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FUNCTIONAL CHANGES IN THE SKIN MICROCIRCULATION IN SMOKING AND NON-SMOKING SPORTSMEN AND SEDENTARY CONTROLS MEASURED BY MEANS OF LASER DOPPLER FLOWMETRY

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Abstract

Introduction: Systematic physical training causes functional and structural adaptive alterations in the circulatory system. Therefore, the aim of the paper is to assess adaptive alterations in skin microcirculation, as well as to compare a response of the skin vascular bed in smoking and non-smoking sportsmen and sedentary controls.

Material and methods: The study covered 42 sportsmen, including 31 non-smokers, and 11 sportsmen who had been smoking at least 10 cigarettes a day for at least last three years. A control group was made up of 31 persons, non-smokers, who led sedentary lifestyles. Skin microcirculation was measured with the use of the laser Doppler flowmeter Perifluks 4001 made by Perimed (Sweden). Measured parameters were: rest flow and hyperemic reaction to specific stimuli: the reactive hyperemia after loosening the cuff, the thermal hyperemia. The author analyzed the frequency of signals recorded with the use of laser Doppler flowmetry in the interval between 0.0095 and 2 Hz, in conditions of the rest flow. The chosen time constant was 0.03 s, every blood flow signal was recorded at the frequency of 32 Hz.

Results: All of the skin microcirculation parameters studied were highest in non-smoking sportsmen (HR – 63.55±9.03 Hz; PP – 15.56±7.13P U; Obiol – 3.96±0.98 PU; RH – 51.39±31.33 PU; MAX RH – 82.48±42.12 PU; TH – 171.34±78.12 PU; MAX TH – 225.27±91.77 PU) in comparison both to the group of smoking sportsmen (HR – 75.18±5.42 Hz; PP – 8.88±4.03 PU; Obiol – 3.04±0.8 PU; RH – 30.15±24.03 PU; MAX RH – 55.85±31.61 PU; TH – 131.75±45.21 PU; MAX TH – 179.79±57.91 PU) and the control group (HR – 74.97±9.06 Hz; PP – 10.92±4.81 PU; Obiol – 3.21±0.63 PU; RH – 35.70±14.76PU; MAX RH – 61.06±32.14 PU; TH – 158.59±93.94 PU; MAX TH – 208.39±103.44 PU). The analyses of the Doppler signal frequency showed a significant increase of activity of the endothelium as well as significantly decrease sympathetic oscillations in the group of non-smoking sportsmen.

Conclusions: Functional adaptation of microcirculation under the influence of endurance training manifests itself in increase values of the rest flow, a greater reservoir of the vascular bed and a more efficient response of blood supply from the skin to the occlusive and thermal stimuli as well as a lower rest HR. The study confirmed a harmful effect of nicotine on skin microcirculation. Endurance training does not compensate this harmful effect.

Key words: regular physical training, skin microcirculation, laser Doppler flowmetry

Introduction

Systematic physical training causes functional and structural adaptive alterations in the circulatory system. They are observed not only when we exercise but also at rest. Intensive physical activity brings about an increase in density of capillaries in trained muscles as well as in the number of vessels to one muscle fibre. As a result, the area of gas exchange becomes larger and energy substrates which are transported with blood are more readily available (1). In well trained people, we observed changes in blood vessels tonus, a decrease in the vascular peripheral resistance (2-4) as well as an increase in the diameter of the small and large arteries (5). The causes of these changes should probably be identified as a change in the nervous regulation of the circulatory system functions due to a decrease in the level of adrenaline and noradrenalin in blood as a result of training as well as due to a decrease in sympathetic stimulation (6). An additional factor which decreases the muscle coat tonus of blood vessels is a lower activation of the rennin-angiotensin system which occurs as a result of endurance training (7). Also, the vascular endothelium – until recently regarded only as a physical border between intra and extra vascular environments – is a place where post-training alterations are manifested. It has been shown that an increase in the blood flow during effort leads to an increase in shear stress, which in consequence stimulates the vascular endothelium to produce vasodilatating factors (2, 3, 5, 8, 9). This is mainly EDRF – the Endothelium Derived Relaxing Factor (nitric oxide NO). An increase in NO production under the influence of values of shear stress occurs probably by the stimulation of nitric oxide synthase gene expression (eNOS) (10). Animal testing has revealed that physical training which brings about an increase in blood flow may cause not only functional but also structural adaptations of the vascular wall with the consequential increase in the vessel diameter. While a reduction in local blood flow by 70% resulted in stricture of the vessel under examination by 21% (7). Physiological activity of the endothelium decreases with age. However, in elderly persons leading sedentary lifestyles who underwent a programme of physical exercises, we observe an improvement in vessel relaxation, and even a return to the correct values which depend upon the endothelium (8, 11, 12). The essence of functional and structural adaptive alterations in larger arteries as well as in the capillary vascular bed gives rise to controversy. Therefore, the aim of the paper is to assess adaptive alterations in skin microcirculation, as well as to compare a response of the skin vascular bed in smoking and non-smoking sportsmen and sedentary controls.

