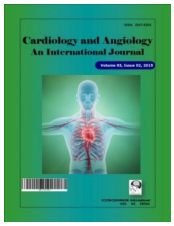

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Effect of Short Systemic Intermittent Hypoxia on Systemic Hemodynamics Blunted in Cutaneous Microcirculation

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Authors' contributions

This work was carried out in collaboration between all authors. Author AP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LP and KC helped at designing the study protocol, managed the analyses of the study results and reviewed the manuscript. All authors have read and approved the final manuscript.

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ABSTRACT

Background: Hypoxic stimulus induces a homeostatic disruption to enhance physiological adaptation. Blood flow in the microcirculation plays an important role in maintaining healthy tissues by delivering oxygen. The cutaneous microcirculations responses to short systemic hypoxia and especially its duration are poorly understood; however the mechanisms of this phenomenon are at the microcirculatory level. The aim of our study was to determine the short systemic intermittent hypoxia's influence on blood flow in skin, local regulatory mechanism fluctuations and changes of systemic hemodynamic parameters in humans.

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Place and Duration of Study: Research was performed in University of Latvia, Institute of Cardiology and Regenerative Medicine, Ojāra Vacieša Street 4, Riga, Latvia, between May 2016 and December 2016.
Methodology: Twelve healthy subjects (n=12, 25.1±2.9 years old) participated in this study. After 20min of acclimatization 10 min of basal resting period in normoxia (FiO2=21%) was recorded. Intermittent hypoxic air breathing was made, corresponding 5 min of acute systemic hypoxic (FiO2=12%) period followed by 5 min of normoxic period, were repeated four times, after hypoxia, 10min of recovery period followed in normoxia. Heart rate variability and systemic hemodynamic parameters and regional blood flow were evaluated. To register skin blood flow laser-Doppler flowmetry was used and evaluation of local factor influences to cutaneous circulation was made by wavelet analysis; fluctuations in the frequency intervals of 0.0095–0.021, 0.021–0.052, and 0.052–0.145 Hz correspondingly represented endothelial, sympathetic, and myogenic activities.
Results: Intermittent acute hypoxia increased systemic hemodynamic parameters, but it didn't significantly change skin blood flow and local regulatory factor activities.
Conclusions: The main findings of study are that intermittent acute hypoxia increase systemic hemodynamic parameters, but didn't change skin blood flow and local endothelial, sympathetic, and myogenic activities.

Keywords: Intermittent systemic hypoxia; microcirculation; spectral analysis; skin blood flow; heart rate variability.

1. INTRODUCTION

Hypoxic stimulus induces a homeostatic disruption to enhance physiological adaptation. Recently suggested hypothesis is that skin microvascular function can mirror the state of microcirculation in other microvascular beds [1, 2], including cardiac muscle [3]. Blood flow in the microcirculation plays an important role in maintaining healthy tissues and organs by delivering oxygen and nutrients [4]. The influence of hypoxia on skin blood flow is poorly understood. It has been suggested that this stimulus causes vasodilation in human skin [5], but the mechanisms of this phenomenon are at the microcirculatory level, such that moderate decrease in oxygen delivery causes dilation of the terminal arterioles, thereby allowing a more homogeneous distribution of oxygen through the capillary network and the diffusion of oxygen to the target tissue [6].

In contrast, it is known that acute exposure to hypoxia evokes changes in local vasodilator and neural vasoconstrictor factors that significantly influences vascular tone. In healthy human studies [7], mild – to – moderate systemic hypoxia doesn't blunt the sympathetic vasoconstriction via α – adrenergic receptors. However, recent evidence suggests that the responsiveness of the sympathetic adrenergic system can be modulated by factors associated with the cutaneous active vasodilator system [8]. During systemic hypoxia, when sympatho – adrenal influence on vascular tone is eliminated, blood flow in the forearm is controlled by local vasodilator mechanisms [9].

A skin has a very compliant circulation, an increase in skin blood flow results in large peripheral displacement of blood volume [10], which could be controlled by local [11] and systemic [12] regulatory factors.

The same effect is observed in cutaneous vasculature [5]. Moreover, when oxygen delivery falls below a critical value, oxygen utilization becomes delivery dependent and decreases in a linear fashion [13]. During normoxia, oxygen is supplied to the tissue mostly by arterioles, whereas in hypoxia, oxygen is supplied to tissues by capillaries through a nitric oxide (NO) concentration – dependent mechanism that controls capillary perfusion and tissue pO_2 [14].

