Preservation of pressure-induced cutaneous vasodilation by limiting oxidative stress in short-term diabetic mice

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Abstract

Objective: Pressure-induced vasodilation (PIV) allows skin blood flow to increase in response to locally applied pressure and may be protective against pressure ulcers. We previously showed that PIV was absent in 1-week diabetic mice exhibiting no neuropathy. Our aim was to determine whether the diabetes-induced PIV alteration could be prevented.

Methods and results: Diabetic mice received no treatment or a daily treatment with either sorbinil, alagebrium or alpha-lipoic acid (LPA) for 1 week. Laser Doppler flowmetry was used to evaluate PIV as well as endothelium-dependent vasodilation following iontophoretic delivery of acetylcholine (ACh). The effect of each treatment on oxidative stress was examined by plasma 8-isoprostane assay. LPA was the sole treatment to prevent both PIV and ACh vasodilation alterations, with a significant reduction of oxidative stress in diabetic mice. Both PIV and ACh-vasodilation were abolished in LPA-treated diabetic mice following injection of N\textsuperscript{-}N\textsuperscript{-}nitro-l-arginine (p < 0.05). In contrast, alagebrium and sorbinil prevented neither diabetes-induced PIV abolition nor endothelial alteration.

Conclusions: LPA treatment significantly reduced the oxidative stress and was able to preserve endothelial nitric oxide availability in the cutaneous microcirculation and then to preserve the PIV response in diabetic mice. LPA treatment could play a key role in limiting the risk of pressure-induced cutaneous ulcer during diabetes.

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1. Introduction

Diabetic foot ulceration represents a major medical, social, and worldwide economical problem. Although several risk factors for diabetic ulcer formation are known, the underlying pathophysiological mechanisms remain unclear [1]. The major risk factor leading to diabetic foot ulcer is poor glycemic control, which results in neuropathic and vascular changes. Prevention of diabetic foot ulcerations is mainly managed by limiting pressure loads [2].

A novel relationship exists between cutaneous mecano-sensitivity and vasodilation, referred to as pressure-induced vasodilation (PIV) [3,4]. When an external pressure is applied on the skin, the cutaneous microarteries vasodilate. This allows a rise in skin blood flow in healthy subjects that in turn delays the occurrence of ischemia. In contrast, when PIV is absent [5,6], skin blood flow decreases leading to tissue ischemia. This cutaneous PIV appears as a protective vascular response to an increase in local pressure. PIV involves capsaicin-sensitive afferent nerve fibres activation, leading to the release of calcitonin gene-related peptide (CGRP) that acts at the endothelial level [3,5]. In addition, Fromy et al. [5] demonstrated a major role for nitric oxide (NO) in PIV development, since PIV disappeared with an acute inhibition of NO synthase (NOS) using N\textsuperscript{-}N\textsuperscript{-}nitro-l-arginine (L-NNA) in rats.

We already reported that PIV was absent in diabetic patients [7,8]. Furthermore, we have shown that PIV was totally abolished in 1 week diabetic mice developing an
endothelial dysfunction, suggesting endothelial NO bioavailability decrease but not neuropathy [9]. This study suggested that 1-week diabetic skin was already at risk for late diabetic complications such as pressure ulcers, demonstrating that neuropathy is not essential for the development of pressure-induced ulcerations. Early detection and appropriate treatment of these ulcers may prevent up to 85% of amputations [1].

It is assumed that most diabetic complications are due to elevated glucose levels that activate pathological molecular pathways, such as the polyol, the non-enzymatic glycation and the oxidative stress pathways [10,11]. These biochemical pathways supposedly play an important role in the development of diabetic vascular complications, via a reduction in vascular NO bioavailability [12,13]. We suggested that early reduction in NO levels could be involved in alteration of the PIV response in 1-week diabetic mice [9].

Thus, the aim of this study was to determine whether PIV alteration observed in diabetic mice could be prevented at an early stage of diabetes, thus protecting the skin against pressure load. For this purpose, we used treatments that act on glucose-activated biochemical pathways and known to improve diabetic vascular dysfunction: sorbinil, alagebrium, and alpha-lipoic acid (LPA). Sorbinil, an aldose reductase inhibitor, has been shown to prevent diabetes-induced endoneurial blood flow impairment in experimental diabetic rat [14,15]. Alagebrium is an advanced glycation breaker and has been shown to improve arterial elasticity in experimental diabetic rat [16,17] and to reduce expression of AGE receptors in diabetic rats [17]. LPA has potent antioxidant properties and has been shown to improve the diabetes-induced endothelial dysfunction [18–20].

