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## Evaluation of age-related peripheral arterial vessel reactivity in test with oral administration of nitric oxide (NO) donor in patients with type 1 diabetes and healthy volunteers

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### Summary

Evaluation of vascular reactivity becomes increasingly more often applied in assessing vascular susceptibility to atherosclerosis and vascular damage in diabetes. "Gold standard" test for vasodilation assessment includes administration of exogenous nitric oxide (NO) donor. The aim of this work was to analyze the course and effect of the test in type 1 diabetes and non-diabetes subjects in different age groups.

71 healthy men and 45 male patients with type 1 diabetes were examined. Evaluation of hemodynamic reaction was conducted with the means of ultrasound color Doppler examination.

It has been demonstrated that the degree of vascular reaction to the administration of NO reduces with age: in subjects <30 years old the change in early diastolic velocity (Vr1) is 236%, while in those >45 years old 102%. The response to stimulus occurred at different times in different subgroups. After NO administration the reaction occurs most rapidly in young men (70-130 sec.) and the slowest reaction was noted in healthy subjects aged <46 years (100-230 sec.). Changes are more pronounced in patients with diabetes, particularly in subjects <30 years, with the change in Vr1 158%, whilst in those aged >45 years it was merely 78%. The reaction in the group of patients with diabetes occurred in those aged < 30 years (100-160 sec.), and in subjects >46 years (120-240 sec.). These differences were statistically significant.

Reliable evaluation of vascular reaction to the action of pharmacological stimulus requires constant spectral analysis. Age- and co-morbidities related modifications to pharmacological test are justified, especially in elderly subjects and in patients with diabetes.

**Key words:** Doppler ultrasound • arterial vessels • nitric oxide

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### Background

Evaluation of vascular reactivity becomes increasingly more often applied in prognosing individual susceptibility to the development of the atherosclerosis process [1-3]. Abundant evidence proves that vascular response to stimuli in the form of temporary ischaemia and to the administered nitric oxide (NO) may reflect the degree

of arterial damage, also observed in diabetes and metabolic disorders [4-6]. Diagnostics may be also conducted at the preclinical stage of the disease, in the period when changes are still morphologically imperceptible in image examination [6, 7]. The fundamental test to specify vasodilative potential consists of sublingual administration of exogenous NO donor, typically nitroglycerine (NTG) preparation and further in assessing relative dilation of

peripheral vessels based on the ultrasound Doppler image. The dilatation is independent of the endothelium activity (non-endothelium dependent dilatation) and is comparable to the dilatation observed in the function tests revealing intrinsic endothelium activity, i.e. the dilation dependent on the secretive function of the endothelium (flow mediated dilatation, FMD) [1]. Methodology of conducting functional tests presented in literature focuses on recording the vasodilative effect, i.e. the changes in the vessel diameter at rest in response to stimulus. Conducting an arbitrary functional test with the exception of the changes in the diameter of the vessel parallelly generates changes in the Doppler spectral waveform, i.e. the hemodynamic effect relevant to the test [8]. Changes in the spectrum display two characteristic hemodynamic response patterns: one induced by the increased flow and the other typical of the test independent of endothelium activity.

The aim of this work was to analyse the effect of oral administration of NO donor in different age groups of male subjects with different metabolic status which may affect vasculature, therefore the degree and progression of hemodynamic response were analysed in healthy subjects and in patients with type 1 diabetes.

## Materials

Two groups of subjects were enrolled: healthy individuals and patients with type 1 diabetes. The group of volunteers (group I) consisted of 71 healthy non-smoking men with unremarkable medical history and low vascular risk evaluated according to the SCORE scale (Systematic Coronary Risk Evaluation, 2003) [8]. The group of patients with type 1 diabetes (group II) consisted of 45 men treated with insulin preparations, with stable

Subjects in both groups were divided into 3 age subgroups (A, B, C):

- group A: aged from 18 to 30 years old,
- group B: aged from 31 to 45 years old,
- group C: aged from 46 to 60 years old.

As expected, patients with diabetes displayed significantly higher values of the sudden vascular incident index (subgroups: 1A and 1B < 1%, subgroup: 1C - 1%, 2A < 1%, 2B 1-4% and 2C 2-12%).

Demographic and clinical data and the distribution of the risk of the cardiovascular incident occurrence are shown in table 1.

## Methods

The evaluation of vascular reactivity (hemodynamic effect) was conducted based on ultrasound examination with colour waveform imaging (USG-CD). The General Electric Logiq 7 system with linear, broadband probe of 10-14 MHz frequency (type M12L) was used. The operating system enables recording of the complete examination in the form of initial data, as well as performs secondary analysis (measurements, image enlargement, filtration of the Doppler signal, changes in angle correction, forms of recording spectral wavelength).

