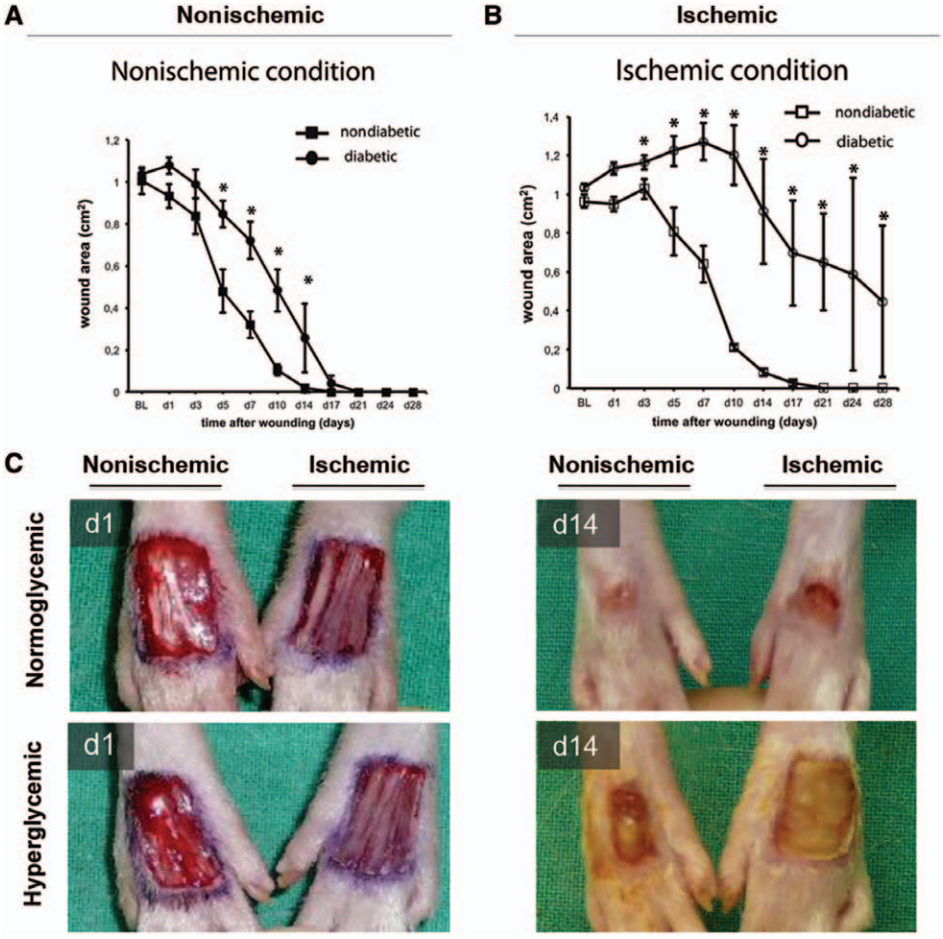

Fig. 4. Time course of wound closure in normoglycemic (square) and hyperglycemic (circle) animals under nonischemic (A) and ischemic (B) conditions. Mean ± SEM; *$P < 0.05$ vs normoglycemic animals; $n = 7$/group. Morphology of wounds in normoglycemic and hyperglycemic animals, under nonischemic and ischemic conditions, at day 1 and day 14 after wound infliction (C).

Table 1. Time of Wound Closure (Days) in Normoglycemic and Hyperglycemic Animals in Nonischemic and Ischemic Conditions ($n = 7$/Group)

	Normoglycemic	Hyperglycemic
Nonischemic	15.1 ± 0.4	17.2 ± 0.5*
Ischemic	19.3 ± 0.8†	36 ± 3.3*†

Data are presented as mean ± SEM.
*$P < 0.05$ versus normoglycemic animals.
†$P < 0.05$ versus nonischemic wounds.

Table 2. Wound Contraction at the Time of Complete Wound Closure (%) in Normoglycemic and Hyperglycemic Animals in Nonischemic and Ischemic Conditions ($n = 7$/Group)

	Normoglycemic	Hyperglycemic
Nonischemic	84 ± 2	70 ± 2*
Ischemic	74 ± 3†	49 ± 9*†

Data are presented as mean ± SEM.
*$P < 0.05$ versus normoglycemic animals.
†$P < 0.05$ versus nonischemic wounds.