2. Methods

2.1. Materials

Male Swiss mice (20–30 g, 12 weeks) purchased from Charles Rivers Laboratories (Les Oncins, France) were kept on a 12:12-h-light/dark cycle with food and water available ad libitum. The present investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Diabetes was induced by a single i.p. injection of streptozotocin (STZ; 200 mg kg$^{-1}$; Sigma, Saint Quentin Fallavier, France) in citrate buffer (pH 4.5) during the fasting state. Non-diabetic mice received equivalent doses of citrate buffer solution to be used as controls. Hyperglycaemia occurred 2 days after STZ injection and was verified using an Accu-Check Active glucometer (Roche, Lyon, France). We included the mice in the diabetic group when blood glucose was >16 mmol/l 2 days after the injection. When hyperglycaemia was confirmed (2 days following STZ injection), diabetic mice were randomly assigned to receive no treatment for untreated diabetic group or a daily treatment for treated diabetic groups during 1 week. Mice were randomized into five groups: (1) untreated non-diabetic mice (control group), (2) untreated diabetic mice (diabetic group), (3) diabetic mice treated with sorbinil (70 mg kg$^{-1}$, daily per os [25]; sorbinil was a kind gift of Pfizer (Groton, CT, USA), (4) diabetic mice treated with alagebrium (1 mg kg$^{-1}$, i.p. injection once a day [16]; alagebrium was a kind gift of Alteon Pharma ( Parsippany, NJ, USA), (5) diabetic mice treated with LPA (100 mg kg$^{-1}$, i.p. injection once a day [26]; Sigma, Saint Quentin Fallavier, France).

Within each group (control, untreated diabetic mice, treated diabetic mice with sorbinil, treated diabetic mice with alagebrium, treated diabetic mice with LPA), 3 separate sets of mice were used to assess (1) PIV and blood sampling for plasma 8-isoprostane assay, (2) ACh response by iontophoresis and blood sampling for fructose-amine assay, (3) SNP response by iontophoresis and MNCV measurements. Then, mice were killed by an overdose of thiopental.

Treated diabetic groups with preservation of the PIV response underwent further studies to determine the origin of the prevention of PIV absence due to diabetes. Since endothelial NO is the main contributor of PIV development, N$^\text{G}$-nitro-L-arginine (L-NNA) (Sigma, 20 mg kg$^{-1}$, i.p.) was injected 30 min prior to the start of the experiment, in order to inhibit NOS. PIV and the endothelium-dependent vasodilation were then assessed in specific groups.

Hair from the top of the skull to the back of the mice was removed with a depilatory lotion to present a hairless area for the skin laser Doppler flow measurements, local pressure application, and iontophoretic deliveries. This was performed 2 days before the experiments to prevent skin irritation, which may confound the results.

For the experiments, mice were anesthetized by i.p. injection of thiopental sodium (65 mg kg$^{-1}$). The level of anesthesia was determined by testing eye reflexes and tail pinch.

2.2. Assessment of the cutaneous microcirculation

After general anesthesia, mice were settled in an incubator (MMS, Chelles, France) warmed to maintain a stable cutaneous temperature (35.0±0.5 °C). Mice were placed in the prone position followed by a 20-min resting period to stabilize the blood pressure and cutaneous temperature. Noninvasive blood pressure measured using the tail cuff method (IITC, Woodland Hills, CA, USA) was recorded before and after the experiments to verify blood pressure stability.

2.3. Assessment of PIV

Skin blood flow in response to local pressure application was measured by laser Doppler flowmetry (LDF). This
method was described by Fromy et al. [4] using a weightbridge that was adapted to hold a laser Doppler probe at one end (PF415; Periflux; Järfalla, Sweden). The probe was connected to a laser Doppler flowmeter (PF5000 Master; Periflux; Perimed, Järfalla, Sweden). The weightbridge was carefully equilibrated with the probe placed in the middle of the hairless skull of the mouse, and an external pressure was increased progressively at 132 Pa min$^{-1}$ (1 mm Hg min$^{-1}$) through the laser Doppler probe using a syringe pump. The LDF signal was digitized with a 20-Hz sampling frequency using a computerized acquisition system (Biopac, Santa Barbara, CA, USA). Data collection started with a 1-min control period before the onset of increasing pressure. The LDF signal was averaged every 30 s to reduce the instantaneous variability of the signals as a result of vasomotion.

