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Recovery of 0.1 Hz microvascular skin blood flow in dysautonomic diabetic (type 2) neuropathy by using Frequency Rhythmic Electrical Modulation System (FREMS)

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ABSTRACT

Synchronized oscillation of smooth muscle cells tension in arterioles is the main control system of microvascular skin blood flow. An important autogenic vasomotion activity is recognized in 0.1 Hz oscillations through power spectrum analysis of laser Doppler flowmetry. Severe dysautonomia in diabetic neuropathy is correlated with loss of 0.1 Hz vasomotor activity, hence with impaired blood microcirculation. FREMS is a novel transcutaneous electrotherapy characterized by sequences of electrical stimuli of high voltage and low pulse duration which vary both in frequency and duration. We have evaluated the changes in laser Doppler flow in the volar part of the forearm before, during and after FREMS. Normal controls ($n = 10$, 6 females, age range 21–39 years) demonstrated significant 0.1 Hz vasomotion power spectra at baseline conditions associated with large oscillations of adrenergic cutaneous sweat activity sampled from the hand; people with diabetes type 2 and severe dysautonomia ($n = 10$, 5 females, age range 63–75 years) displayed a significant decrease of 0.1 Hz vasomotion power spectra. During FREMS application we observed an increase ($p < 0.05$) of 0.1 Hz vasomotion power spectra only in the diabetic group, despite persistence of adrenergic cutaneous sweat activity suppression in this group. However, after the application of the stimuli, the relative energy values around the 0.1 Hz peak remained significantly higher than preapplication values in the diabetic group ($p < 0.05$). From these findings, we suggest that FREMS is able to synchronize smooth cell activity, inducing and increasing 0.1 Hz vasomotion, independently from the autonomic nervous system.

1. Introduction

Smooth vascular muscles dilate and contract rhythmically to deliver oxygen to tissues surrounding capillary beds. Studies focused on contraction generation [1], and on determining the effect of external factors on the intensity of the contraction [2] in individual arteries [3–5]. Periodic blood flow changes through the skin can be evaluated non-invasively in humans [6,7]; the rhythmicity of these oscillations is considered to be a fundamental feature of tissue perfusion. Increases in skin flow are associated with decreased blood oxygen levels implying increased tissue oxygenation. This way, vasomotion can control oxygen consumption, since the rate of vasomotor activity can change oxygen consumption by 2–8-fold, depending on the fraction of open microvessels. Recently, the control of vasomotion has been partially elucidated [8]. The initiation of vasomotion and its control are thought to be mediated, at least partially, by calcium flux in endothelial cells, that is in turn related to nitric oxide-mediated release of cGMP, which activates an intracellular calcium channel inside the sarcoplasmic reticulum of smooth muscle cells. In fact, synchronization could be observed also in de-endothelialized cells if a cGMP agonist (8-bromo-cGMP) is used [9]. Hence, vasomotion is regulated in terms of rhythmicity and synchronization by nitric oxide released by endothelial cells, which in turn increases cGMP that prompts depolarization in smooth muscle cells. After depolarization, smooth muscle cells remain entrained to generate vasomotion. When a sufficient number of cells become activated at the same moment the current will propagate depolarizing all cells coupled via gap junctions [10,11]. In fact, skin vasomotion impairment has been observed in various clinical and experimental conditions (including chronic venous insufficiency, diabetic polyneuropathy, etc.). Moreover, in diabetes, there is an impairment of cutaneous vasomotion, characterized by decreased baseline wave amplitude, and also after elicitation of shear stress (suprasystolic occlusion) [12–14].

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Typically, this impairment is characterized by a reduction of low-frequency (=0.1–0.01 Hz) microcirculatory fluctuations [15], as compared to normal controls. Sympathetic discharge to the vessels is also important for the initiation of vasomotion, probably mediated by $\alpha 1$ adrenergic receptors; impaired vasomotion in people with diabetes is held to be related to the impaired sympathetic discharge, and this is considered to be an ominous sign [13,16].

Very few attempts were made to obtain clinical amelioration of peripheral vasomotion. Recently, chronic intermittent electrical stimulation has been shown to be able to significantly improve the endothelial dysfunction of precapillary arterioles of ankle flexor muscles in ischemic rats with an improvement of nitric oxide production and reversal of the vasoconstrictor response to acetylcholine and restoration of vasodilation induced by bradykinin [17,18].