SMA pixel/mm², day 7, $P = 0.003$) in comparison to nonischemic/normoglycemic wounds. Interestingly, ischemic/hyperglycemic wounds were completely devoid of myofibroblasts during the whole time of wound closure.

DISCUSSION

The negative impact of diabetes on wound repair is well known in clinical practice. In diabetic patients with combined arterial insufficiency of the lower extremities, ulcers are more frequent and have a less favorable prognosis compared with nondiabetic patients.[23–25] The synergistic interaction between diabetes and ischemia in chronic wounds appears intuitive, but there are no specific data on the type of interaction. Because diabetic ulcers are a very heterogeneous group involving multiple pathophysiological processes,[26,27] the interpretation of clinical

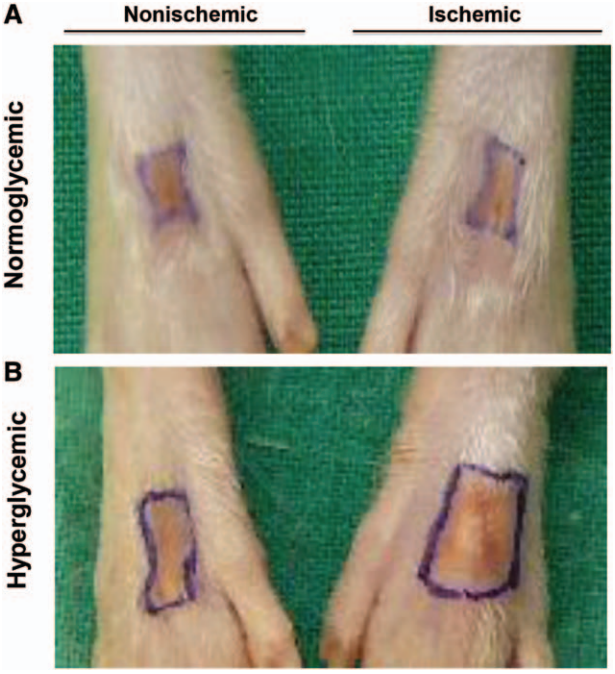

Fig. 5. Morphology of scars in normoglycemic (A) and hyperglycemic (B) animals, under nonischemic and ischemic conditions, after complete wound repair. Note that the surface of the scar, that is, hairless skin, is significantly larger in hyperglycemic animals compared with normoglycemic animals. This difference indicates that the wound surface proportion closed by contraction is significantly smaller in hyperglycemic animals.

observations is difficult. To date, there has been no experimental model that would allow addressing the question of the type of the interaction between diabetes and ischemia.

We have developed a single and reproducible animal model that takes into account most of the problems encountered in wound repair research. Our model allows (1) the creation of homogeneous and comparable wounds, (2) the production of a large full-thickness wound in an area of tightly fixed skin where both wound contraction and epithelialization can be quantified, and (3) the selective and standardized infliction of arterial ischemia. By resecting the arteries only on one side, we obtain a nonischemic control limb in the same individual.

By injecting streptozotocin 2 weeks before wound infliction, we induce a hyperglycemic condition free from long-term effects such as neuropathy or microangiopathy. This allows to specifically investigate (1) the effects of hyperglycemia per se, when comparing hyperglycemic and normoglycemic wounds in the nonischemic condition and (2) the interaction and synergy between the 2 factors when comparing the delay in ischemic wounds of hyperglycemic and normoglycemic animals.

We observed that hyperglycemia alone has only slight effect on wound healing. Wound closure was, however, significantly delayed in ischemic wounds compared with nonischemic wounds, in both normoglycemic rats and hyperglycemic rats. Interestingly, this delay was almost 5-fold longer in hyperglycemic rats compared with normoglycemic rats for a same level of ischemia (~19 vs 4 delayed days). We then demonstrate that hyperglycemia not only increases but also potentiates the deleterious effect of ischemia on wound repair in a synergistic way.