2.4. Assessment of endothelium-independent and -dependent responses

Skin blood flow was recorded using a laser Doppler multifiber probe (481-1, Perimed, Järfalla, Sweden) during transcutaneous iontophoresis applied to a 1.2-cm$^2$ area on the hairless back of the mice. Endothelium-independent response was assessed using cathodal SNP iontophoretic delivery (67 mmol/l; Nitriate; SERB, Paris, France) with a current application of 100 μA for 20 s. Endothelium-dependent response was assessed using anodal ACh iontophoretic delivery (5.5 mmol/l; Sigma, Saint Quentin Fallavier, France) with a current application of 100 μA for 10 s. The iontophoresis technique was chosen to assess the in vivo cutaneous microvascular function in order to avoid any systemic effects.

2.5. Assessment of nerve function

Motor nerve conduction velocity in sciatic-tibial fibers was assessed by stimulation at the exposed sciatic notch and knee while recording the M-wave (compound muscle action potential) from the tibial-innervated dorsal interossei foot muscles [21]. During recording, the temperature of the site surrounding the nerve was kept constant at 37 °C.

2.6. Biochemical assays

2.6.1. Plasma 8-isoprostane

Plasma was obtained by centrifugation of blood collected in tubes containing EDTA. Plasma samples were snap frozen in liquid nitrogen, and stored at −80 °C. Measurement of the isoprostane 8-epi-PGF$_{2\alpha}$, purified from plasma was made using a commercially available enzymatic immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA), according to the manufacturer’s instructions. The performance characteristics, as assessed by the manufacturer for intra- and inter-assay CVs, were less than 10%.

2.6.2. Fructosamine

Serum was obtained by centrifugation of blood collected in dry tubes. Serum samples were stored at −20 °C. Fructosamine was determined by a nitroblue tetrazolium colorimetric test, based on the ability of the ketoamine group of glycated proteins, to reduce tetrazolium salts under alkaline conditions [22].

2.7. Data analysis

Data are expressed as means±S.E.M. They were first subjected to Bartlett’s test for homogeneity of variance. One-way ANOVA was followed by the Student–Newman–Keuls multiple range test to estimate the significance of differences for between-group comparisons. An unpaired Student’s $t$-test was performed to compare LPA-treated diabetic group to LPA-treated diabetic group following NOS inhibition. Significance was defined as $p<0.05$.

3. Results

3.1. Animals

At the time of experimentation, all of the diabetic mice significantly lost weight compared to control mice ($p<0.05$) (Table 1). Only treating diabetic mice with LPA had a significant effect on weight gain compared with untreated mice.

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Glycemia (mmol/l)</th>
<th>Fructosamine (μmol/l)</th>
<th>MABP (mm Hg)</th>
<th>Basal LDF (a.u.)</th>
<th>MNCV (m s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31 ±1</td>
<td>8.9 ± 0.3</td>
<td>167 ± 9</td>
<td>98 ± 3</td>
<td>113 ± 7</td>
<td>48 ± 5</td>
</tr>
<tr>
<td>Diabetic</td>
<td>22 ± 1*</td>
<td>29.7 ± 1.2*</td>
<td>331 ± 24*</td>
<td>93 ± 3</td>
<td>123 ± 10</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>Diabetic + Sorbinil</td>
<td>22 ± 1*</td>
<td>24.7 ± 1.5*</td>
<td>253 ± 7*</td>
<td>105 ± 3</td>
<td>92 ± 8</td>
<td>53 ± 4</td>
</tr>
<tr>
<td>Diabetic + Alagebrium</td>
<td>20 ± 1*</td>
<td>26.9 ± 1.4*</td>
<td>235 ± 9*</td>
<td>112 ± 5</td>
<td>115 ± 17</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Diabetic + LPA</td>
<td>27 ± 1*</td>
<td>24.5 ± 1.7*</td>
<td>261 ± 8*</td>
<td>99 ± 3</td>
<td>170 ± 27</td>
<td>53 ± 2</td>
</tr>
</tbody>
</table>