The technique of conducting pharmacological test conformed with the standards established in the literature [1,9-11]. The examination was carried out on the right brachial artery, in patients lying supine. The arteries were assessed in the area of elbow joint, above the articular space, to obtain optimum conditions in order to record spectral waveform using the Doppler pulsation wave (PW) technique. The impact of functional test on the vessel was measured based upon the changes in values of measurements carried out at baseline and after the test.

Hemodynamic effect was analysed based upon the values of blood flow velocity in four characteristic waves of Doppler spectrum:

- at the systolic stage - velocity measurement: maximum systolic (Vs),
- at the diastolic stage - velocity measurement: early diastolic (Vr1), maximum diastolic (Vr2) and late diastolic (Vr3).

At the first stage of the examination parameters of blood flow at rest were recorded (time 0, t0). At the second stage

**Table 1.** Material: Demographic and clinical data in the studied group.

Studied groups	Group I			Group II		
	A	B	C	A	B	C
Age intervals (years)	18÷30	31÷45	46÷60	18÷30	31÷45	46÷60
Number of subjects in the group	21	21	29	15	15	15
Average age of the group	24,1	38,5	52,2	24,6	39,0	52,1
Mean cholesterol level (mmol/l)	4,5	4,6	4,6	4,6	5,2	5,5
Mean glucose level in the blood (mmol/l)	5,4	5,2	5,6	6,2	6,4	6,8
Mean values of systolic blood pressure (mmHg)	118	120	122	119	121	126
Risk of cardiovascular incident SCORE (%)	0	0	<1%	<1%	1-4%	2-12%



**Figure 1.** Recording of the resting spectral waveform in the brachial artery.

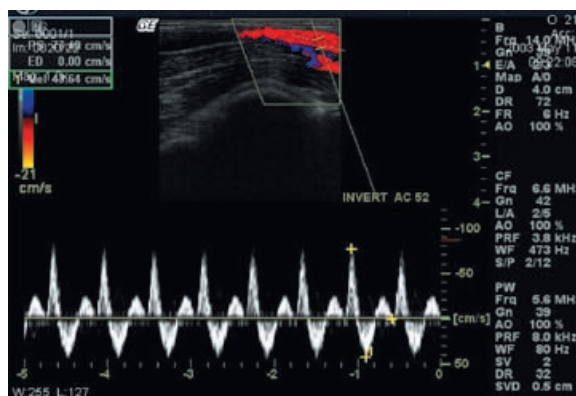
one dose of the NO donor in aerosol (400 µg, Nitromint preparation) was administered sublingually, followed by continuous recording of spectral waveform for 360 seconds (6 minutes). Velocities were analysed every 15 sec. Relative changes in velocity values were accepted for analysis as reflecting the degree of vascular response.

Mean values, standard deviation (SD) and standard error (SE) were calculated for all groups. As the distributions of the measured parameters did not differ significantly from the normal distribution, t-Student test was used to compare parameters for independent tests.

**Results**

High-resistance flow is characteristic of brachial arteries. Spectral waveform of such nature was initially registered in all subjects (Fig. 1). In both groups hemodynamic effect of the test was observed as the increase in the early diastolic velocity (Vr1), which was reflected in the deepening of the negative wave of the Doppler spectrum. The wave corresponds with the intracardial flow wave closing the aortic valve (Fig. 2). No significant differences were observed in the remaining parameters of the spectral waveforms (Vs, Vr2 and Vr3).

In group I changes of velocity Vr1 were statistically significant, and the mean difference for all groups was 20,9 cm/s (difference of approx. 170%); detailed data depicting the scales of hemodynamic effect of the NO test and its progression in time are presented in table 2. Significant differences were observed in changes of early diastolic velocity (Vr1) in all age groups. The decrease in the reaction with age (the differences between the IA subgroup and groups IB and IC was statistically significant) was a frequent phenomenon. In the group of patients with diabetes (group II) the increase in velocity Vr1 was also observed in response to NO. The recorded increase in velocity Vr1 was smaller than in group I, but also statistically significant – the average difference was 13,9 cm/s (difference of approx. 104%). In the group of patients with diabetes differences in changes of velocity Vr1 in separate age groups were noted. The decrease in hemodynamic effect with age was observed (differences between group IIA and groups IIB and IIC were statistically significant).



**Figure 2.** Spectral waveform in the brachial artery after the action of pharmacological stimulus. Noticeable increase in early diastolic velocity.

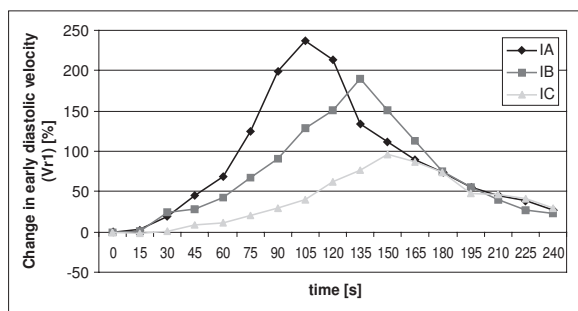
**Table 2.** Results: Progression of the vasodilative reaction after the administration of NO.