Various studies aimed to establish the effect of several types of cutaneous electrical stimulation (TENS) on vasomotion [19–21]. The most important difference with traditional TENS – which is a fixed train of electrical pulses – emerged in its two main stimulation modalities, i.e., low and high frequency. Although low frequency TENS showed better ability than high frequency to increase skin blood flow, none of these approaches clarified the possible interaction between stimulation and the physiological characteristics of microcirculation. A recent paper by Chen et al. has reconsidered possible effects of TENS over skin blood flow [22].

A new method of transcutaneous electric stimulation has been recently proposed which, differently from traditional TENS, is characterized by sequences of specific voltage-controlled electrical impulses (regulated at perception threshold), which vary in both frequency (F) and width (W); this is usually defined as Frequency Rhythmic Electrical Modulation System (FREMS) [23].

FREMS has been specifically designed to directly interact with excitable cell firing frequency range. Previous FREMS application over striate muscles has been able to induce a reproducible influence over spinal excitability through a firing pattern of pulses with frequency increasing from 1 to 40 Hz, thus simulating the temporal recruitment resulting from motoneuron activity [23].

The aim of the study was to correlate the clinical effectiveness of FREMS stimulation (Aptiva, Lorenz Biotech Spa – Italy) applied over the skin of the volar surface of the forearm, with temporal and spectral characteristics of the blood flow signal, measured with laser Doppler flowmetry (Periflux System 5000 Perimed). The acquisition has been performed in the volar part of the forearm before, during, and after FREMS stimulation.

In particular, we qualitatively analyzed the temporal effect of the stimulation on sympathetic activity and the relationship between the temporal stimulation pattern and instantaneous blood flow. A quantitative analysis has been also performed, with the aim to assess the effect of FREMS stimulation to vasomotion waves in the blood flow. A vasomotion index, expressed as the fraction of spectral energy of the blood flow signal, which concentrates in the frequency range associated to vasomotion (around 0.1 Hz), has been evaluated before, during, and after the stimulation.

2. Methods

2.1. Participants

Two groups of participants were recruited, one consisting of healthy volunteers, the other of patients with type 2 diabetes-related polyneuropathy, whose sensitive conduction from the sural nerve could be evoked. Group size, age and gender distribution are as follows:

- group A (control group) consisted of 10 healthy volunteers, age range 21–39 years (mean = 30.4, SD = 5.46); 6 were women and 4 were men
- group B (diabetes-2 group) consisted of 10 patients with type 2 diabetes and symptoms suggesting polyneuropathy and dysautonomy (orthostatic hypotension, disturbances of intestinal peristalsis, hypoesthesia of lower limbs and chronic symmetric painful syndrome), aged 63–75 years (mean = 68.3, SD = 3.36); 5 were women and 5 were men.

To characterize each group according to quantitative pathophysiological parameters, these two groups underwent the following tests:

1. Electroneurography of lower limbs, using a Medelec Synergy N-EP – EMG/EP Monitoring System 2 channel apparatus (Oxford Instruments Medical BP546, France), detecting common peroneal, posterior tibial and sural nerve conduction on both sides. For each subject, this parameter has been calculated as the mean of the above six conduction velocities.
2. Autonomic cardiovascular reflexes: Valsalva ratio and maximal heart rate increment during the hand-grip test (%). ECG signal was recorded through two electrodes, one in the precordial region (4th intercostal space in V3), the other on the palm of the left hand. We provided the recording system an ECG signal preamplifier (CED 1902 isolated pre-amplifier) connected to a multi-channel polygraph (CED 1401plus) and directed by a CED Signal 1.9 acquisition software (Cambridge Electronic Design Limited Science Park, England).
3. Evaluation of peripheral arteriolar elasticity, through an HDI/PulseWave CR-2000 (Hypertension Diagnostics Inc., Minnesota, USA), able to noninvasively apply a Pulse Contour Technique providing an indirect quantitative estimate of arteriolar elasticity (SAEI in milliliters per mmHg $\times 100$) [24].