Material and methods

The study covered 42 sportsmen, training at least 2 hours a day, five times a week (the academic basketball premier league, the national volleyball premier league). An additional criterion was the specified characteristic curve of training load. Persons qualified for the study, apart from specialist exercises characteristic for the phase of starts, 3-5 hours a week, for at least 8 weeks, devoted to endurance training engaging minimum 1/6 of the skeletal muscles. According to Huonker et al. it is the minimum training time necessary for getting and maintaining adaptive functional alterations in the circulatory system. Extending the endurance effort above 5 hours a week leads to structural alterations in the cardiovascular system (10). The tested group included 31 non-smoking sportsmen (median age 21.42±1.99) and 11 sportsmen smoking at least 3 years minimum 10 cigarettes a day (median age 21.27±1.68). The control group consisted of 31 non-smoking volunteers (median age 20.94±3.62). All of the people examined were healthy, they had a normal blood pressure. The body mass index (BMI) was below 25 $kg \cdot m^{-2}$. The participants were asked not to take part in physical activities and to avoid products that influence the circulation (coffee, tea, Coca-Cola, alcohol).

The tests were performed in horizontal position on the back, in a constant temperature of 21±1.2°C and humidity of 40 – 60%, after ca. 20 minutes of adaptation. The microcirculation was evaluated with the use of a laser Doppler flowmeter (Perifluks 4001, Perimed, Sweden). The measurements were performed with a flowmeter containing a neon – helium laser emitting the light of 632.8 μm (red light). The flowmeter is based on Doppler's effect, when a bundle of light is reflected by the mobile elements of the blood, thus changing its frequency. This effect allows a measurement of the blood flow, which is the product of number of the moving erythrocytes in the given volume times their mean velocity (13,14). The optode was placed on the skin of the back of the hand between the first and second metacarpal bones using special both sides adhesing ring. The place of measurements (dorsal part of the hand) practically eliminates the risk of hemodynamic disturbances related to circulation disorders. The flow was measured in conventional Perfusion Units score (PU), in proportion to the energy of the Doppler signal.

PU = CMXC x VEL

Where:

PU – perfusion expressed in perfusion units

CMXC – number of mobile blood elements

VEL – median speed of blood cells movement

1 PU corresponds the voltage of 10 mV at the outlet.

The course of the experiment:

1. The procedure was started after 20 minutes of stabilization of the circulatory system in horizontal position.
2. Blood pressure (RR) measurement (mmHg) – on the brachial artery and heart rate (HR) measurement (one minute).
3. Registration of the rest flow (RF) in horizontal position, on a dominating upper limb, registration time 2 min.
4. Registration of the flow after occluding the arm with the cuff of the manometer filled with air up to pressure exceeding the formerly measured systolic pressure by 50 mmHg, biological zero (BZ) - registration time 2 min.
5. Registration of the reactive hyperemia (RH) after loosening the cuff, registration time 2 min.
6. Stabilization of the blood flow back to rest flow level.
7. Rising the optode's temperature up to 44°C, using the built-in heating module, 1 min.
8. Registration of the thermal hyperemia (TH), registration time 2 min.
9. Stabilization of the blood flow back to rest flow level.

