

# Role of Nitric Oxide in the Visible Light-Induced Rapid Increase of Human Skin Microcirculation at the Local and Systemic Level: I. Diabetic Patients

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## Abstract

**Objective:** This study aimed to reveal the effects of polychromatic visible (pVIS) or pVIS + near IR (nIR) light similar to some components of solar light on skin microcirculation and microvascular response to the vasodilators acetylcholine (ACh) and nitroglycerine (NG), in the extremities of patients with diabetic microangiopathy.

**Background Data:** The mechanisms behind light-induced increases in microcirculation as well as extracellular effects of terrestrial pVIS and pVIS + nIR light remain unknown.

**Materials and Methods:** In 24 subjects with type 2 diabetes mellitus local microcirculation was measured in the skin of the foot before and after exposure to both types of light. In another 26 patients systemic microcirculation was studied in the back of the hand before and after exposure of the lumbar-sacral area to light energy. Two different types of light therapy were performed by using two devices: Q-light, which delivers pVIP (385–750 nm) and pVIS nIR light (385–1700 nm) with a power density of 40 mW/cm<sup>2</sup>, which is similar to summer sunlight at noon in Central Europe.

**Results:** At 2 min after irradiation (12 J/cm<sup>2</sup>) of the forefoot with pVIS or pVIS + nIR light, a rise in local blood flow volume (Qas) was observed, on average by 39% and 31%, respectively. The maximal effect (+41–47%) had developed in all patients at 30 min, and it then decreased and disappeared completely 24 h post-irradiation. We obtained similar results after irradiation of the sacral area in Qas of the skin of the hand. Both types of microcirculation also increased following a second exposure to the light sources. Enhancement of microcirculation was accompanied by a decrease in the microvascular response to ACh and NG solutions administered intracutaneously by iontophoresis.

**Conclusion:** Both types of irradiation stimulated microcirculation at the local and systemic levels through a mechanism of enhancement of endothelium-dependent and endothelium-independent vasodilation, in which nitric oxide plays a major role.

## Introduction

WE HAVE KNOWN FOR MANY YEARS that the wound healing, anti-inflammatory, and analgesic effects of visible (VIS) and near-infrared (nIR) light from laser and non-laser sources are associated with improvement of microcirculation. This effect develops both in the area irradiated (i.e., locally), and in distant tissues and organs (i.e., at the systemic level).<sup>1–10</sup> It is the microcirculatory network, where the blood's transport and regulatory functions are realized, that provides tissue respiration, trophics, detoxification, and reg-

ulation of many processes. Disturbances of microcirculation at the local and systemic levels have been proven to play an important role in the development of many diseases, including such diverse pathologies as atherosclerosis, hypertension, diabetes mellitus, and chronic inflammatory processes.<sup>11</sup> However, the mechanisms of light-induced enhancement of microcirculation thus far are only spoken of theoretically. Experts in phototherapy explain the improvements in microcirculation as resulting from the release of endogenous vasodilators such as histamine and prostacyclin,<sup>5,10,11</sup> and release of cytokines.<sup>8</sup> However, the most im-

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TABLE 1. CHARACTERISTICS OF PATIENTS WITH TYPE 2 DIABETES MELLITUS IN THE FOUR STUDY GROUPS

Groups	n	Age, y (mean $\pm$ SD)	Women/ men (%)	Duration of DM, y (mean $\pm$ SD)	Percentage of patients taking oral anti- hyperglycemics (oral drugs/insulin)	Co-morbid disease (% of patients)	
						Hypertension	Ischemic heart disease
I	11	61.3 $\pm$ 1.5	91/9	10.6 $\pm$ 1.7	80/20	86	57
II	13	64.6 $\pm$ 0.9	85/15	11.2 $\pm$ 1.3	92/8	85	46
III	13	61.7 $\pm$ 1.1	85/15	11.4 $\pm$ 1.9	85/15	88	50
IV	13	64.3 $\pm$ 1.0	85/15	10.5 $\pm$ 2.0	88/12	100	75
Average	50	63.0 $\pm$ 1.2	86/14	10.9 $\pm$ 1.0	86/14	90	57

portant overall regulator of vascular tone is nitric oxide (NO), which is produced by many of the body's cells, including endothelial, blood, and mast cells, as well as by neurons.<sup>12,13</sup> In numerous studies done in the last 15 years the role of this compound has been substantiated not only as the primary vasodilator, neurotransmitter, and modulator of cellular immunity, but also as an initiator of transduction of intra- and intercellular signals.<sup>14-17</sup>

In 1997, a hypothesis was put forward<sup>18</sup> that the nitric oxide-synthesizing enzyme NO-synthase (NOS) and its co-enzyme guanylate cyclase absorb energy in the visible part of spectrum and can be activated by this light energy. This should lead to an increase in NO synthesis and to a rapid transduction of light energy into NO-producing cells and the surrounding cells. Although research describing light-induced NO production *in vitro* has already started to appear in the literature,<sup>19-23</sup> activation of NO synthesis *in vivo* and participation of NO in improvements in microcirculation remains unproven.