However, control of cutaneous microcirculation is primarily mediated by the autonomic nervous system [15] and these rhythmic variations in cutaneous blood flow are under the influence of autonomic innervation of the skin microvasculature [16].

Vascular tone represents the balance between local vasodilator mechanisms, which attempt to secure adequate blood flow for metabolic demand and neuronal vasoconstrictor reflexes attempting to maintain arterial pressure [17]. The regulation of cutaneous vascular tone has impact on vascular vasomotion and blood volume distribution as a challenge to hypoxia.

Hypoxic vascular responses are not uniform across vascular beds and the mechanisms of hypoxic vasodilation appear to be tissue specific [17]. Nevertheless, the vascular response to hypoxia is mediated by autonomic nervous system [15], but also the endothelial cell lining of blood vessels, as well as the underlining smooth muscle cells independent from endothelium [18] can alert vascular tone in complex ways [17].

The aim of our study was to determine the short systemic intermittent hypoxia's influence on blood flow in skin, local regulatory mechanism fluctuations and changes of systemic hemodynamic parameters in humans.

2. MATERIALS AND METHODS

2.1 Subjects

Twelve subjects (7 male and 5 female) participated in this study (Table 1). Subjects were non-smokers, were not taking any medications, and did not have any cardiopulmonary, neurological, metabolic or peripheral vascular diseases. The subjects were familiarized with the experimental procedures and provided written informed consent according to the Declaration of Helsinki. The study protocol was approved by the Scientific Investigation Ethics Commission of the University of Latvia Institute of Experimental and Clinical Medicine.

2.2 Experimental Condition and Protocol

Procedures were performed while the subject was in sitting position. After 20 min of acclimatization in a quiet temperature-controlled room (25°C), 10 min of basal resting period (R1) in normoxia (FiO_2=21%) was recorded. Intermittent hypoxic air breathing was made, corresponding 5 min of acute systemic hypoxic (FiO_2=12%) period (H1-4) followed by 5 min of normoxic (FiO_2=21%) period (N1-4), were repeated four times. After intermittent hypoxia 10 min of recovery period (R2) in normoxia (FiO_2=21%) followed. The dorsal forearm skin (non-glabrous) blood flow measurement area was 10 cm distally from the elbow joint. Recording was made continuously using laser-Doppler flowmetry (moorLDI2, Moor Instruments, Devon, UK) at a frequency of 40 Hz as it was done in our previous research [19]. Measuring place was heated locally by local heating probe till 33°C (thermo-neutral) and held for whole experiment. Heart rate and RR intervals were registered beat-to-beat, through a heart rate monitor (Polar S810i) with a 1000 Hz sampling frequency, fastened by an elastic band across chest in heart level. Polar S810i is a valid method to record RR interval and to obtain a valid analysis of HRV [20]. Analysis of HRV measurements were conducted with the Kubios HRV Analysis Software 2.1 (MATLAB, the Biomedical Signal Analysis Group, University of Kuopio, Kuopio, Finland). To simulate systemic hypoxia (normobaric hypoxia) a hypoxicator (GO2Altidude, Biomedtech, Melbourne, Australia) which has an air separation system employing semi-permeable membrane technology [21] was used, continuously pumping air at a flow rate of 20 l*min−1 into an air bag which was connected to a facial mask to deliver lower atmospheric O_2 concentration to the subjects (GO2Altitude, Biomedtech, Melbourne, Australia). Gas concentrations in the bag (oxygen mixture at 12%) were monitored by an oxygen sensor (Cambridge Sensotec, Cambs, UK). Arterial blood oxygenation (SpO_2) was recorded online with a pulse oximeter (GO2Altitude, Biomedtech, Melbourne, Australia). Continuous arterial blood pressure, total peripheral resistance and cardiac output were measured with non-invasive Finameter monitoring system (FinameterMIDI, FMS, Amsterdam, Netherlands). Regional blood flow was measured using strain gauge venous occlusion plethysmography (EC6, Hokanson Inc., Bellevue, USA).