Frequency of signals received by means of the laser Doppler fluximetry between 0.01 up to 2 Hz during basic flow was also analysed. In this range five groups were singled out: I – frequency band between 0.01-0.02 Hz; II – frequency band between 0.021-0.05 Hz; III – frequency band between 0.051-0.145 Hz; IV – frequency band between 0.15-0.5 Hz; V – frequency band between 0.51-2 Hz. In each band range there is a different factor which determines blood flow oscillation. I – shows vascular oscillations depending on the endothelium metabolic activity (EF); II – shows the effect of the sympathetic system on skin flow (SF); III – illustrates oscillations resulting from the arteriola basic systolic tonus which occurs due to discharges of particular myocytes forming a circular layer of the vessel muscle coat, this response is often referred to as myogenic and it is independent of the sympathetic system (MF); IV – breath frequency (BF); V – heart frequency (HR) (15,16). A time constant of 0.03 s was selected, and every blood flow signal was taken at the frequency of 32 Hz.

The values of the examined parameters were presented as median ± standard deviation (SD). Statistic analysis was performed with the use of the Tukey test, assuming $p<0.05$ to be significant.

Results

In their own material, the author observed faster pulse at rest in smoking sportsmen - 75.18±5.42 PU, compared to not active persons 74.97±9.02 PU and non-smoking sportsmen - 63.55±9.02 PU. The skin perfusion measured at rest was significantly bigger in sportsmen (15.56±7.13 PU) than in control group (10.92±4.81 PU) and in smokers (8.88±4.03 PU). The values of biological zero were close to zero in all tested persons. Median flow during the post-occlusive hyperemic reaction and its maximal values were significantly increase in non-smoking sportsmen group: RH – 51.39±31.33 PU; MAX RH – 82.48±42.13 PU. In control group the values were: RH – 35.70±14.76 PU; MAX RH – 61.06±32.13 PU, and in smoking sportsmen: RH – 30.15±24.03 PU; MAX RH – 55.85±31.61 PU.

The perfusion curves during the reactive hyperemia had normal course in all subjects time from releasing the cuff to maximal microperfusion was on the average 1.1±0.7 s in non-smokers sportsmen, 1.29±0.7 s in smokers and 1.3±0.9 s in the controls

Groups of non-smoking sportsmen and smoking sportsmen differed significantly statistically from each other as regards parameters: HR, RF, BZ, RH, BF, SF, EF.

There were no statistically significant differences between smoking sportsmen and the control group in respect of any parameter (except BF), and all the values were decrease in the group of smoking sportsmen.

The group of non-smoking sportsmen and the control group differed significantly statistically from each other as regards all the parameters except values obtained in thermal hyperemia.

The results are shown in tables.

Table 1. *Selected parameters of microcirculation in smoking, non-smoking sportsmen and control group (mean value-x and standard deviation-SD)*

Parameters		Smoking sportsmen n=11	Non-smoking sportsmen n=31	Control group n=31
RF (PU)	x	8.88	15.56	10.92
	SD	4.03	7.13	4.81
BZ (PU)	x	3.04	3.96	3.21
	SD	0.8	0.98	0.63
RH (PU)	x	30.15	51.39	35.70
	SD	24.03	31.33	14.76
MAX RH (PU)	x	55.85	82.48	61.06
	SD	31.61	42.13	32.13
TH (PU)	x	131.75	171.34	158.59
	SD	45.21	78.12	93.94
MAX TH (PU)	x	179.79	225.27	208.39
	SD	57.91	91.77	103.44
HR (Hz)	x	75.18	63.55	74.97
	SD	5.42	9.02	9.06
EF (Hz)	x	0.014	0.018	0.014
	SD	0.006	0.004	0.003
SF (Hz)	X	0.04	0.025	0.037
	SD	0.01	0.009	0.002
MF (Hz)	X	0.079	0.089	0.081
	SD	0.02	0.03	0.02
BF (Hz)	X	0.33	0.25	0.23
	SD	0.11	0.09	0.06

RF - rest flow in horizontal position.
BZ - biological zero
RH - median value of the flow during post-occlusive reactive hyperemia.
MAX RH - perfusion at the peak intensity of the signal during the reactive hyperemia.
TH - median value of the flow during thermal hyperemia.
MAX TH - perfusion at the peak intensity of the signal during the thermal hyperemia.
HR - heart rate.
EF - endothelium frequency
SF - sympathetic frequency
MF - myogenic frequency
BF - breath frequency