In recent years phototherapy has been performed with polychromatic VIS (pVIS) light and nIR light emitted by various lasers and non-laser sources.<sup>24-26</sup> These combinations have been shown to be more efficient than the use of monochromatic radiation. Apart from this, after exploration of the immunosuppressive and carcinogenic effects of UV rays, which are a minor component of the terrestrial solar spectrum, it has become apparent that further study is needed of the effects on humans and animals of exposure to pVIS and nIR energy, which are dominant components of the terrestrial solar spectrum.

Taking into account all of the above we carried out a series of trials whose results are described in this study, as well as in another article in this same journal, in which first indirect, and then direct, evidence is presented to elucidate the role of NO in light-induced enhancement of microcirculatory blood flow in patients with diabetes mellitus (DM) and in healthy volunteers after irradiation with the entire spectrum of visible light (385-750 nm), and with a combination of pVIS and nIR energy (385-1500 nm). Because of the possibility of rapid NO synthesis by NOS, in the present work we studied the dynamics of a rise in microcirculatory blood flow in the skin of diabetic patients immediately after one and two phototherapy sessions, as well as the reactivity of microvessels to two intracutaneously administered vasodilators, an NO donor, nitroglycerine (NG), and to an inductor of its synthesis by endothelial cells, acetylcholine (ACh). In these ex-

periments we compared the effects of phototherapy with those of the more classic vasodilators.

## Materials and Methods

### Patients

The Ethics Committee of I.P. Pavlov State Medical University approved the design of this study in 2005, but it did not recommend testing the effects of NG as a vasoactive agent in the compromised vasculature of diabetic patients.

Inclusion criteria for these trials were: those with type 2 DM aged 40-80 y of both genders, duration of DM of at least 2 years, and the presence of diabetic polyneuropathy. Exclusion criteria were: having type 1 DM, age over 80 y, and patients with diabetic ulcers, vasculitis, or collagen vascular diseases.

We examined 50 patients with type 2 DM with a mean age 63.0  $\pm$  1.2 y, 86% women and 14% men, whose duration of DM was on average 10.9  $\pm$  1.0 y (Table 1). Compensation of DM was confirmed by assessing circadian fluctuations of glycemia and the content of glycosylated hemoglobin (HbA<sub>1c</sub>) according to WHO criteria. For a day before exposure to light, and for 2 d after light treatment, the patients did not take any medications that affect vasodilatation or platelet aggregation.

Study participants were randomly divided into four groups (Table 1). In those in groups I and II, microcirculation was measured in blood vessels of the dorsal surface of the foot before and after irradiation with pVIS or pVIS + nIR light, respectively. In those in groups III and IV, microcirculation was measured in the blood vessels of the skin on the back of the hand before and after irradiation of the lumbar-sacral region with pVIS or pVIS + nIR light, respectively. In these patients, the fact that the area tested was distant from the area of light exposure allowed us to record changes in systemic microcirculation.

### Study of microcirculation

Assessment of the microcirculation in the blood vessels of the skin of the upper and lower extremities was performed using a high-frequency ultrasound Doppler device (Minimax-Doppler-K; Minimax AG, St. Petersburg, Russia) operating at a frequency of 25 MHz, which allowed us to measure the blood flow volume (Q<sub>as</sub>, in mL/sec/cm<sup>3</sup>) in the studied area to a depth of 5 mm.<sup>27</sup> Unlike ultrasound as-

assessment of larger vessels, where blood flow parameters are recorded in a single artery or vein, for ultrasound assessment of microcirculation, its integral hemodynamic parameters are fixed.

In addition to the total blood flow rate, we also recorded the changes in the tested areas after intracutaneous iontophoresis for 1 min using acetylcholine solution (ACh, 0.3%, 1 mL) or nitroglycerine solution (NG, 0.1%, 1 mL), which induce endothelium-dependent and endothelium-independent vasodilation, respectively.<sup>28-34</sup> As an agonist of muscarine receptors of endothelial cells, ACh stimulates these cells to produce the vasodilating factors nitric oxide (NO), prostacyclin, and endothelium-dependent hyperpolarization factor. NG, which is an NO donor, was used to reveal endothelium-independent dilation of blood vessels. The blood flow rates in the zone of administration of the vasoactive agents was assessed before the first and second phototherapy sessions, and 90 min after them. These measurements were performed every minute for 7 min after iontophoresis. In addition to the seven individual assessments of blood flow volume, the mean value over the entire 7-min period was also calculated.