This way, the groups were thus characterized (Table 1):

- control group, healthy participant, by young age, no systemic disease, normal peripheral nerve conduction measures, normal cardiovascular autonomic reflexes, and high values of small arteriole elasticity,
- diabetic group, patients characterized by advanced age, type 2 diabetes mellitus, moderate dysautonomy, as shown by values of the cardiovascular autonomic reflexes, symmetric distal polyneuropathy, as shown by peripheral nerve conduction measures, and significant reduction of elastic compliance of peripheral arterioles.

2.2. FREMS stimulation

In the past 5 years, in the institute of endocrinology at Sacco Hospital in Milan, a new transcutaneous electrostimulation technology, defined FREMS (Frequency Rhythmic Electrical Modulation System) has been developed [25,23,26,27].

Differently from other applications, i.e. TENS, it is characterized by quasi-rectangular negative electrical impulses with a defined maximal voltage according to the stimulus perception threshold (up to 300 V). The instrument can deliver different stimulation patterns, composed of impulses that have variable time-width (W) between 10 and 40 μs, whereas the frequency (F) of generated impulses (number of single impulses per second) varies from 1 to 1000 Hz. These two parameters are modulated in a pre-set format in order to supply a stimulation sequence.

In this study, the selected stimulation sequence is composed of different phases which are defined as period of time (T) in which only one parameter, W or F, varies. The stimulation can be described

Table 1
Characterization of the subjects.

Group	Age [year]	Valsalva ratio	Handgrip test	Capillary impedance [ml/mmHg]	Nerve conduction velocity [m/s]	
					MCV	SCV
Normal ($n = 10$)	35.4 ± 8.83	> 1	> 30%	12.58 ± 2.33	51	58
Diabetic ($n = 10$)	66.4 ± 9.7	< 1	< 30%	18.07 ± 3.81	39	42

as a three-phase sequence of pulses, in which a first phase is a pulse sequence with frequency increasing from 1 to 19 Hz, a middle phase exhibits a reciprocal oscillation of frequency and width of the pulses and a final phase is again a pulse sequence with frequency increasing from 1 to 40 Hz.

The features of the system allow the following actions:

1. High voltage stimulation is able to activate nervous cutaneous fibers without reaching pain threshold, due to brief impulse duration. Furthermore, it is possible to stimulate for several minutes without inducing thermo-electrical or galvanization effects in the tissue, also because of the use of non-ionized electrodes. A stimulation protocol lasts about 30 min.
2. Induction of "firing" stimulation: the impulse sequences are able to generate activating mechanisms and/or biological function modulation in the tissues according to a specific correspondence between stimulation frequency and functional event.

2.3. Experimental setting

All participants underwent polygraphic examination to estimate the microcirculatory effects of the FREMS stimulation sequence. This study was approved by the local Ethical Committee. Participants were 20 volunteers (10 healthy, 10 patients with diabetes mellitus, type 2) who agreed to participate in the study after receiving complete information on the purpose of the research and the procedures involved and signed the informed consent form. During the 24 h preceding the session, participants abstained from alcohol, smoke, coffee, tea, or other drugs, except oral hypoglycemics or insulin. Each participant lied on a comfortable bed, in a silent environment, with constant temperature and isolated from external noise and stimulation.

We recorded from the volar skin surface of the upper right limb, through polygraphy, the following measures in the time domain:

1. Variation of CC skin conductance, expressed in $\mu\Omega^{-1}$, obtained through a CED 2502 (CED Model 2502 Skin Conductance unit), with a 250 Hz sampling frequency. As CC reflects the degree of palmar sweating, which is in turn the index of systemic catecholaminergic activation, we assumed this recording to represent the index of sympathetic activity acting on target tissues (sympathetic outflow). The anatomic basis of this assumption resides in the facts that the adrenergic innervation of the upper limb is totally provided by fibers originating in the inferior cervical ganglion and, adrenergic fibers involved in sweat gland innervation are limited to the palm. Skin conductance has been measured using 3cm-spaced electrodes positioned on the palm of the hand.
2. Blood flow in the stimulated tissue, obtained through a Periflux System 5000 (Perimed) equipped with a PF 5010 LDPM Unit, with a sampling frequency of 500 Hz. Probe has been positioned in the central third of the forearm, between the radius and the ulna.
3. Skin temperature at the volar forearm surface, with a 10 Hz sampling frequency. Temperature was measured using the same Periflux instrument as with blood flow (PF 5020 Temp Unit).

Actual positioning of the various devices, on a sample subject, is shown in Fig. 1.