2.3 Spectral Analysis

Based on previous researches [19,22-24] spectral analysis of skin blood flow oscillations can be used as a valid tool for evaluation of different local and systemic regulation influences to microvascular bed. To evaluate it was used wavelet analysis; the wavelet transform of each period was calculated. Using frequency bands from previous studies [19,23,24], the frequency spectrum was separated into five frequency intervals: 0.0095 to 0.021 Hz, 0.021 to 0.052 Hz, and 0.052 to 0.145 Hz, which are related to endothelial, sympathetic, and myogenic activity.

2.4 Statistical Analysis

The Kolmogorov–Smirnov test was used to establish normality of the data. The differences between all repeated normoxic and hypoxic episodes were analyzed by ANOVA. Significance was accepted at $P<.05$ and all values are expressed as mean±standard deviation for parametric data or median (25 percentile; 75 percentile) for nonparametric data.

Table 1. Characteristics of study subjects

Parameters			
Subjects			
Total, n		12	
Male, n		7	
Female, n		5	
Age, years		25.1±2.9	
Weight, kg		69.4±11.4	
Height, m		1.74±0.09	
Body mass index, kg/m2		22.8±2.7	
Parameters	**Before IH (FiO_2=21%)**	**After IH (FiO_2=21%)**	**P value**
HR (min-1)	63.6±8.5	64.6±8.8	.383
SBP (mmHg)	123.0±14.5	126.8±13.7	.110
DBP (mmHg)	65.9±10.5	69.1±8.4	.027
MAP (mmHg)	87.3±12.3	91.0±9.8	.137
SV (ml)	100,2±15,0	99.1±18.0	.470
CO (l/min)	6.3±1.7	6.4±1.7	.365
TPR (dyn*s/cm5)	900.3±295.1	907.6±272.8	.204

Values are mean±SD. IH: intermittent acute hypoxia; HR: heart rate; SBP: indicates systolic blood pressure; DBP: diastolic blood pressure, MAP: mean arterial pressure, SV: stroke volume; CO: cardiac output; TPR: total peripheral resistance. P<.05 significance level

3. RESULTS

Table 1 shows anthropometrical data of respondents (males, n=7; females, n=5). Hypoxia significantly (P<.05) increases diastolic blood pressure (DBP); however the other parameters are stable and don't changes (Table 1).

Table 2 shows systemic cardiovascular parameters. Intermittent acute hypoxia significantly increases cardiac output (CO), but after exposure of hypoxia it returns in previous level (R1=6.0±1.5 vs. R2=6.2±1.6; P>.05). Heart rate (HR) increases in hypoxia (Table 2) and its change have the same dynamics as CO; however stroke volume (SV) stays stable in all experiment just significant decrease can be seen in compare to R1 state (Table 2). Systolic blood pressure (SBP) doesn't changes in all experiment; the same can be observed in DBP and mean arterial pressure (MAP). Total peripheral resistance (TPR) has the same tendency as arterial blood pressures. TPR decreases in beginning of the test; however in late phases of experiment its returns in R1 level. After the test TPR increases significantly. Forearm flow (RP), resistance (RVR) and conductance (RVC) are stable in all experiment, except between H3 and R2 phases (Table 2).

Evaluation of HRV data in intermittent acute hypoxia shows, that RR interval significantly (P<.05) changes in time of test, comparing before (R1) and after (R2) normoxia periods (Table 3). In analysis of time-domain parameters (SDNN, RMSSD and SDNN/RMSSD ratio) significant change can be observed just in later phases of intermittent hypoxia (Table 3). Analysis of frequency-domain parameters shows that there are no significant change in parameters in early phase of intermittent hypoxia, but after test half the HF, HFnu decreases and LFnu increases (Table 3). Significant increase can be observed in LF/HF ratio comparing R1 and R2 conditions.

Skin blood flow and spectral analysis of data (Table 4) shows that intermittent acute hypoxia don't change cutaneous blood flow in all experiment; just between H2 and N2 episodes significant change can be observed. There are no significant changes in endothelial (metabolic), neurogenic (sympathetic) and myogenic regulatory activities. However, neurogenic activity is stable in all experiment, but significant change can be observed between H3 and N4 episodes (Table 4).

4. DISCUSSION

The main findings of study are: 1) intermittent acute hypoxia increase systemic hemodynamic parameters, 2) didn't change skin blood flow, and 3) didn't change local regulatory factor activities.