**Irradiation procedure**

To administer the therapy with pVIS (385-750 nm) and pVIS + nIR (385-1700 nm) light, we used two Q-light devices (Biotechnology and Photomedicine AG, Rorschacherberg, Switzerland). The spectral range of these devices simulates significant portions of the terrestrial solar spectrum, and their power density (40 mW/cm<sup>2</sup>) is similar to the natural pVIS and nIR light present in the summertime at noon in Central Europe. However, the light energy produced by these devices has a high degree of polarization (95%), like that of lasers. According to recent studies,<sup>38-41</sup> irradiation with this type of light for 5-8 min (12-19.2 J/cm<sup>2</sup>) induces in the human body anti-inflammatory and wound healing effects. In our trials the energy density was 12 J/cm<sup>2</sup>, the time of exposure was 5 min, and the diameter of the irradiated area was 20 cm.

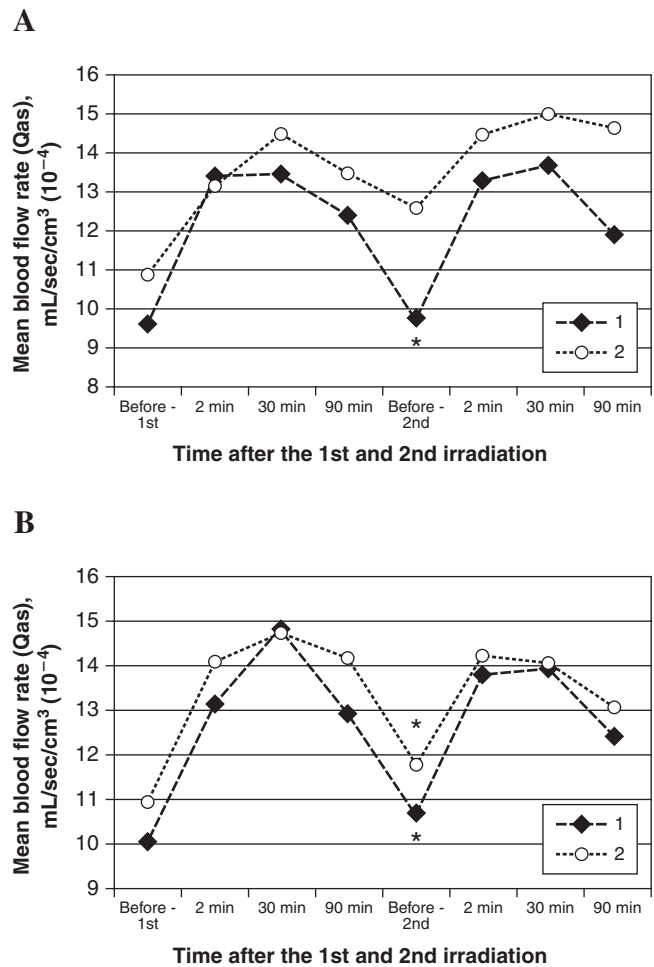
**Statistics**

Evaluation of the statistical significance of the changes seen was performed using the parametric method for matched (dependent) pairs of results (Students *t*-test). Correlation of the effects was calculated using Pearson's correlation coefficient.

**Results**

**Changes in local and systemic microcirculation**

As soon as 2 min after the initial irradiation of the dorsal foot surface with pVIS light, a rise in local skin microcirculation (Qas) was observed in 100% of the irradiated subjects in group I, ranging from 23-88%, and on average 39%. In those receiving pVIS + nIR light irradiation, increases were seen in 77% of patients in group II, ranging from 18-71%, and on average 31% (Fig. 1 and Table 2). The maximal Qas increases (by 41% and 47% in groups I and II, respectively) occurred 30 min after irradiation. After 90 min the effect decreased, and it had disappeared completely after 1 d (before the second irradiation). The second treatment session with



**FIG. 1.** Effect of two daily exposures to pVIS (A) and pVIS + nIR (B) light on the skin blood flow rate (Qas) in the microvessels of diabetic patients after local irradiation of the foot (1), and in the hand after irradiation of the lumbar-sacral zone (2). The difference in the Qas pre- to post-irradiation was not statistically significant (*p* > 0.05). The other differences were statistically significant (*p* < 0.05-0.001).

pVIS or pVIS + nIR light led to an equally rapid (2 min) increase in local blood flow rates (by 38% and 37% in groups I and II, respectively), with maximal effect seen after 30 min (increases of 42% and 38% in groups I and II, respectively).