Particular care and cooperation from the subject were used to reduce movement artifacts. The few artifacts occurring in the signals were corrected by selecting an adequate analysis window. All the instruments were calibrated, according to manufacturer's specifications, before starting the experiments. Skin conductance variation has been evaluated using as reference the condition of no ionic flow through the skin, which has been assigned a value of zero.

We then applied FREMS stimulation electrodes between the laser Doppler flow probes. The cathode was applied 5 cm above the laser probe, while the anode was applied 5 cm below the probe. Three stimulation sequences were applied separated by pauses. After obtaining stabilization of the observed measures, we recorded continuously the above mentioned parameters, as shown in Fig. 2.

2.4. Data analysis

We observed a large variability between the different participants, as concerns the absolute values of the measured parameters. For this reason, we focused on the analysis of the variation of the parameters on each subject before, during, and after the application of the stimulus.

The analysis has been carried out both in the temporal and in the spectral domain. As concerns the former, a qualitative analysis has been carried out to assess the changes in CC conductance and in flow during the stimulation sequence. The measure of conductance, reflecting sympathetic activity, has been used to analyze the relationship between the stimulus and nervous activity. Visual inspection of the flow signals suggests that a typical shape of the signal is present in the control group. The reproducibility of the signal can be visually enhanced by measuring the temporal increments $\delta v(t_i)$ of the low-pass filtered flow signal $v_l(t_i)$, obtained through averaging the actual signal $v(t_i)$ over a time window of 2 s:

$$\delta v(t_i) = v_l(t_i) - v_l(t_{i-1}) \quad (1)$$

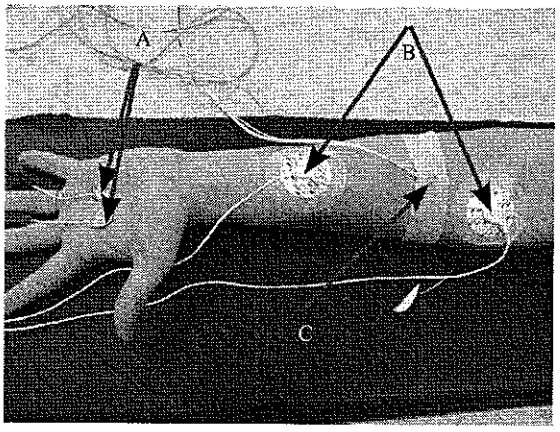

Fig. 1. Experimental setup: (a) SEI electrodes connected with the CED Model 2502 Skin Conductance unit. (b) Stimulating electrodes (3M Red Dot) driven by FREMS, Aptiva 1.5 Lorenz Biotech S.P.A. (c) Laser Doppler probe Perimed PROBE 457.

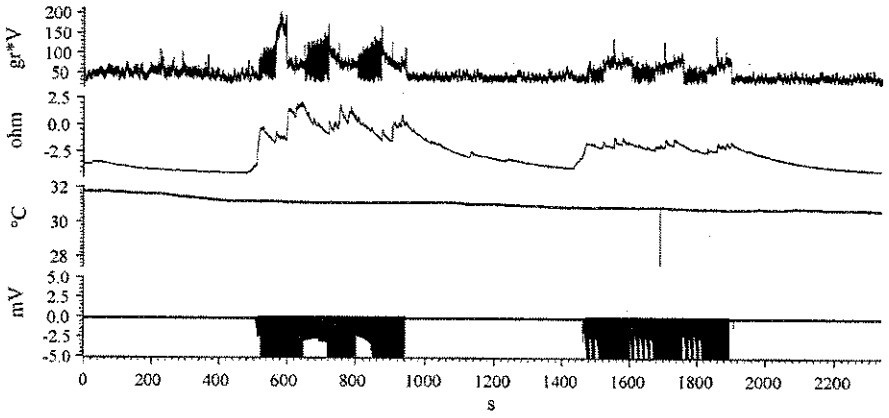

Fig. 2. Polygraphic sampling (from top to bottom): blood flow (expressed in arbitrary units), CC skin conductance, temperature of the stimulated tissue, administration of two consecutive FREMS stimulation sequences.

which is a rough approximation of the first derivative of the signal.