Table 2. Systemic and regional blood flow and resistance

Systemic parameters										
Parameters	**R1**	**H1**	**N1**	**H2**	**N2**	**H3**	**N3**	**H4**	**N4**	**R2**
SpO_2 (%)	97.5±1.2	88.9±2.5*†	96.1±1.6	88.1±2.5*†	96.3±1.1	88.2±2.8*†	96.3±1.4	88.4±2.6*†	97.0±1.1	97.6±1.2
CO (l/min)	6.0±1.5	6.4±1.5*	5.7±1.3*	6.1±1.3	5.8±1.3†	6.3±1.5*	6.0±1.5	6.4±1.6*†	6.1±1.7	6.2±1.6
HR (min^{-1})	62.2±7.5	67.6±9.3*	61.9±7.4	65.8±7.8*	62.0±7.2	67.2±7.6*†	63.8±6.8	67.7±7.3*	64.4±8.4†	63.3±7.8
SV (ml)	95.9±18.0	94.3±17.3	92.7±17.1*†	93.0 ±17.0*	93.1±16.4*†	93.3±15.9*†	94.0±17.1†	94.9±17.4†	94.4±18.3†	97.5±18.0
SBP (mmHg)	122.6±15.1	121.1±14.2	120.0±13.3†	119.6±14.0†	121.2±12.8†	122.2±13.6†	123.9±14.3†	125.2±14.4	125.3±15.7	126.8±14.4
DBP (mmHg)	65.5±10.9	64.6±9.3†	64.9±8.8†	65.3 (63.4; 71.2) †	66.1±9.2†	67.3 (65.8; 72.4) †	69.8 (66.5; 71.1)	71.0 (66.8; 72.0)	71.5 (65.1; 72.8)*	68.9±8.8
MAP (mmHg)	86.9±12.8	85.5±11.1†	85.3±10.5†	85.0±11.4†	86.8±10.7†	87.4±10.8†	90.0 (88.1; 94.6)	91.4 (89.0; 94.7)	92.2 (86.4; 95.8)	90.9±10.3
TPR ($dyn*s/cm^5$)	928.6±291.8	852.9±234.6*†	947.7±279.4	887.2±254.4†	956.8±279.4	885.1±267.8†	944.8±291.6	743.5 (722.1; 985.3) †	945.7±284.1	935.4±267.7
Regional vascular parameters										
Parameters	**R1**	**H1**	**N1**	**H2**	**N2**	**H3**	**N3**	**H4**	**N4**	**R2**
RP (ml/min (100g))	3.26±1.14	3.09±0.99	2.83±1.08	2.91±0.93	2.27 (2.16; 3.48)	3.07±1.06	2.71±0.97	2.86±1.13	2.80±1.13	2.81±0.96
RPR (mmHg* ml*min-1 (100g)-1)	30.5±13.0	31.1±12.2	34.9±15.0	32.3±11.8	34.5±11.5	31.4±10.2†	36.8±13.2	35.6±12.7	36.9±13.8	35.6±10.9
RVC (ml*min-1 (100g) *mmHg-1)	0.032 (0.028; 0.048)	0.032 (0.027; 0.049)	0.034±0.015	0.036±0.015	0.033±0.014	0.036±0.014†	0.031±0.012	0.030 (0.022; 0.036)	0.032±0.015	0.031±0.012

** P<.05 (versus R1), † P<.05 (versus R2), n = 12. R1: baseline in normoxia; H1-4: intermittent acute hypoxias test, hypoxia (FiO_2=12%) each 5 minute episode; N1-4: intermittent acute hypoxias test, normoxia (FiO_2=21%) each 5 minute episode; R2: recovery in normoxia; SpO2: Haemoglobin saturation with oxygen (%); CO: cardiac output (l/min); HR: heart rate (min-1); SV: stroke volume (ml); SBP: systolic arterial blood pressure (mmHg); DBP: diastolic arterial blood pressure (mmHg); MAP: mean arterial blood pressure (mmHg); TPR: total peripheral resistance (dyn*s/cm5); RP: regional (forearm) blood flow, (ml/min (100 g)); RPR: regional (forearm) peripheral resistance (mmHg* ml*min-1 (100 g)); RVC: regional (forearm) vascular conductance (ml*min (100 g)*mmHg-1)*