Table 2. *Significance of differences between microcirculation parameters in particular groups*

Parameter	Smoking sportsmen Non-smoking sportsmen	Smoking sportsmen Control group	Non-smoking sportsmen Control group
RF (PU)	p=0.000*	p=0.997 (NS)	p=0.000*
BZ (PU)	p=0.004*	P=0.580 (NS)	p=0.007*
RH (PU)	p=0.006*	p=0.794 (NS)	p=0.036*
MAX RH (PU)	p=0.104 (NS)	p=0.91 (NS)	p=0.042*
TH (PU)	p=0.357 (NS)	p=0.620 (NS)	p=0.813 (NS)
MAX TH (PU)	p=0.350 (NS)	p=0.657 (NS)	p=0.756 (NS)
HR (Hz)	p=0.000*	p=0.997 (NS)	p=0.000*
EF (Hz)	p=0.007*	p=0.964 (NS)	p=0.000*
SF (Hz)	p=0.000*	p=0.478 (NS)	p=0.000*
MF (Hz)	p=0.347 (NS)	p=0.975 (NS)	p=0.237 (NS)
BF (Hz)	p=0.006*	p=0.000*	p=0.531 (NS)

NS – statistically not significant.
* - p<0,05

Discussion

The impact of physical activity on skin perfusion (functional congestion) was first reported by Benedict and Parmenter in 1929 (cit. in 17). They noticed that after a prolonged march the skin temperature is lower than before the effort. In 1942 Christensen et al., using water pletysmography, found obstruction of the blood vessels in 4 persons followed by significant increase of the skin perfusion after 2 – 3 min (cit. in 17). Ducloux et al. stated that in the group of sportsmen a significant increase of skin perfusion occurs during physical effort, compared to untrained persons. Moreover, sportsmen decrease skin flow outside the area of muscle activity unlike the people who are not well trained (17).

The effect of training on basic perfusion in the vascular bed is not clear. The majority of authors think that dynamic muscle training causes an increase in rest flows (13, 14, 18). However, Franzoni at al. did not show such differences (8). Researchers do not give reference values, only Oimomi at al. state that the rest flow value should be within 10-20 PU (19). Results of my own studies prove that a thesis regarding higher values of flow at rest in sportsmen is most often explained by an increase in the shear stress. An increase in shear stress stimulates nitric oxide (NO) and prostacycline production by the vessel endothelium leading to the vasodilatating effect. Recent examinations suggest that nitric oxide has a fundamental role in vascular control and prove that physical effort causes the increased production of this substance in young and healthy people, improves impaired functions of the endothelium in people with metabolic and vascular illnesses as well as reducing a decrease in the level and bioavailability of nitric oxide which depends on the age (4, 5, 21, 20). On the basis of a number of experiments it was established that the vessel endothelium constitutes an „interface" between the blood stream in a vessel and a response and adaptation of the vessel wall. The endothelium is capable of registering mechanical load caused by blood flow and responding to it through quick changes in vessel wall functions. These responses consist in relaxation and stricture of vessel smooth muscles (3, 22).

An analysis of frequency of signals received in laser Doppler flowmeter measurements in the frequency band 0.01-0.02 Hz proved that there was a significantly increase activity of the endothelium in the group of non-smoking sportsmen in comparison both to the persons who do not train and to the smoking sportsmen. Significantly decrease rest flows in smokers show a decrease in nitric oxide and prostacycline production which is the fact well documented in the literature on the subject (14, 17). Decrease activity of the adrenergic system under the influence of systematic physical effort is also of importance. Lower sympathetic activation leads to lower stimulation of smooth muscle coat of blood vessels and in consequence to an increase in flows (6). An analysis of frequency of signals received in laser Doppler flowmeter measurements in the frequency band 0.021-0.05 Hz revealed that there were significantly decrease sympathetic oscillations in non-smoking sportsmen than in the control group. This increased sympathetic activity observed in smoking sportsmen can be explained with the stimulated sympathetic nervous system by nicotine (23).