At 2 min after irradiation of the patients' lumbar-sacral area with pVIS light (group III), a statistically significant increase in the blood flow rate (Qas) was recorded in the microvessels of the skin of the hand in 87% of the subjects. The mean Qas increase was 20%, with individual variability from +10% to +44% (Fig. 1 and Table 2). The distance of the area of blood flow testing from the irradiated zone indicated that there was a systemic effect. The maximal increase (32%) was achieved at 30 min post-irradiation. The Qas value remained elevated for 90 min (24%), then decreased somewhat at 24 h (15%), then rose again, more markedly, after the second irradiation session. The first irradiation session in the pVIS + nIR group (group IV) of the lumbar-sacral area of these patients was also accompanied by a rise in systemic microcirculation, as assessed in the skin of the hand (Fig. 1 and Table 2). Although the increases appeared with the same incidence

TABLE 2. COMPARISON OF THE CHANGES IN LOCAL AND SYSTEMIC BLOOD FLOW RATES (QAS) IN SKIN MICROVESSELS OF THE EXTREMITIES OF DIABETIC PATIENTS AFTER IRRADIATION WITH pVIS AND pVIS + NIR LIGHT

Blood flow type	Area of irradiation	Area of blood flow testing	Type of light used	Mean increase in Qas (%) post-irradiation						
				Time after first session				Time after second session		
				2 min	30 min	90 min	24 h	2 min	30 min	90 min
Local	Dorsal surface of foot	Dorsal surface of foot	pVIS	+39	+41	+28	+2 <sup>a</sup>	+38	+42	+24
			pVIS + nIR	+31	+47	+28	+6 <sup>a</sup>	+37	+38	+23
Systemic	Lumbar-sacral area	Back of hand	pVIS	+20	+32	+24	+15	+33	+38	+34
			pVIS + nIR	+29	+35	+30	+8 <sup>a</sup>	+30	+29	+19

<sup>a</sup>The difference from the pre-irradiation Qas level was statistically insignificant ( $p > 0.05$ ). Other differences were statistically significant ( $p < 0.05$ – $0.001$ ).

(in 87% of the irradiated subjects), its variability (from 10% to 83%) and the mean changes seen after 2 min (+29%) exceeded the corresponding parameters seen after the use of pVIS light alone. However, 24 h later the blood flow rate returned to the initial levels, and the increases seen after the patients' second irradiation session were less significant than those seen after the first session.

#### Changes in microcirculation after local iontophoresis of vasodilating agents

As can be seen in Table 3, the subcutaneous administration of ACh solution to the dorsal foot surface prior to light exposure produced nearly immediate (1 min later) increases in the local microcirculation, on average by 18% in the group I and by 14% in the group II. The maximal increases (46% and 41% in groups I and II, respectively) were seen at 2 min, while the mean Qas increases seen at 7 min after ACh iontophoresis were 19.4% and 20.4%, respectively. At 90 min after light exposure, in parallel with the rise in local blood flow, the vascular response to ACh administration was significantly lower than seen that prior to light exposure in group I (+12.4%), and it also remained at pretreatment levels (+19.7%) in group II. However, at 90 min after the second exposure decreases in microvascular reactivity to ACh were seen not only in group I, but also in the group II, as compared to the reactivity seen prior to the second session (Table 3).

In compliance with Ethics Committee recommendations, we did not study the reactivity of foot skin of diabetic patients to NG, because it is a strong vasoactive agent. As will be shown below, we did perform this testing in the back of their hands, where the pathology of the microvascular network was less severe.

Indeed, the blood flow rate in nonirradiated hands of patients in groups III and IV was  $10.9 \pm 0.2$  mL/sec/cm<sup>3</sup>, which was 10% higher than that seen in the foot skin microvessels of the patients in groups I and II ( $9.9 \pm 0.4$  mL/sec/cm<sup>3</sup>). However, the reactivity of microvessels in

nonirradiated hands injected with ACh turned out to be the same as that seen in the feet: the mean increase in Qas at 7 min after ACh injection iontophoresis was 18.7% in group III and 20.4% in group IV, with maximal increases (36% and 34%) seen after 2–3 min (Table 4). The microvascular reactivity to ACh iontophoresis in the groups irradiated with pVIS or pVIS + nIR was significantly lower than that seen before irradiation (increases of 14.3% and 11.6%, respectively; Table 4). This same tendency toward decreases in the microvascular reactivity to ACh iontophoresis was also seen after the second session, compared to levels seen prior to the second session.