The phenomenon of increased sensibility to ischemia caused by hyperglycemia has been investigated in ischemic tissues other than skin.[13–17] Hyperglycemia has been associated with impaired outcome in patients with ischemic stroke and myocardial infarction.[13–16] The impact of hyperglycemia on stroke size has been studied in animal models and revealed that hyperglycemic animals presented 94% larger infarct areas (140% in streptozotocin-induced hyperglycemia and 48% after dextrose infusion) compared with normoglycemic animals.[28] In a recent study, we found a significantly higher rate of limb necrosis in hyperglycemic rats compared with normoglycemic rats with the same level of lower extremity ischemia (71% vs 29%).[17]

The abovementioned studies showed the negative impact of hyperglycemia on the tolerance to ischemia and the development of necrosis in ischemic tissues. However, the mechanisms of tissue survival and wound healing are different, and our finding on wound healing deserves to be highlighted.

We here revealed a common mechanism through which both ischemia and hyperglycemia delay wound repair. We demonstrate that both factors delay wound repair mainly by decreasing wound contraction. This can be correlated with a reduced α-SMA expression, hallmark of myofibroblast,[20–29] even if there is a controversy about the direct relationship between expression of α-SMA and wound contraction.[30] Moreover, when hyperglycemia is combined with ischemia, effects of ischemia are amplified, resulting in an elimination of myofibroblast expression.

Hyperglycemia seems to impair wound reperfusion in ischemic condition at later stages of the wound repair process. This is in line with previous studies in which hyperglycemia has been associated with endothelial cell dysfunction, provoking an imbalance between vasoconstricting and vasodilating substances[26,31] as well as decreased arteriogenesis and angiogenesis.[32,33] However, the statistical reperfusion difference in ischemic wounds between normoglycemic and hyperglycemic animals is observed from day 14, beyond the time point in which myofibroblast differentiation is supposed to occur.