The objective evaluation of the spectral properties of the signal in the frequency region related to vasomotion has been obtained by evaluating the ratio R_v between the average energy in a narrow band centered around the 0.1 Hz frequency (from $f_l = 0.09$ Hz to $f_h = 0.11$ Hz) normalized with respect to the average energy of the whole spectrum:

$$R_v = \frac{(1/(f_h - f_l))\sum_{f=f_l}^{f_h} PSD(v)}{(1/f_s)\sum_{f=0}^{f_s} PSD(v)} \quad (2)$$

where f_s is the maximum frequency in the spectrum and $PSD(v)$ is the power spectral density of the signal.

The spectrum has been evaluated over an observation time of about 6 min, respectively before the application of the stimulus, during the stimulation, and after the stimulation. Observation time has been selected as the minimum length of the available data set, in order to have equal duration of all samples, excluding major artefacts. The estimation has been performed using classical FFT estimation, with a standard Hanning windowing technique. The length of the observation window was selected so to have enough samples to allow an accurate estimation of the frequency components belonging to the 0.1 Hz frequency range, and a time span short enough to assume stationarity of the signal.

The evaluation of the significance of the measured differences has been performed using two-tailed, paired t-test, assuming as reference value the condition prior to the application of the first stimulation. In detail, we evaluated:

- Average flow increase during the stimulation in each participant, compared to the flow before the first stimulation.
- Flow increase after the stimulation, compared to the flow before the stimulation.
- R_v increase after the stimulation, compared to the R_v before the stimulation.

3. Results

A preliminary analysis of the acquired data has been carried out to assess the presence of temperature variation during the measurement, as temperature may highly impact blood flow. In this analysis we did not observe any significant change in temperature along the whole dataset, so we can assume that no temperature-dependent effect occurred in our data.

The different behavior CC conductance variation in the two groups of participants was particularly evident in a visual inspection of the temporal variation of this measure, as shown in Fig. 3. The upper part of the figure reports the typical behavior occurring in all controls. The shape of the curve exhibits a sharp conductance increase immediately after the beginning of the stimulation period, followed by a series of close peaks and by an exponential decay. A few more characteristics appear in most controls. A small increase may appear also before starting stimulation, probably due to an expectation of the stimulation by the participants. Moreover, the amplitude of the response during the second stimulation appears to be lower than the one during the first stimulation, which might be interpreted as a result of adaptation. However, the size of the control sample is not sufficient to extract statistically significant measures to describe the shape of the curve.

On the contrary, curve shape in patients with diabetes may reveal two different conditions: in participant A in Fig. 3, there is a small increase in conductivity, although sensibly lower than in healthy participants. In other cases, for instance, in participant B, there is no reaction at all when the stimulation is applied.

The effect of the stimulation on flow, as measured with laser Doppler flow, in all healthy participants, shows a very similar behavior. Fig. 4 shows the response, averaged over all healthy participants, to the FREMS stimulation, for each of the three repetitions of the stimulus. The individual responses have been synchronized with respect to the start of the stimulatory impulses, and the plot shows the average and standard deviation of the individual responses $\delta v(t_i)$, in all healthy participants, for each value of t_i. The greater and faster response during the first stimulus is to be noted, suggesting the effect of sympathetic efflux losing its power with stimulus repetition, in contrast with a dramatic decrease of the standard deviation in the subsequent course of the response.

The mean increase of blood flow in healthy participants was 35% whilst in the diabetic group was only 25%. In addition, the healthy control group showed a simultaneous increase of CC conductance, despite the absolute value was participant-dependent, while we could not observe any increase in the diabetic group. The increase of the flow corresponds to an increase of the various frequency components that appear in the spectrum. However, both during and after the stimulation, the relative energy of the components is changed. While the peak corresponding to the systolic beat accounts for a large part of the energy, a comparison of the spectra shows a relative increase of the energy related to the frequency band around 0.1 Hz, which is the frequency band most affected by the stimulation.