$p<0.05$ vs. control $^\dagger$ $p<0.05$, $^{**}$ $p<0.001$ vs. diabetic. $n=10$ in each group. MABP, mean arterial blood pressure; LDF, laser Doppler flowmetry; MNCV, motor nerve conduction velocity.
diabetic mice ($p<0.001$). In all of the diabetic groups, blood glucose and fructosamine levels were significantly increased compared with control mice (Table 1). Only treating diabetic mice with sorbinil and alagebrium significantly decreased plasma fructosamine levels compared to untreated diabetic mice ($p<0.05$), showing the efficiency of both treatments. As expected, there was no change in MNCV between all groups (Table 1). Neither the treatments nor the diabetes induction significantly changed basal MABP and basal LDF levels (Table 1).

3.2. PIV assessment

In the untreated control group, we observed an increase in LDF in response to the local pressure corresponding to a PIV of $42 \pm 8\%$. In contrast, in the untreated diabetic group, we did not observe an increase in LDF in response to local pressure application (Fig. 1). Treating diabetic mice with sorbinil or alagebrium did not prevent PIV alteration ($p<0.01$ vs. control) (Fig. 1). However, LPA was the sole treatment to prevent the alteration of PIV in diabetic mice in comparison with the untreated diabetic mice ($p<0.05$). In addition, LPA treatment completely preserved PIV, since no difference was observed between LPA diabetic group and control group. The PIV observed in diabetic mice treated with LPA was completely abolished after an acute i.p. injection of L-NNA ($p<0.05$) (Fig. 1).

3.3. Assessment of endothelium-independent responses

No difference in endothelium-independent vasodilation in response to SNP was observed between groups (data not shown).

3.4. Assessment of endothelium-dependent responses

In the control group, we observed an increase in LDF in response to iontophoretic delivery of Ach, corresponding to a maximal percentage vasodilation of $59 \pm 15\%$ (Fig. 2). Diabetes causes a significant decrease in ACh-induced vasodilation ($p<0.05$ vs. control group) that was not statistically improved by treating diabetic mice with either sorbinil or alagebrium (Fig. 2). LPA was the sole treatment to significantly prevent the alteration of the ACh-dependent response in diabetic mice in comparison with untreated diabetic mice ($p<0.05$). In addition, LPA treatment completely preserved the ACh-dependent vasodilation in comparison with the control group. This response was completely abolished after an acute i.p. injection of L-NNA ($p<0.05$).

3.5. Plasma 8-isoprostan assay

Plasma 8-isoprostan level was significantly higher in all of the diabetic groups (treated and untreated) than the control group (Fig. 3). However, LPA was the sole treatment to significantly decrease 8-isoprostan level in treated diabetic mice compared to untreated diabetic mice ($p<0.05$), showing the efficiency of LPA treatment.

4. Discussion

Since hypoxia and reduced nerve blood flow have been suggested as key factors involved in the pathogenesis of neuropathy [23,24], several long-term diabetic studies have been interested in improving the vascular and nervous functions during diabetic neuropathy with treatments.

Fig. 1. Percentage of vasodilation during local pressure application after 1 week of diabetes in untreated non-diabetic mice (control) and in diabetic mice untreated or treated daily during 1 week with sorbinil (n=9), alagebrium (n=9), alpha-lipoic acid (LPA, n=9) and in LPA-treated diabetic mice with N-nitro-L-arginine (L-NNA) injected 30 min prior to the start of the experiment (diabetic+LPA+L-NNA, n=7). ***$p<0.001$, **$p<0.01$ vs. control. †$p<0.05$ vs. diabetic. ‡$p<0.05$ vs. diabetic+LPA. LDF, Laser Doppler Flow.
However, only few studies were interested in early diabetes vascular alterations (1–2 weeks) and focused mainly on arterioles that provide circulation to the region of the sciatic nerve related to late neuropathy [29] or in isolated arteries [30]. Although impaired microcirculation has been observed in diabetic patients with and without neuropathy [31–33], this impairment has not been clearly linked to pressure-induced ulcers during diabetes. Previously, we demonstrated that PIV was abolished in 1-week diabetic mice exhibiting endothelial dysfunction but not neuropathy suggesting that diabetic skin was already at risk for late diabetic pressure ulcers. In the current study, we provide evidence that 1-week diabetes-induced PIV abolition can be prevented by LPA, a potent antioxidant. Since the injection of L-NNA, a NOS inhibitor, abolished the PIV response in LPA-treated diabetic mice, we demonstrated that LPA could preserve NO synthesis and/or could prevent NO degradation. This increased NO availability is likely responsible for PIV preservation in short-term diabetes.