Table 3. RR interval and heart rate variability

	Time-domain			
	RR interval (ms)	**SDNN (ms)**	**RMSSD (ms)**	**SDNN/RMSSD**
R1	975.0±137.1	82.0±30.3	82.2±54.5	1.20±0.46
H1	938.3±141.0*	76.6±25.4	74.7±49.6	1.22±0.40
N1	925.3±155.0*	80.1±31.4	75.2±54.5	1.37±0.60
H2	961.2±141.9	79.8±31.3	61.9 (44.3; 97.4)	1.15±0.32
N2	939.5±150.4*	80.1±33.0	51.0 (40.6; 138.3)	1.29±0.58
H3	945.0±120.8	84.9±31.6	77.8±52.1	1.17 (0.82; 1.48)
N3	918.7±141.3*†	68.7±24.3*	67.7±51.2*	1.27±0.50†
H4	923.5±113.9*†	70.8±24.1	68.7±49.8	1.25±0.51
N4	872.6±124.9*†	61.9±19.9*	55.7±37.4*†	1.31±0.41†
R2	947.5±138.0	66.6 (54.2; 73.4)	55.4 (36.6; 76.1)	1.20±0.36
	Frequency-domain			
	LF (ms^2)	**HF (ms^2)**	**LF nu(n.u.)**	**HF nu(n.u.)**	**LF/HF**
R1	1172.1 (846.7; 3883.3)	3530.2±3753.4	46.3±28.1	53.7±28.1	0.69 (0.40; 2.98)
H1	1776.3 (779,8; 3557.0)	1480.1 (776.0; 5724.7)	45.7±25.8	54.3±25.8	0.94 (0.36; 1.26)
N1	2115.8±1542.5	1651.6 (509.4; 5829.3)	48.7±27.8	51.3±27.7	1.12 (0.31; 2.62)
H2	1818.3±1300.8	1760.3 (987.9; 4296.2)	41.4±22.0	58.6±22.0	0.76 (0.30; 1.39)
N2	1823.5±1346.5	1784.1 (705.8; 7368.2)	44.3±22.4	55.7±22.4	1.13±1.00
H3	1898.0±1340.7	3267.1±3951.9	46.9±26.7	53.1±26.6	0.88 (0.43; 3.07)
N3	1552.1±1086.9	1598.4 (441.1; 4317.6)	47.9±27.1	52.1±27.1	0.79 (0.41; 3.35)
H4	1787.5±1375.7	1664.6 (547.9; 3236.7)	46.4±27.6	53.6±27.6	0.94 (0.36; 2.54)
R2	1822.8±1000.2	1751.2 (562.1; 3047.8)	50.6±25.4	49.4±25.4	0.83 (0.70; 3.41)*

**P<.05 (versus R1), †P<.05 (versus R2); R1: baseline in normoxia; H1-4: intermittent acute hypoxias test, hypoxia (FiO_2=12%) each 5 minute episode; N1-4, intermittent acute hypoxias test, normoxia (FiO_2=21%) each 5 minute episode; R2: recovery in normoxia; SDNN: the standard deviation of normal to normal RR intervals; RMSSD, the root mean square differences of successive RR intervals; SDNN/RMSSD, ratio; LF: low frequency; HF: high frequency; LF nu: low frequency (normalized units); HF nu: high frequency (normalized units); LF/HF, ratio; n=12*

Intermittent systemic hypoxia increases systemic hemodynamic parameters (Table 2), which results in significant cardiac output and heart rate increase, even more – this phenomenon changes in the same pattern as intermittent hypoxias phases and it is presented during all experiment. It is known that the autonomic nerve system (ANS) plays a crucial role in modulation of the oscillatory behavior of the cardiovascular system [25]. Fluctuations of cardiac output are dependent on two parameter relationship: heart rate and stroke volume. The change in heart rate is mediated by autonomic influence. The baroreceptor and chemoreceptor reflexes markedly influence autonomic control of circulation, especially in situations involving stimuli such as marked changes in blood pressure or O2 concentration [26]. Our data (Table 2) suggest that fast adaptation of cardiac output is mediated by change of heart rate, because stroke volume was unchanged during all experiment.