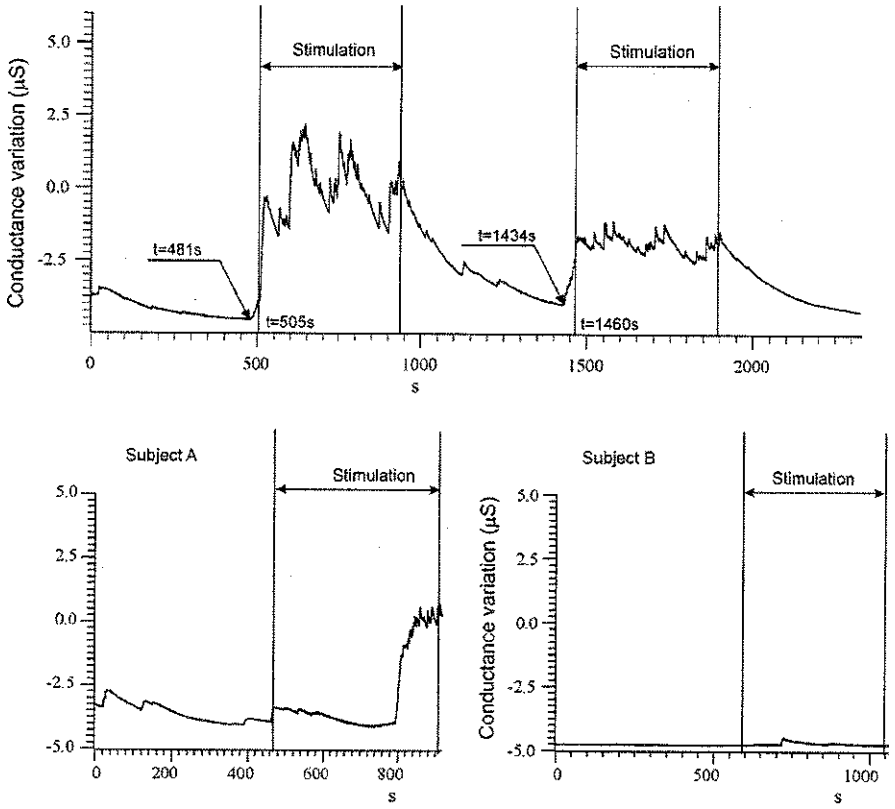

Fig. 3. CC conductance: top, typical aspect of the curve for a healthy participant during two consecutive stimulation periods, bottom, the two different behaviors observed in people with diabetes mellitus, type 2.

The measured range of variability of R_v is between 1.5 and 2.9 for participants with diabetes, while in healthy participants it varies from 2.3 to 5.5.

During the application of the electrical stimulation, we have observed an increase of the values of the R_v parameter both in normal and in participants with diabetes mellitus, type 2. However, the increase in healthy controls is around 12%, while in cases with diabetes mellitus the increase is around 50%. A statistical evaluation of the differences, due to the high variability of the parameters, shows that the increase is significant ($p < 0.05$) only in the group of patients with diabetes mellitus, type 2, while in the normal group, the increase resulted not to be statistically significant.

After the stimulation, the values of this parameter tends to return toward the initial values. However, a different behavior can be observed in the two groups. In the control group, the values of R_v after the stimulation are in the same range of the values before the stimulation. The average difference between the values before the stimulus and after the stimulus is actually slightly negative (the values of the parameter decrease with stimulation). However, this difference proved to be not significant. Fig. 5, on the left, shows a box plot of the R_v values obtained in the control group.

In the case of the diabetic group, on the contrary, the effect of the stimulation persists also after the stimulation itself has ceased. Post-stimulation R_v values are still significantly higher than pre-stimulation ones ($p < 0.05$). The plot on the right side of Fig. 5 shows an increase in the average value both during the stimulus and after the stimulus has ceased.

4. Discussion

In this study, after administration of FREMS, we observed an increment of R_v in both groups, i.e., in the ratio between the average energy in a narrow band centered around the 0.1 Hz frequency with respect to the average energy of the entire spectrum.

The R_v measure well describes the most important characteristics concerning the pathophysiological meaning of vasomotion activity, in agreement with the current model of blood flow, which is viewed as the result of spatial recruitment of several vasomotor units (pre-capillary sphincters) oscillating at the same frequency, with the power of each range of frequency in the whole spectrum reflecting the number of vasomotor units which are synchronously contracting at a given frequency.