Flow values observed during the occlusion should be close to zero; slightly higher values are the result of the accidental movement of blood cells. A significant increase is observed in illnesses where the endothelium functions are impaired, e.g. in diabetes (14, 24). In the persons examined, values of biological zero were within the range regarded as correct.

We may assess a functional state of microcirculation with the use of stimuli which force the vascular bed to maximum perfusion. One of the more often used tests is a skin reactive hyperaemia test in response to occlusion (hyperaemic test) as well a hypethermic test which tests skin microcirculation reactivity to a thermal stimulus. A hyperaemic test assesses a myogenic response of vessels which occurs after the occlusive cuff is loosened. A direct cause of this local hyperaemia is a vasodilatating effect of metabolites collected during the ischemia on the arteriola smooth muscles and pre-capillary sphincters. A possible decrease in maximum values proves the impaired vascular reactivity and it is evident from the literature that such impairment occurs in illnesses where the endothelium functions and structure are altered (diabetes, arterial hypertension, arteriosclerosis). A decrease in maximum flows correlates well with the time how long these illnesses last. Microcirculation response is significantly higher in non-smoking sportsmen, both for average and maximum values. This shows that vessel myogenic response in sportsmen is efficient and first of all that certain reservoirs exist in the vascular bed which can be used in case of increased flows. Wang at al. proved greater arterial reactivity to ischemia in persons training Tai Chi (25). Hornig at al. confirmed that the use of training programmes caused an increase in hyperaemic reactivity (26). Yamazaki at al. showed that in the case of responses provoked in people who train, these responses are quicker, while skin microcirculation response to an endurance load is slower (27). It was supported by our own studies where the time required to achieve maximum values was the shortest in the group of non-smoking sportsmen. Perfusion curves are significantly different in healthy persons and in those suffering from an illnesses of blood vessel endothelium. The perfusion curves during the reactive hyperemia had normal course in all subjects. They were sharp (short time from loosening the cuff to achieving maximum flow – close to zero), two-phase (a characteristic drop in hyperaemia once the maximum was achieved, and next again an increase in perfusion). A one-phase or flat curve indicates aberrations in the blood vessel endothelium (14, 24).

Under normal conditions only a part of microcirculation is active, the remaining part constitutes a functional reservoir activated when perfusion increases. Vasodilatation under the influence of thermal stimulus is a result of releasing skin blood vessels from the effect of sympathetic impulsation. When the skin is warmed up to 44°C by the heating module of the probe, the vascular bed is activated up to the maximum vasodilatating response and has a perfusuion which is several times greater in healthy people. In the persons examined an increase in perfusion in response to a thermal stimulus observed was in conformity with the references in the literature. A difference between values in non-smoking sportsmen, smoking sportsmen and the control group was not statistically significant. However, in non-smoking sportsmen highest average and maximum values were observed.

Increase average and maximum values in hyperaemic and hyperthermic reactivity show that the sportsmen examined have greater reservoirs of the vascular bed. The above, as well as achieving maximum values in a shorter time constitute to a functional adaptation of the skin vascular bed in response to endurance training. Lower values, both average and maximum ones in both provoking tests, were noted in the group of smoking sportsmen in comparison to non-smokers. This can be explained with a definitely harmful effect of nicotine which impairs the endothelium functions, increases sympathetic activation and causes a loss of so called vascular reservoir (24, 28).

In the tests held we also observed a significant decrease in rest systole in the group of non-smoking sportsmen in comparison to two other groups. Sportsmen's bradycardia is a well-known fact (1, 29), but the causes of these changes are still unclear. A number of possible mechanisms are taken into consideration: a decrease in sympathetic activation, an increase in the effect of sympathetic innervations, changes in the heart conducting system or alterations in electrolytes concentration (26).

Skin microcirculation in sportsmen is not well known and this problem requires further examination.

Conclusions

1. On the basis of the results presented we may draw a conclusion that the functional adaptation of microcirculation under the influence of endurance training manifests itself in higher values of the rest flow, a greater reservoir of the vascular bed and a more efficient reactivity of blood supply to cells to the occlusive and thermal stimuli as well as a lower rest HR. This thesis has to be proven in further studies.
2. The tests proved a harmful effect of nicotine on skin microcirculation. Endurance training does not compensate this harmful effect.

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