In order to go further in the assessment of the endothelial function in the cutaneous microcirculation, we used the iontophoretic method, which gives an opportunity to study local in vivo endothelium-dependent vasodilation. The ACh-dependent vasodilation was completely preserved by LPA treatment, and it was greatly reduced with L-NNA in diabetic mice. This is concordant with previous findings using NOS inhibitor in in vitro experimental studies and in vivo endoneurial studies on LPA treated diabetic rats [18,34]. Since ACh is mainly mediated by the endothelial L-arginine/NO pathway [35], all these results confirm that the prevention of diabetes-induced endothelium-dependent vasodilation alteration relies on an increased availability of endothelial NO that effectively preserves PIV development. In a previous study, Fromy et al. [5] demonstrated a major role for endothelial NO in PIV development. Since PIV disappeared when acutely inhibiting NOS using L-NNA in normal rats, inhibition of neuronal NOS with 7-nitroindazole caused only a small decrease of PIV and the absence of

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**Fig. 2.** Percentage of vasodilation in response to iontophoretic delivery of acetylcholine (ACh) after 1 week of diabetes in untreated non-diabetic mice (control) and in diabetic mice untreated or treated daily during 1 week with sorbinil, alagebrium, alpha-lipoic acid (LPA) and in LPA-treated diabetic mice with N-nitro-L-arginine (L-NNA) injected 30 min prior to the start of the experiment (diabetic + LPA + L-NNA). *p<0.05 vs. control. †p<0.05 vs. diabetic. ‡p<0.05 vs. diabetic + LPA. n=10 in each group except for L-NNA group, n=7. LDF, Laser Doppler Flow.

**Fig. 3.** 8-Isoprostane plasma level measured after 1 week of diabetes in untreated non-diabetic mice (control, n=6) and in diabetic mice untreated (n=6) or treated daily during 1 week with sorbinil (n=3), alagebrium (n=5), alpha-lipoic acid (LPA, n=5). ***p<0.001, **p<0.01, *p<0.05 vs. control. †p<0.05 vs. diabetic.
involvement in PIV for neurokinines and substance P excludes inflammatory response [5]. In addition, we showed that PIV was abolished in 1-week diabetic mice exhibiting endothelial dysfunction. Neither endothelium-independent response nor neuropathy was detected suggesting that NO-dependent response to external pressure was reduced [9]. Therefore, this study reports that LPA-induced PIV preservation was largely due to a beneficial effect on NO synthesis and/or prevention of NO degradation that allow an increase in NO bioavailability leading to endothelium-dependent vasodilation preservation.

Diabetes mellitus is characterized by increased production of reactive oxygen species (ROS) with a sharp reduction in antioxidant defense and altered cellular redox status [10,11,36,37] that can lead to damage of cells and enzymes as well as an increased lipid peroxidation. These consequences of oxidative stress can promote the development of microvascular complications of diabetes mellitus. In our study, oxidative stress level was evaluated by measuring plasma 8-isoprostane resulting from oxidation of arachidonic acid, thus reflecting the increase in ROS production in diabetes [38]. Indeed, O’Byrne et al. [39] have shown an inverse relationship between NO synthesis and ROS activity by measuring radiolabelled nitrite and plasma 8-isoprostane. A significant reduction of the oxidative stress was observed with LPA treatment in diabetic mice. Vascular studies showed an impairment of ACh-induced vasodilation, with a generation of superoxide associated to reduced NO availability in the vasculature of diabetic animals [11,13,18–20].