Intracutaneous infusion of NG solution into the skin on the hands of the patients in groups III and IV before irradiation enhanced the blood flow rate only half of that seen with ACh administration: its mean increase amounted to only to 8.7%, whereas in the case of ACh this figure was 18.7% (Table 4). Nevertheless, 90 min after the first irradiation session NG administration we saw an increase in microcirculation, on average by 6.4% after exposure to pVIS light and by 9.1% after exposure to pVIS + nIR light (Table 4). This effect of NG and irradiation was apparently less than that seen before irradiation, not only by the criterion of the mean increase in blood flow, but also according to the maximal amplitude value (in group III). A similar effect was also seen 90 min after the second irradiation session in both groups III and IV.

Table 5 illustrates correlations of light-induced increases in blood flow rate in microvessels of the lower and upper extremities, with decreases in the microvascular reactivity to vasoactive agents at the local and systemic levels. The correlation coefficient was  $-0.60$  ( $p < 0.05$ ) in case of ACh, and  $-0.85$  ( $p = 0.05$ ) in case of NG. As a rule, the pronounced rise in Qas was accompanied by significant reductions in the effects of the vasoactive substances. On the other hand, when light irradiation did not enhance microcirculation at the local and systemic levels, no decrease in blood flow response to ACh and NG was seen. Moreover, it should be noted that at 24 h after the first pVIS session, when local blood flow rate returned to its pre-irradiation value, significantly ele-

TABLE 3. CHANGES IN LOCAL SKIN MICROCIRCULATION (QAS) IN THE DORSAL FOOT SURFACE OF DIABETIC PATIENTS AFTER INTRACUTANEOUS ACETYLCHOLINE (ACh) IONTOPHORESIS BEFORE AND AFTER IRRADIATION OF THE FOOT WITH pVIS OR pVIS + NIR LIGHT

Time with regard to ACh iontophoresis	Qas (mL/sec/cm <sup>2</sup> ) prior to iontophoresis and mean increases up to 7 min post-iontophoresis (% increase or decrease in Qas value over that present prior to iontophoresis)																	
	pVIS (group I)					pVIS + nIR (group II)												
	Prior to	1 min	2 min	3 min	4 min	5 min	6 min	7 min	Qas mean increase	Prior to	1 min	2 min	3 min	4 min	5 min	6 min	7 min	Qas mean increase
1. Before the first session	9.6	+18	+46	+35	+16	+12	+8	+1	+19.4	10.1	+14	+41	+40	+27	+15	+4	+2	+20.4
2. 90 min after the first session	12.4	+7	+25	+27	+15	+9	+3	+1	+12.4 <sup>a</sup>	12.8	+20	+39	+30	+24	+13	+8	+4	+19.7
3. 24 h before the second session	10.2	+24	+50	+53	+40	+25	+16	+3	+30.1 <sup>b</sup>	10.7	+14	+42	+40	+23	+13	+2	+1	+20.7
4. 90 min after the second session	11.7	+28	+42	+37	+22	+17	+10	+5	+22.9 <sup>a,b</sup>	12.4	+12	+29	+32	+23	+4	+3	-1	14.6 <sup>a</sup>

<sup>a</sup>The differences in Qas mean increase between terms of testing 1-2 and 3-4 were statistically significant ( $p < 0.05$ ).

<sup>b</sup>The differences in Qas mean increase between terms of testing 1-2 and 3-4 were significant ( $p < 0.01$ ). The other differences were not statistically significant ( $p > 0.05$ ).



TABLE 4. CHANGES IN SYSTEMIC SKIN MICROCIRCULATION (QAS) ASSESSED IN THE BACK OF THE HANDS OF DIABETIC PATIENTS AFTER INTRACUTANEOUS ACETYLCHOLINE (ACh) OR NITROGLYCERINE (NG) IONTOPHORESIS BEFORE AND AFTER IRRADIATION OF THE LUMBAR-SACRAL AREA WITH pVIS OR pVIS + NIR LIGHT