Studied group	Decrease in Vr1 after NO (cm/s)	Mean max reaction time and range (s)
I A	-28,4	102 (70-130)
I B	-20,2	134 (90-195)
I C	-14,2	155 (100-230)
II A	-16,9	134 (100-160)
II B	-12,7	158 (110-210)
II C	-12,1	174 (120-240)

In group I hemodynamic effect of the test achieved its peak value within 70-230 seconds after the drug administration. It was most rapid in subgroup IA, within 70-130 seconds, and in subgroup IB between 90 and 195 second. Noticeably slower response was observed in subgroup IC, after approx. 100-230 sec. In group II the greatest hemodynamic effect occurred within 100-240 seconds after the drug administration. Most rapidly in subgroup IIA, after 110-170 seconds, noticeably slower in subgroups II B and IIC, where mean was 110-210 and 120-240 seconds. Differences in the reaction time between subgroups IIA, IIB and IIC were statistically significant.

**Discussion**

Function tests evaluating vascular reactivity to various stimuli are increasingly applied in the diagnostics of vascular system [1-6, 13, 14]. Endothelium-derived nitric oxide is one of the most powerful vasodilators responsible for physiological blood flow in human organism. Pharmacological tests involving administering NO donor that exceeds secretive capability of the endothelium serve to reveal the dilative functional reserve of the vascular wall. Results of tests revealing endothelium activity may be interpreted more fully against the background of pharmacological test results [1, 16, 17]. The action of nitric oxide consists in triggering the dilation of smooth muscles in vascular walls and in decreasing peripheral resistance [17, 18]. The consequence of increasing the relaxation of the vascular wall is the rise of early diastolic velocity (Vr1).



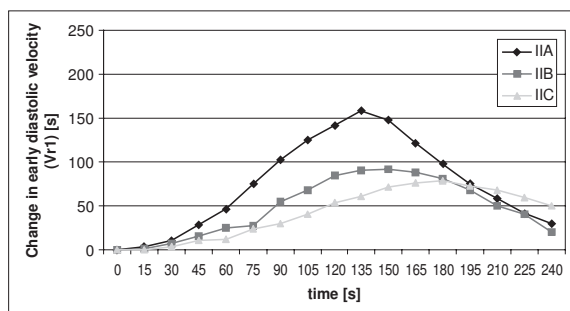
**Figure 3.** NTG Test Dependence of the time of occurrence of the reaction and its persistence in group I.

The protocol of conducting pharmacological test established in literature assumes taking measurement of the test (vasodilative) effect at approx. 90 second after the application of NTG dose less frequently in the consecutive, arbitrarily assumed periods [10, 15, 16]. Presented results prove that such protocol may lead to unreliable results, particularly in the elderly, in whom the response to stimulus is delayed. The prompt response of vascular system in younger subjects may result from better preparation distribution by the circulatory system, more efficient NO diffusion in tissues, high vascular flexibility and more efficient autoregulatory mechanisms on the level of capillaries [1].

Hence, it seems purposeful to modify the protocol of pharmacological test with exogenous NO. The obtained results depicting the change in hemodynamic effect with age both in the group of volunteers and in patients with type 1 diabetes are similar to variability of the vasodilative effect in comparable groups most widely discussed in literature. This similarity suggests depicting the same phenomenon dependent on the relaxation of the muscular coat of arterial vessel walls. Noticeably higher variability of recorded velocities Vr1 facilitate these measurements and yields reliable results. Individually differentiated time of the occurrence of vascular response requires continuous recording of the progression of pharmacological test between 1 and 6 minute and secondary data analysis with regard to values obtained in separate age groups. The optimal procedure involves spectral analysis enabling determination of the maximum decrease in early diastolic velocity individually and conducting parallel measurement of artery diameter.

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**Figure 4.** NTG Test Dependence of the time of occurrence of the reaction and its persistence in group II.

At present the recording of long sequences of spectral waveform with the option of its secondary analysis is possible only when using most technologically advanced systems. One should expect, however, that in the nearest future the technology of storing large quantities of initial data will be transferred to the level of middle range systems, making wider application of the proposed solution possible.

We have also shown that the age-related changes in vasodilation assessment protocol should be implanted in cases when diabetes subjects are studied. Diabetes is often considered as 'vascular disease' due to its damaging impact on vasculature, thus proper use of vascular assessment methods in these group of individuals is of utmost importance.

## Conclusions

- Vascular reaction to the administration of nitric oxide observed on the basis of hemodynamic effect decreases with age.
- The observed decrease in vascular reaction to nitrate stimulus is even more pronounced in patients with diabetes than in healthy individuals.
- Vascular response occurs at a different time after the action of nitrate stimulus both in the group at low vascular risk and in patients with diabetes, which should justify the proposed modification of the pharmacological test protocol.

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