Increase in heart rate could be explained by increased sympatho-vagal tone [27], which provides adaptation to different stressors. The autonomic influence to heart was evaluated by heart rate variability (HRV) [28]. It is proposed in

literature that acute hypoxia impacts cardiac ANS activity, with reactions determined by the intervening of O2 concentration, i.e., exposure to FiO2=12% increases resting HR by enhanced sympathetic tone [29], which corresponds with our previous data [19], and suppressed parasympathetic activity [29]. However, our intermittent acute hypoxia data (Table 3) sowed, that this signal didn't change the sympatho-vagal tone during hypoxic test; this observations are supported by other researchers [28]. This could be explained by short exposure to acute hypoxias stimulus [28], which didn't cause significant change in internal environment. However, the change of HR must be due to increased activity of ANS, specifically sympathetic nervous system. Other reasonable explanation of lack of sympathetic activity is due to HRV analysis sensitivity; because HRV represents more parasympathetic tonus [30]. It is suggested that cardiac vagal activity is the predominant contributor of HF component. The LF rhythm mainly reflects baroreflex function of the heart [31] and the LF/HF ratio mirrors the sympatho-vagal balance, so just reflects sympathetic modulations [25], but not direct systems influence. However, our data (Table 3) suggest that there is observed significant sympathetic effect on heart, which was caused by intermittent hypoxia, just comparing the LF/HF ration before (R1) and after (R2) the test itself, but still the data doesn't explain the fluctuation phenomena of HR in time of intermittent hypoxias test. Our suggestion is that this change of HR is caused by ANS, but the regulatory effect was not represented in HRV change.

Observed increase in cardiac output or in other words increase of total blood flow to body is blunted in peripheral tissue, because our data shows that there was not increase in forearm blood flow (Table 2) or cutaneous blood flow (Table 4). Only reasonable explanation is that this induced physiological adaptation processes was executed in redistribution of blood flow toward the organs with the greatest metabolic needs [32] like brain, heart or respiratory muscle vascular beds. However, the major functional rearrangements in response to acute or intermittent hypoxia are an activation of external respiration, heart functions, and vascular shifts [33].

The microvasculature responds to external factors and change in internal environment of the body as integral system [33]. As a rule, shift occurring at a certain site reflects the changes in microvessels of the entire systemic circulation [33], which agrees with widely accepted hypothesis, that skin microvascular function can mirror the state of microcirculation in other microvascular beds [1,2], including cardiac muscle [3]. It is known, that in microcirculation level exist spatial heterogeneity in blood flow, which could be explained by different oxygen requirements of the tissues [34]. Our previous data [19] supported hypothesis that acute continuous hypoxia changes skin blood flow; however data of intermittent acute hypoxia (Table 4) didn't support that observations. This leads us to conclusion, that acute intermittent hypoxia doesn't changes the skin blood flow, and effect of hypoxic stimulus is dependent of hypoxias duration and type. However, it is possible that local tissue regions with higher oxygen consumption are served by vessels with higher blood flow, thereby providing a balance between oxygen supply and oxygen

Table 4. Skin blood flow and spectral analysis

	Skin blood flow (PU)	Endothelial activity (PU^2/Hz)	Neuronal activity (PU^2/Hz)	Myogenic activity (PU^2/Hz)
H1	65.1±32.2	3.6 (1.0; 7.0)	3.6 (2.3; 12.0)	4.0 (2.6; 10.6)
N1	66.2±30.8	6.4 (1.8; 16.1)	4.4 (2.8; 14.0)	3.3 (2.4; 9.2)
H2	64.5±24.1*	6.3 (2.4; 8.6)	5.5 (2.5; 13.5)	4.5 (2.8; 13.7)
N2	67.5±26.0	4.9 (3.2; 9.5)	4.5 (2.9; 13.8)	4.2 (2.3; 9.2)
H3	70.3±24.6	3.0 (1.6; 5.7)	6.6 (3.0; 15.7)†	4.9 (3.7; 14.0)
N3	72.6±26.2	4.7 (2.7; 10.6)	6.3 (4.0; 12.7)	4.2 (3.3; 9.8)
H4	69.3±21.1	3.7 (2.1; 11.0)	5.9 (5.4; 14.1)	7.2 (3.1; 8.9)
N4	70.2±24.1	4.1 (2.1; 9.3)	5.9 (2.7; 8.4)	4.8 (2.5; 10.5)

**P<.05 (H2 versus N2); †P<.05 (H3 versus N4). n=12. H1-4, intermittent acute hypoxias test, hypoxia (FiO_2=12%) each 5 minute episode; N1-4, intermittent acute hypoxias test, normoxia (FiO_2=21%) each 5 minute episode*

demand [34], which caused larger change in blood support to areas of increase metabolic demands. In thermoneutral conditions, skin blood flow is 300-500 ml/min, it is about 10% from total body's blood flow; however, it can vary in large amplitudes depending on different stress stimuli [10]. The intermittent acute hypoxia was not long enough in thermoneutral resting conditions to cause large disturbance in oxygen supply and demand to skin tissues.