Vasodilator and time points		Qas (mL/sec/cm <sup>2</sup> ) prior to iontophoresis and mean changes up to 7 min after iontophoresis (% increase or decrease over the pre-iontophoresis Qas value)																	
		pVIS light (group III)					pVIS + NIR light (group IV)												
		Prior to	1 min	2 min	3 min	4 min	5 min	6 min	7 min	Qas mean increase	Prior to	1 min	2 min	3 min	4 min	5 min	6 min	7 min	Qas mean increase
ACh	1. Before the first session	10.9	+20	+34	+36	+20	+14	+6	+1	+18.7	10.9	+27	+34	+33	+23	+13	+9	+4	+20.4
	2. 90 min after the first session	13.4	+14	+36	+25	+16	+7	+4	-2	+14.3 <sup>b</sup>	14.1	+10	+27	+22	+12	+9	+4	-3	+11.6 <sup>b</sup>
	3. 24 h before the second session	12.6	+25	+46	+37	+18	+12	-1	-2	+19.3	11.8	+34	+50	+30	+19	+14	+9	0	+22.3
	4. 90 min after the second session	14.6	+25	+36	+29	+15	+6	+4	-3	+16.0 <sup>a</sup>	13.0	+27	+42	+34	+23	+11	+2	-3	+19.4
NG	1. Before the first session	11.2	+3	+15	+22	+15	+6	+1	-1	+8.7	10.9	+13	+18	+23	+15	+10	+3	-1	+11.6
	2. 90 min after the first session	13.3	+3	+13	+15	+10	+4	+2	-2	+6.4 <sup>c</sup>	13.7	+6	+13	+22	+13	+7	+2	+1	+9.1 <sup>c</sup>
	3. 24 h before the second session	12.2	+11	+14	+28	+28	+11	+4	-1	+13.6 <sup>d</sup>	11.8	+10	+23	+28	+23	+13	+5	-1	+14.4
	4. 90 min after the second session	14.6	+2	+6	+15	+17	+10	+5	-1	+7.7 <sup>a</sup>	13.0	+6	+15	+21	+14	+11	+5	-5	+9.6 <sup>b</sup>

<sup>a</sup>The differences in Qas mean changes between time points 1-2 and 3-4 were statistically significant (<sup>c</sup> $p < 0.05$ ), (<sup>b</sup> $p < 0.01$ ).

<sup>b</sup>The differences in Qas mean changes between time points 1-2 and 3-4 were statistically significant (<sup>d</sup> $p < 0.05$ ).

The other differences were not statistically significant (<sup>c</sup> $p < 0.07$ ; remainder  $p > 0.05$ ).

TABLE 5. CORRELATION OF LIGHT-INDUCED INCREASES IN THE LOCAL AND SYSTEMIC BLOOD FLOW RATE (Q<sub>as</sub>) IN THE HAND AND FOOT SKIN MICROVESSELS OF DIABETIC PATIENTS, AND DECREASES IN THE RESPONSE TO VASODILATING SUBSTANCES ACETYLCHOLINE (ACh) AND NITROGLYCERINE (NG)

Blood flow	Time points	<i>pVIS</i> light			<i>pVIS</i> + <i>nIR</i> light		
		Q <sub>as</sub> mean increase (%)	Mean decrease in response to <sup>a</sup>		Q <sub>as</sub> mean increase (%)	Mean decrease in response to <sup>a</sup>	
			ACh	NG		ACh	NG
Local (dorsal foot surface)	90 min after the first exposure	+28%	-36% (-41%)		+28%	No (no)	
	24 h before the second exposure	+2% <sup>b</sup>	No (no)	ND	+6% <sup>b</sup>	No (no)	ND
	90 min after the second exposure	+24%	-24% (-21%)		+23%	-29% (-24%)	
Systemic (back of the hand)	90 min after the first exposure	+24%	-24% (no)	-26% (-32%)	+30%	-43% (-21%)	22% (no)
	24 h before the second exposure	+15%	No (no)	no (no)	+8% <sup>b</sup>	No (no)	No (no)
	90 min after the second exposure	+34%	-17% (-22%)	-43% (-39%)	+19%	-13% (-16%)	-33% (-25%)

<sup>a</sup>The decrease in vasodilatory response to iontophoresis with ACh and NG after irradiation was evaluated by assessing the reduction in the Q<sub>as</sub> mean increments at 7 min post-iontophoresis of vasodilators compared to the Q<sub>as</sub> increment before irradiation. Figures in parentheses indicate the decrease in the maximal Q<sub>as</sub> increment after irradiation compared to that seen before irradiation.

The difference with the Q<sub>as</sub> previous value was not statistically significant (<sup>b</sup>*p* > 0.05). The other differences were statistically significant (*p* < 0.05–0.001).

ND, no data.

vated microvascular reactivity to ACh was seen in the patients in group I (+30.1%), which was 1.6-fold higher than the level seen before light treatment (Table 3). As a consequence, the vascular response to ACh 90 min after the second session was nearly twice as high in these patients than after the first session. Similarly, at 24 h after the first *pVIS* light treatment of the sacral area of patients in group III, microvascular reactivity to NG in hand skin was 1.6-fold higher than that seen before irradiation (Table 4). At 24 h after irradiation with *pVIS* + *nIR* light, there was only a tendency toward an increase in microvascular reactivity, and in this context combined *pVIS* + *nIR* irradiation was less effective than *pVIS* light alone.