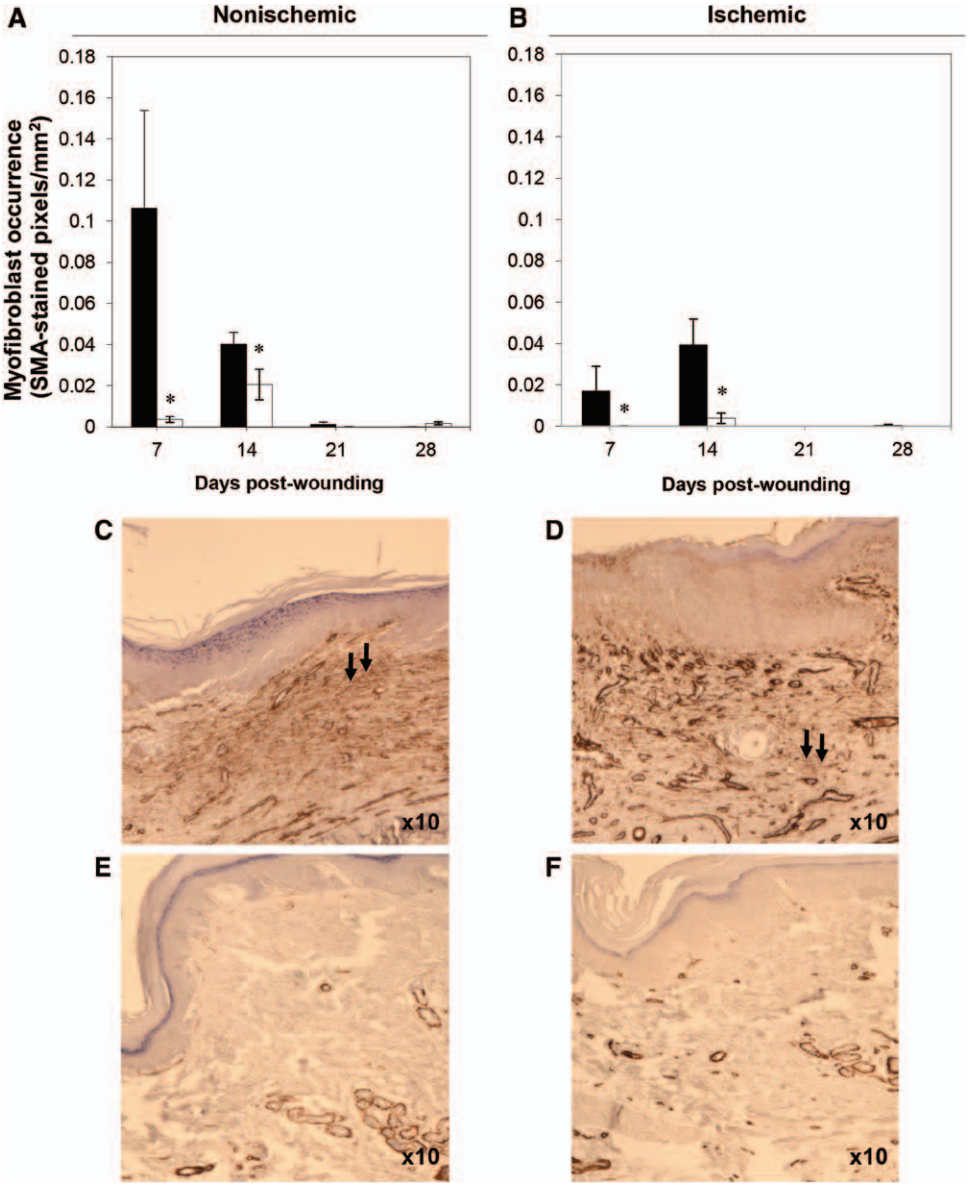

Fig. 6. Time course of myofibroblast expression in normoglycemic (black bar) and hyperglycemic (white bar) animals under nonischemic (A) and ischemic (B) conditions. Mean ± SEM; *$P < 0.05$ vs normoglycemic animals; $n = 4$/time point/group. Staining for α-SMA at day 7 in normoglycemic (C, E) and hyperglycemic wounds (D, F) in nonischemic (C, D) and ischemic (E, F) conditions. Positive cells are represented by brown staining (double arrows).

Therefore, in this model, the blood flow difference seems not to be the mechanism through which hyperglycemia impairs myofibroblast differentiation in ischemic wounds.

CONCLUSIONS

In conclusion, we present a new animal model to study diabetic wound repair under conditions of persistent ischemia. Our results show that hyperglycemia alone has an impact on the wound healing process as observed in normoxic condition. Moreover, it exacerbates the inhibitory effect of ischemia on wound repair, in a synergistic way, specifically on myofibroblast differentiation and wound contraction. Our results therefore highlight the importance of glycemic control in patients with ischemic wounds. Similar to the management of ischemic stroke patients, glycemic control should be a crucial part of the treatment of ischemic wounds, specifically when associated with arteriopathy that cannot be treated surgically. Our study opens ways for new therapeutic strategies for the management of diabetic wounds that could be able to restore myofibroblast expression, such as mechanical stimulation, oxygen therapy, or admin-

istration of growth factors. Additional studies are required to elucidate the molecular pathways that could be targeted specifically.