Various studies have been conducted to determine the influence of the autonomic nervous system over specific frequency ranges of vasomotor unit oscillations. Wavelet analysis of laser Doppler flow tracks showed that the noradrenergic and cholinergic innervation of arteriolar endings may affect oscillation frequency ranges above 0.1 Hz, whereas within the 0.1 Hz range, the oscillations reflect an endogenous, spontaneous muscular activity. This oscillation is probably initiated by transmembrane calcium ion flux and transmitted over the vascular tissue through a "syncytial" modality through gap-junctions [10,28–33]. Reduction or absence of this latter activity, i.e., "vasomotion", has been indicated as an ominous hallmark in diabetic neuropathy; however, its relationship with autonomic impairment has not yet been clarified [12].

We consider likely that the effect of the stimulation on flow is the result of direct excitation of the smooth muscle cells composing the vasomotor units. In this case, the induction of reproducible blood flow waves, through their synchronization, would be independent from autonomic nerve influence. The indirect, but strong confirmation of this assumption is the evidence of FREMS ability to induce 0.1 Hz oscillations in normal controls, as well as in patients with autonomic dysfunction, the latter group suffering from a serious catecholaminergic activity impairment.

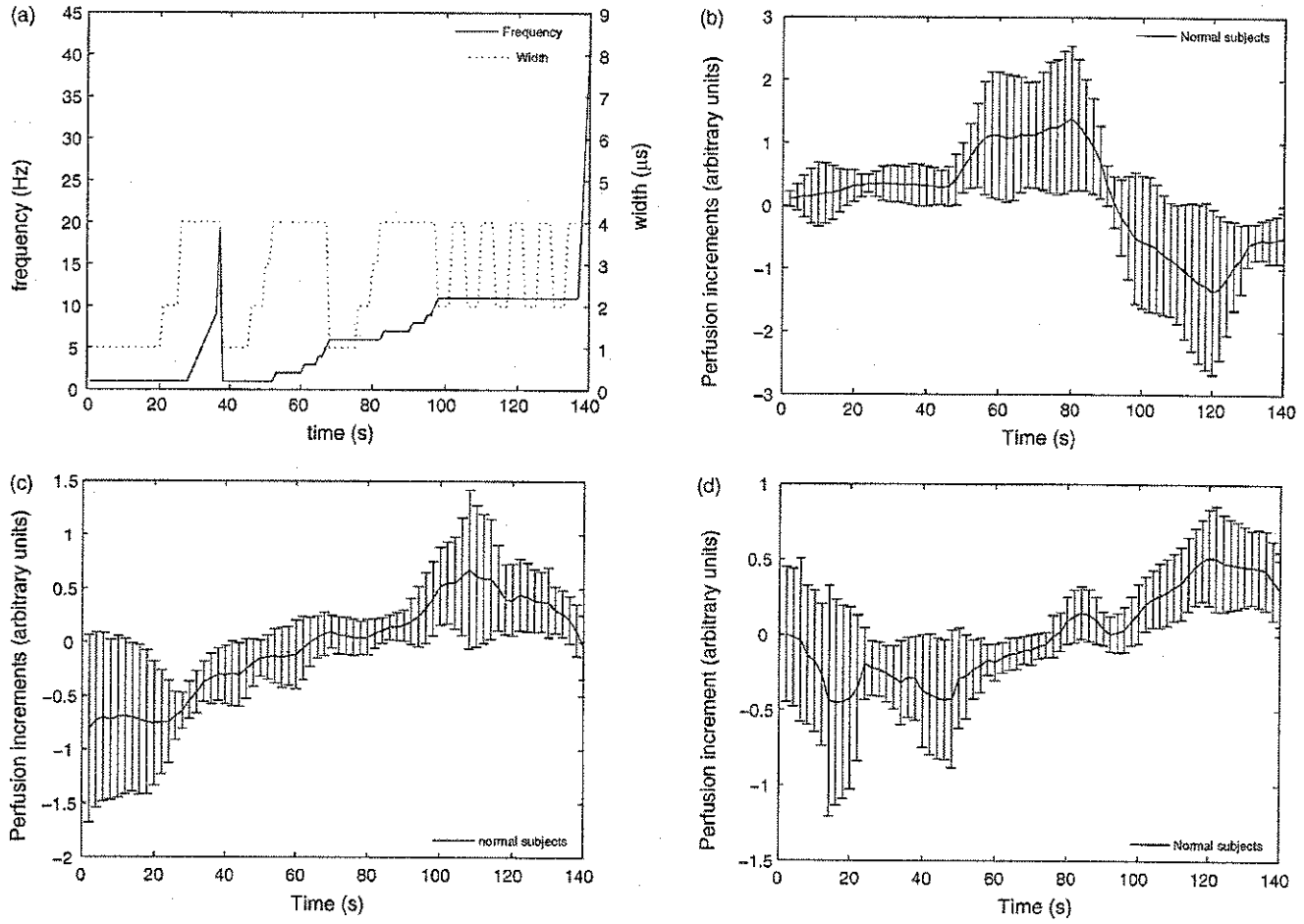

Fig. 4. Relationship between FREMS stimulus and blood flow in the normal group. The plot (a) shows one FREM stimulus. Plots from (b) to (d) show the flow increments $\delta v(t_i)$ (mean value and standard deviation in the group of healthy participants) during the first, second, and third FREMS stimulus, respectively.

Low frequency TENS is more effective than high frequency TENS in increasing blood flow. FREMS acts at lower frequency ranges than conventional TENS. We showed that increasing amplitude of flow waves is related to the increase of FREMS pulses from 1 to 19 Hz,which somehow are comparable to those of low frequency TENS. Current views of the mechanisms of low frequency TENS mediating an increase in skin blood flow (i.e., the putative effect on peripheral nerve fiber groups III and IV [34] or on autonomic nervous system-mediated substance P release [35]) should receive reconsideration.