It is known, that microvasculature plays the key role in the complex compensatory adaptive responses of the body to various regimes of hypoxic stimulation [33]. The increased CO was divided to periphery and caused total peripheral resistance (TPR) fluctuation (Table 2) in the same pattern as intermittent hypoxias phases, but mean arterial pressure (MAP) didn't change in all experiment. This resistances change could be controlled via ANS, to compensate CO fluctuations and to stabilize MAP. Control of cutaneous microcirculation is primarily mediated by the sympathetic nervous system [15]. The rhythmic variations in cutaneous blood flow are made by autonomic innervation of the skin microvasculature [16]. If there was ANS activity's change it could be seen in cutaneous neuronal regulation. The spectral analysis of skin blood flow is useful tool to non-invasively evaluate endothelial, sympathetic and myogenic regulatory activities [35]. The neurogenic and myogenic tones must be evaluated as inversely proportional to the amplitudes of the neurogenic and myogenic rhythms in microvessels [36]. The hypothesis was that challenge to intermittent hypoxia could cause fluctuations in output of ANS to skin. It is known, that during resting and thermoneutral conditions the skin is under tonic vasoconstrictor influence [10]; however the effect of acute hypoxic stimulus is still unclear. Our regional and skin blood flow data (Table 2; Table 4) and systemic hemodynamic (CO and TPR) data (Table 2) were controversial. The change of spectral power in neurogenic frequency band (0.02-0.06 Hz) would support assumption that increased neurogenic activity would compensate increased systemic hemodynamic caused flows generated pressure on the blood vessel walls, but our data (Table 4) didn't show any change in sympathetic tonus to skin.

On the other hand the skin blood flow can also be modulated by non-neurally mediated events, one of which may be vascular cutaneous autoregulation [37] which is executed by local factors. The vascular responses to hypoxia are mediated by the endothelial cell lining of blood vessels as well as the underlining smooth muscle cells, independent of the endothelium [18]. Our spectral analysis data (Table 4) allowed us to test the other hypothesis: skin's unresponsiveness to increase of systemic hemodynamics could be mediated by local factors; however, myogenic and endothelial activities (Table 4) were not change in our experiment. This phenomenon, lack of skin's response to increased hemodynamic challenge could not be explained by our results; never less, the idea of physiological adaptation processes, which could be executed in redistribution of blood flow toward the organs with the greatest metabolic needs [32] could be the reason of it.

Our results may be clinically relevant to understand the regulatory mechanisms of microvasculature, specifically of skin in short term intermittent hypoxic conditions. It could give a deeper incites in adaptation mechanisms of vascular bed of periphery and its connection with systemic hemodynamic.

Finally, it could be pointed out that the small sample size (n=12) was the reason for limited interpretation of gender specific influence on local vascular blood flow and its regulation. However, in literature exists controversial data for gender specific influence on vascular reactivity in small conduit artery level [38] and cutaneous skin in local cooling [39] or acetylcholine induced-vasodilation [40]. Second limitation was short time (5 min x 4 times) of intermittent hypoxic exposition, it is well known that time and degree of hypoxia can influence vascular responses. Another limitation is local regulatory factor evaluation just with spectral analysis in normoxic and acute hypoxic conditions without provocation tests. To simplify the evaluation of response of skins vascular bed there was not used any kind of vasoactive drug (acetylcholine or sodium nitroprusside) or provocative physiological tests (local cooling, heating, or occlusion) to stimulate local vascular reactions. This kind of tests could give us deeper incites in local adaptive mechanisms.

5. CONCLUSION

In conclusion, short term intermittent systemic hypoxia causes sympathetic stimulation to heart which results in increased heart rate and larger

cardiac output which could be the fastest adaptation to hypoxic stimulus, however, the blood flow and local regulatory mechanisms was not changed in so short hypoxic conditions.

CONSENT

The subjects were familiarized with the experimental procedures and provided written informed consent according to the Declaration of Helsinki. All authors declare that 'written informed consent was obtained from the participants (or other approved parties) for publication of this research paper.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and by the Scientific Investigation Ethics Commission of the University of Latvia Institute of Experimental and Clinical Medicine and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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