LPA is known to exert significant antioxidant activities in vivo and in vitro, not only by deactivating reactive oxygen and nitrogen species but also involving chelating transition metal ions [40]. The reduced form of LPA (DHLA) can regenerate and extend the metabolic life spans of the well-known antioxidants vitamins C and E, glutathione, and Coenzyme Q10 via reduction of their radical or oxidized forms [40]. Thus, the synergistic interactions of antioxidant play an important role in the prevention of diabetes complications [41]. Jones et al. [42] showed that pre-incubation of endothelial cells with LPA increased vitamin C activity, decreased ROS, and generated NO. Moreover, it has been shown that treatment with LPA or other antioxidants protected cultured endothelial cells against the oxidative stress induced by AGEs [43]. Altogether, these effects contributed to preserve PIV by reducing NO quenching with ROS leading to an increased NO bioavailability. Indeed, Pricci et al. [44] suggested that short-term hyperglycemia effect was linked to the interaction between superoxide and NO to form peroxynitrite. Therefore, reducing oxidative stress should subsequently limit NO scavenging and improve NO bioavailability, thus preserving PIV development. In our study, it appears that PIV preservation is inversely related to oxidative stress.

At the onset of diabetes, sorbinil and alagebrium had no beneficial effect on PIV preservation and ACh responses. SNP vasodilation was unchanged in all groups, demonstrating intact smooth muscle cell vasodilation capacity strengthening the absence of the beneficial effect on endothelial responses. These results suggested that both related pathways (polyol and AGEs) were not mainly involved in PIV alteration in 1-week diabetes mice. Furthermore, these two treatments did not demonstrate a significant decrease in oxidative stress level. In contrast to long-term diabetes [14,15,25,27,28], we did not observe a prevention of endothelial dysfunction by inhibiting aldose reductase during 1 week of diabetes. Therefore, we could suggest that aldose reductase enzyme was not sufficiently activated after 1 week of hyperglycemia to impair both endothelial function and PIV. Another potential explanation might be linked to the possible inhibition of glucuronate reductase by sorbinil [45], which would deplete cells of vitamin C and thereby contribute to increased oxidative stress. This might counteract any beneficial effect of aldose reductase inhibition on oxidative stress as well as endothelial function in 1-week diabetic mice. Furthermore, sorbinil may also exert its action by decreasing the amount of fructose that is more reactive than glucose to glycation and thus decreases the AGEs formed as well as AGEs-induced oxidative stress [46] but with no beneficial effect on PIV preservation in the treated diabetic mice. It is of interest that the magnitude of this effect was similar in most cases to that of alagebrium, which may reflect the small effect of AGE-induced vascular dysfunction in short-term diabetes. Alagebrium, through its ability to break AGE-crosslinking of matrix proteins, has been shown to exert beneficial cardiovascular effects in animals [16,47] as well as in clinical studies [48,49]. Furthermore, alagebrium has been shown to scavenge free radicals or products of oxidative stress and to inhibit AGEs formation [17,50]. So alagebrium would be an AGE inhibitor as well as an AGEs breaker. Alagebrium beneficial effect on the reduction in plasma fructosamine levels confirmed its ability to reduce AGE formation but did not reduce significantly the oxidative stress in treated diabetic mice. The time course of vasodilatory impairment is consistent with the formation of post-Amadori glycosylation products formed over a period of several weeks in experimental diabetes. Since AGEs accumulate slowly on long-lived proteins 1 week of diabetes was not long enough to alter the cutaneous microcirculation. Alagebrium treatment showed beneficial effects on vascular complications for longer period of treatment than 1 week in experimental diabetes [16]. In addition, long-, but not short-term treatment with AGEs formation inhibitor such as aminoguanidine, prevented and reversed the endothelial NO defects in diabetic rats [51,52]. Consistently, neither sorbinil nor alagebrium was able to preserve endothelial function as well as PIV response in our study, indicating that both polyol and AGEs pathways were not sufficiently activated in short-term diabetes. In accordance, the inhibition of both pathways did not sufficiently reduce the oxidative stress to protect the cutaneous microcirculation from local pressure after 1 week of diabetes.
In conclusion, this is the first study showing that it is possible to preserve the cutaneous PIV response early in diabetes. The results suggest that early diabetes-induced PIV preservation was mainly due to oxidative stress reduction that protects endothelial NO pathway in the cutaneous microcirculation. Therefore, LPA treatment by preserving the cutaneous PIV response should also preserve the normal reaction to pressure strain, which might limit the risk of pressure-induced ulcer in diabetic patients. The study highly contributes to bring a new potential prevention strategy with LPA treatment. Moreover, PIV and ACh investigations could be used as preventive measures to screen regularly all diabetic patients and to identify feet at risk. However, future studies are needed to clarify the underlying mechanisms and future studies will be valuable to determine whether combined supplementation have far better/potent effects than individual supplementation.

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