Taken together, these findings suggest that the light modifies both the endothelium-dependent and the endothelium-independent mechanism of vasodilation. In this context the question arises as to whether the light enhances the release or the synthesis of endogenous vasoactive compounds that rapidly, even prior to iontophoresis with ACh or NG, induce the endothelium-dependent and the endothelium-independent relaxation of blood vessels.

An indirect answer to this question can be found by comparing the degree of the rapid rise of the local and systemic blood flow rates after exposure of skin to light with that seen after intracutaneous administration of ACh and NG. According to the data presented in Table 6, the values of the maximal changes in local blood flow rate following both kinds of light treatment were quite comparable with the effects of the 0.3% ACh solution, although the rate of development of the light's effect was somewhat lower. Indeed, 2 min after intracutaneous ACh iontophoresis the change in

local blood flow was +41–46%, whereas that seen after light treatment varied, ranging from +31–39%. At the systemic level the effect of ACh also developed more quickly; at 2 min it was +34%, while after exposure to light it was only +20–29%. However, the effects of the light exceeded those of the intracutaneous administration of the NG solution. The maximal amplitude of the reaction was +32–35% for the light, and only +22–23% for the NG. Similarly, the increase in blood flow seen at 2 min was higher after exposure to light (+20–29%) compared to that seen with the NG solution (+15–18%). Thus, under the conditions of our study, the light stimulated the endothelium-independent vasodilatation even more than the NG solution.

## Discussion

The progressive rise of morbidity seen in those with DM (6–10% annually) and its high mortality due to the most common complication of the disease, diabetic angiopathy, stimulate constant improvement of ways to treat it. For many years DM therapy has been supplemented by courses of application of visible light and *nIR* monochromatic laser energy to portions of the body surface and, via use of a light guide, of blood in large veins and arteries.<sup>39</sup> For the last decade, the use of incoherent narrow-band radiation from light-emitting diodes has been shown, like laser energy, to stimulate microcirculation.<sup>40,41</sup> Using high-frequency doppler ultrasound, it has been shown<sup>41</sup> that daily irradiation of diabetic patients with incoherent narrow-band (980 nm) and coherent monochromatic (850 nm) *nIR* light with equal power and energy density turn are both effective, both

TABLE 6. COMPARISON OF LOCAL AND SYSTEMIC EFFECTS OF LIGHT ON THE BLOOD FLOW RATES (QAS) IN SKIN MICROVESSELS OF THE EXTREMITIES OF DIABETIC PATIENTS WITH LOCAL EFFECTS OF VASODILATORS

Treatment	Mean increase in Qas (% of the pre-treatment value)			
	Local (dorsal foot surface)		Systemic (back of the hand)	
	At 2 min	At maximum	At 2 min	At maximum
pVIS light	+39%	+41%	+20%	+32%
Acetylcholine	+46%	+46%	+34%	+36%
Nitroglycerine		ND	+15%	+22%
pVIS + nIR light	+31%	+47%	+29%	+35%
Acetylcholine	+41%	+41%	+34%	+34%
Nitroglycerine		ND	+18%	+23%

ND, not determined.

in improving microcirculation and by clinical assessment, whereas the results of irradiation with incoherent visible light (670 nm) were significantly lower.

The work detailed here demonstrates for the first time a rapid and significant improvement in microcirculation at the local and systemic levels after irradiation with the entire spectrum of visible light (385–750 nm), as well as with a combination of polychromatic and nIR irradiation (385–1700 nm). Future research is warranted on the effects of treatment with these two types of light energy, not only on microcirculatory parameters, but also on the overall health status of diabetics. Since the spectral range and power density of the types of radiation used here are similar to those of the predominant components of the terrestrial solar spectrum, it is reasonable to attempt to develop a wider program to study the effects of natural pVIS and nIR light on diabetic patients, and to elucidate the possibilities of using these important environmental factors for physiotherapy and rehabilitative therapy. Such studies seem particularly appropriate, as phototherapy with combined pVIS and nIR light (480–3400 nm), according to our data,<sup>35</sup> induces a rapid decrease in elevated glucose levels and atherogenic lipids, the major contributing factors in the development of microangiopathy in the type 2 diabetics. Such irradiation also produces significant anti-inflammatory, analgesic, and wound-healing effects.<sup>36–38</sup>

When performing irradiation therapy of diabetic patients, it is necessary to take into account the effects detailed here about the peculiarities of the action of pVIS light and its combined use with nIR energy. Whereas their direct local effects on microcirculation were similar, the changes seen in tissues remote from the irradiated zone (the systemic effect) differed. The pVIS light produced a more prolonged stimulatory effect, both on the blood flow rate and microvascular reactivity, which was seen 24 h after the first session. Therefore with the use of daily light therapy, when the elevated microvascular reactivity and blood flow rate are preserved, with repeated use of pVIS light its efficacy may be less than that seen with pVIS + nIR irradiation, as described above.<sup>41</sup>