Regarding statistical significance of the R_v increase in the two groups, and the fact that only the diabetic group showed a persistent effect after the administration of FREMS, it should be said that the induction of blood flow waves appears to be under several influences. The catecholaminergic output modulates blood flow more potently and faster than others, but does so also induce emotional variations in the individual, which are correlated with blood flow variations. A further element of variability is provided by vessel elasticity, which is in turn age-related. Other important factors, such as environmental temperature or intentional movements

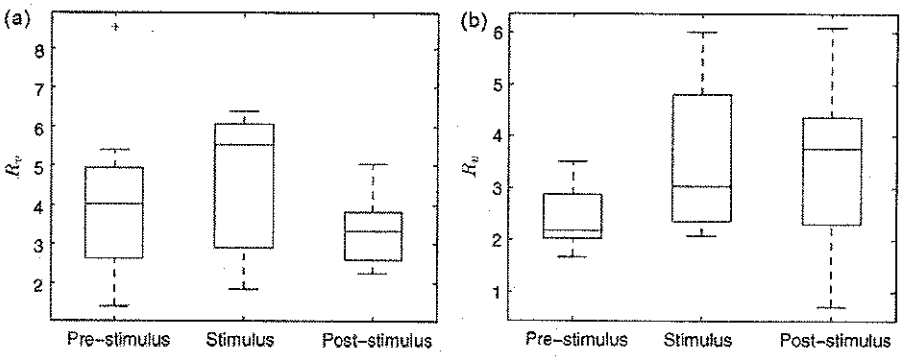

Fig. 5. Box plot of R_v values in the control group (left) and in the group of patients with diabetes mellitus, type 2 (right). Each plot reports the minimum, mean, and maximum values, and the 10th and 90th percentiles (box).

have to be kept under control to ensure acquired data validity. The intensity of a single impulse of FREMS is not able to excite the striatal muscle. Hence, we suggest that the effectiveness of FREMS in the diabetic group only depends on the presence of a high catecholaminergic output in the healthy control group, that in turn is endowed with higher resilience, as shown by its variability and prompt responsiveness to stimulation, as compared with the diabetic group. If we consider the effect of FREMS as a direct consequence of vasomotion unit recruitment, independently from autonomic nervous influence, it should be expected that the most significant effects occur in people with dysautonomia, like our participants with diabetes mellitus type 2, than in healthy controls.

5. Conclusions

Our results indicate that FREMS sequences can induce a well defined pattern of vasomotion. Blood flow increased both in healthy controls and in patients with diabetes mellitus, type 2, after application of FREMS sequences, but 0.1 Hz vasomotor activity increased significantly only in the group of patients with type 2 diabetes mellitus, in which the autonomous nervous system is impaired. This suggests that FREMS can functionally activate the smooth muscle cells of the microcirculation system.

Furthermore, the analysis of the ratio between power spectrum at 0.1 Hz and blood flowmetry during FREMS stimulation may contribute to understanding the role of the autonomic nervous system in vascular activity more in depth.

Conflict of interest

The authors state there are no conflict of interests.

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