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REFERENCES

1. Unwin N, Gan D, Whiting D. The IDF Diabetes Atlas: providing evidence, raising awareness and promoting action. *Diabetes Res Clin Pract.* 2010;87:2–3.
2. Reiber GE, Vileikyte L, Boyko EJ, et al. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diabetes Care* 1999;22:157–162.
3. Velander P, Theopold C, Hirsch T, et al. Impaired wound healing in an acute diabetic pig model and the effects of local hyperglycemia. *Wound Repair Regen.* 2008;16:288–293.
4. Persoon A, Heinen MM, van der Vleuten CJ, et al. Leg ulcers: a review of their impact on daily life. *J Clin Nurs.* 2004;13:341–354.
5. Iversen MM, Tell GS, Riise T, et al. History of foot ulcer increases mortality among individuals with diabetes: ten-year follow-up of the Nord-Trøndelag Health Study, Norway. *Diabetes Care* 2009;32:2193–2199.
6. Escandon J, Vivas AC, Tang J, et al. High mortality in patients with chronic wounds. *Wound Repair Regen.* 2011;19:526–528.
7. Danaei G, Finucane MM, Lu Y, et al; Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Blood Glucose). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. *Lancet* 2011;378:31–40.
8. Wild S, Roglic G, Green A, et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004;27:1047–1053.
9. Jeffcoate WJ, Price P, Harding KG; International Working Group on Wound Healing and Treatments for People with Diabetic Foot Ulcers. Wound healing and treatments for people with diabetic foot ulcers. *Diabetes Metab Res Rev.* 2004;20(Suppl 1):S78–S89.
10. Tobalem M, Uçkay I. Images in clinical medicine. Evolution of a diabetic foot infection. *N Engl J Med.* 2013;369:2252.
11. Berdal M, Jenssen T. No association between glycemia and wound healing in an experimental db/db mouse model. *ISRN Endocrinol.* 2013;2013:307925.
12. Pietramaggiori G, Scherer SS, Alperovich M, et al. Improved cutaneous healing in diabetic mice exposed to healthy peripheral circulation. *J Invest Dermatol.* 2009;129:2265–2274.
13. Lin B, Ginsberg MD, Busto R. Hyperglycemic but not normoglycemic global ischemia induces marked early intraneuronal expression of beta-amyloid precursor protein. *Brain Res.* 2001;888:107–116.
14. Lin B, Ginsberg MD, Busto R. Hyperglycemic exacerbation of neuronal damage following forebrain ischemia: microglial, astrocytic and endothelial alterations. *Acta Neuropathol.* 1998;96:610–620.
15. de Courten-Myers G, Myers RE, Schoolfield L. Hyperglycemia enlarges infarct size in cerebrovascular occlusion in cats. *Stroke* 1988;19:623–630.
16. Eitel I, Hintze S, de Waha S, et al. Prognostic impact of hyperglycemia in nondiabetic and diabetic patients with ST-elevation myocardial infarction: insights from contrast-enhanced magnetic resonance imaging. *Circ Cardiovasc Imaging* 2012;5:708–718.
17. Lévigne D, Tobalem M, Modarressi A, et al. Hyperglycemia increases susceptibility to ischemic necrosis. *Biomed Res Int.* 2013;2013:490964.
18. Hinz B, Celetta G, Tomasek JJ, et al. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. *Mol Biol Cell* 2001;12:2730–2741.
19. Hinz B, Phan SH, Thannickal VJ, et al. The myofibroblast: one function, multiple origins. *Am J Pathol.* 2007;170:1807–1816.
20. Hinz B. Formation and function of the myofibroblast during tissue repair. *J Invest Dermatol.* 2007;127:526–537.
21. Modarressi A, Pietramaggiori G, Godbout C, et al. Hypoxia impairs skin myofibroblast differentiation and function. *J Invest Dermatol.* 2010;130:2818–2827.
22. Alizadeh N, Pepper MS, Modarressi A, et al. Persistent ischemia impairs myofibroblast development in wound granulation tissue: a new model of delayed wound healing. *Wound Repair Regen.* 2007;15:809–816.
23. Sakata J, Sasaki S, Handa K, et al. A retrospective, longitudinal study to evaluate healing lower extremity wounds in patients with diabetes mellitus and ischemia using standard protocols of care and platelet-rich plasma gel in a Japanese wound care program. *Ostomy Wound Manage.* 2012;58:36–49.
24. Wu SC, Driver VR, Wrobel JS, et al. Foot ulcers in the diabetic patient, prevention and treatment. *Vasc Health Risk Manag.* 2007;3:65–76.
25. Dang CN, Boulton AJ. Changing perspectives in diabetic foot ulcer management. *Int J Low Extrem Wounds* 2003;2:4–12.
26. Costa PZ, Soares R. Neovascularization in diabetes and its complications. Unraveling the angiogenic paradox. *Life Sci.* 2013;92:1037–1045.
27. Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet* 2005;366:1736–1743.
28. MacDougall NJ, Muir KW. Hyperglycaemia and infarct size in animal models of middle cerebral artery occlusion: systematic review and meta-analysis. *J Cereb Blood Flow Metab.* 2011;31:807–818.
29. Hinz B. The myofibroblast: paradigm for a mechanically active cell. *J Biomech.* 2010;43:146–155.
30. Dallon JC, Ehrlich HP. Differences in the mechanism of collagen lattice contraction by myofibroblasts and smooth muscle cells. *J Cell Biochem.* 2010;111:362–369.
31. Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. *Int J Vasc Med.* 2012;2012:918267.
32. Ruiter MS, van Golde JM, Schaper NC, et al. Diabetes impairs arteriogenesis in the peripheral circulation: review of molecular mechanisms. *Clin Sci (Lond).* 2010;119:225–238.
33. van Golde JM, Ruiter MS, Schaper NC, et al. Impaired collateral recruitment and outward remodeling in experimental diabetes. *Diabetes* 2008;57:2818–2823.