On the whole, our findings of stimulation of microcirculatory blood flow not only with monochromatic coherent laser light, but also with incoherent narrow-band radiation and polychromatic VIS and nIR light, would appear to be in accordance with the findings of others, indicating that these

types of radiation produce similar beneficial effects in terms of pain relief and accelerated tissue repair.<sup>42</sup>

The pathogenesis of micro- and macroangiopathies in DM is to a significant degree due to systemic endothelial dysfunction that develops under conditions of abnormal glucose metabolism, and consists of an inadequate decrease (or an increase) in the formation of various biologically active substances in the endothelium, most importantly NO.<sup>11</sup> One of manifestations of this pathological state is the disturbance of vasomotor (vasodilatory) reactions of skin microvessels, which is detectable in functional tests using these vasoactive substances.<sup>30–34</sup> According to the data available,<sup>31</sup> and particularly to the observations of one of the authors of the present work in diabetic patients as compared to healthy people, there is a decreased blood flow rate (Qas) in the skin microvasculature, and a disturbance of systemic mechanisms of both endothelium-dependent and endothelium-independent vasodilatory reactions, the disturbance of the latter being the stronger of the two. Our findings confirm these data: whereas in diabetic patients the mean increases in Qas in hand skin were 19.6% in the ACh test and 10.2% in the NG test, but in healthy people these same figures were 24.4% and 26.4%, respectively. This means that the microvasculature's reactivity in diabetic patients compared to healthy subjects was lower by 1.3-fold for ACh, and by 2.6-fold for NG. According to our data, both pVIS and pVIS + nIR irradiation affected both mechanisms of vasomotor reactions, decreasing vasodilatory effects on microvessels, with a parallel rise in blood flow rate.

Interestingly, in spite of the similarity of effects of the two types of radiation on the local microcirculation, the mechanisms of their influence on vasodilatory reactions were different (Table 5). The single exposure to pVIS light significantly decreased the endothelium-dependent reaction to ACh, whereas pVIS + nIR light affected it only after the second session. The pVIS light alone produced increases in microcirculation at the systemic level, accompanied predominantly by a decrease in the reaction to NG (i.e., the endothelium-independent response), whereas after treatment with the pVIS + nIR light there was a greater degree of endothelium-dependent reduction in vasodilatation. The pronounced inverse correlation of the light-induced effects, with an increase in blood flow rates and a partial decrease



in microvascular network reactivity to ACh and NG, suggests that the light itself induces the appearance in the circulation of vasodilators that enhance the endothelium-independent and endothelium-dependent relaxation of skin microvessels, and thereby increase blood flow rates at the local and systemic levels.

Since both ACh and NG through different mechanisms promote the appearance of NG in the circulation, the decrease in the response of skin microvessels to subcutaneous administration of these vasodilators may be a consequence of the light-induced formation of NO. As shown by comparison of the vasodilating reaction of NG (0.1% solution) with that of a therapeutic dose of light (12 J/cm<sup>2</sup>), the latter has a more pronounced effect. This seems to be accounted for by the fact that light activates not only endothelium-independent mechanisms of vasodilation, but unlike NO, it also activates the endothelium-dependent ones. Our findings that the pVIS light produced rapid and significant enhancement of microcirculation at the systemic level are to a great degree due to endothelium-independent NO production. From this theory, it follows that in diabetic patients the light activates a mechanism that is inhibited to a great degree in this population. In accordance with findings of other authors<sup>11,31</sup> we were not able to discriminate which mechanisms were responsible for the decrease seen in endothelium-independent microvascular reactivity in diabetic patients. Since NG works as an NO donor to vascular smooth muscle cells, these mechanisms may be associated with dysfunction of these cells and their low responsiveness.<sup>43</sup> It should also be kept in mind that this low reactivity is a consequence of decreased production of NO or of its increased inactivation and destruction. On the other hand, in patients with cardiovascular pathology NO can be released from nitrate- or nitrite-containing drugs that are regularly taken by that population (e.g., sodium nitroprusside, inorganic nitrite, or glycerol trinitrate). This process is also activated by visible light, as has been shown in an *in vitro* model.<sup>44</sup>

## Conclusion

Both types of irradiation stimulated microcirculation at the local and systemic levels through a mechanism of enhancement of endothelium-dependent and endothelium-independent vasodilation, in which nitric oxide plays a major role.

In our next paper<sup>45</sup> direct evidence will be presented that the rapid enhancement of local and systemic human blood microcirculation induced by pVIS light results from activation of NO synthesis in irradiated skin.

## Disclosure Statement

No competing financial interests exist.

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