

Title: Four Weeks of Hypoxia Training Improves Cutaneous Microcirculation in Trained Rowers

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Short title: Hypoxia training improves cutaneous microcirculation

Summary

Hypoxia training can improve endurance performance. However, the specific benefits mechanism of hypoxia training is controversial, and there are just a few studies on the peripheral adaptation to hypoxia training. The main objective of this study was to observe the effects of hypoxia training on cutaneous blood flow (CBF), hypoxia-inducible factor (HIF), nitric oxide (NO), and vascular endothelial growth factor (VEGF). Twenty rowers were divided into two groups for four weeks of training, either hypoxia training (Living High, Exercise High and Training Low, HHL) or normoxia training (NOM). We tested cutaneous microcirculation by laser Doppler flowmeter and blood serum parameters by ELISA. HHL group improved the VO_{2peak} and power at blood lactic acid of 4 mmol/l (P_4) significantly. The CBF and the concentration of moving blood cells (CMBC) in the forearm of individuals in the HHL group increased significantly at the first week. The HIF level of the individuals in the HHL group increased at the fourth week. The NO of HHL group increased significantly at the fourth week. In colusion, four weeks of HHL training resulted in increased forearm cutaneous blood flow and transcutaneous oxygen pressure. HHL increases rowers' NO and VEGF, which may be the mechanism of increased blood flow. The increased of CBF seems to be related with improving performance.

Keywords: hypoxia training, NO, VEGF, microcirculation, CBF

Introduction

Elite athletes have utilized hypoxia training for several years to improve performance (Dufour *et al.* 2006, Sinex and Chapman 2015). Hypoxia training is conducted by nitrogen dilution or oxygen filtration, simulating high altitudes with low oxygen. Athletes use many different methods of hypoxia training, (Living High and Training High, Living Low and Training High, Living High and Training Low, and similar methods), to optimize performance (Wilber 2007). In order to maintain training intensity and experience enough hypoxia stimulation, Living High, Exercise High and Training Low (HHL) is considered a superior method for elite athletes. HHL is a modified training program to increase body tolerance to hypoxia and strengthen cardiopulmonary functions (Chapman *et al.* 1998, Xu *et al.* 2015).

However, the objective benefits of hypoxia training are controversial (Gore *et al.* 1998, Henderson *et al.* 2001, de Paula and Niebauer 2012). The original method of hypoxia training was designed to increase erythrocyte volume and ultimately to enhance sea level peak oxygen uptake (VO_{2peak}) and endurance performance (Gore *et al.* 2006, Garvican-Lewis *et al.* 2013, Ramos-Campo *et al.* 2015). Indeed, the acclimatization to high altitude results in central and peripheral adaptations that improve oxygen delivery and utilization (Levine and Stray-Gundersen 1997, Garvican-Lewis *et al.* 2013). However, there is substantial evidence showing that improvements in endurance performance at sea level can be obtained through altitude exposure via nonhematological mechanisms, including increases in mitochondrial efficiency, muscle

pH buffer capacity, and mitochondrial uncoupling protein content within the skeletal muscle (Gore *et al.* 2007).

The microcirculation is associated with hypoxia adaptation (Ovadia-Blechman *et al.* 2015, Treml *et al.* 2018). Hypoxia causes cutaneous vasodilation and robust increase in cutaneous blood flow in healthy humans (Simmons *et al.* 2007, Lawley *et al.* 2014). Intermittent hypoxia training had greater positive effect on hemodynamics, microvascular endothelial function, and work capacity of untrained healthy men (Shatilo *et al.* 2008). However, the issue of how a hypoxia stimulus influences the microcirculation and microcirculatory blood flow is still debating and under investigated (Rowell *et al.* 1982, Simmons *et al.* 2007, Siebenmann *et al.* 2017, Treml *et al.* 2018). Pappas *et al.* found that acute systemic hypoxia causes sympathetic stimulation to heart which results in increased heart rate and larger cardiac output, and which could be the reason of forearm skin blood flow increase in acute systemic hypoxia (Pappas *et al.* 2015). One might assume—as also written in the scientific literature—that in hypoxia a decrease in oxygen delivery to the cells resulted in a vasodilation of the capillaries in order to enhance blood flow in the microcirculation (Delashaw and Duling 1988). The role of the cutaneous microcirculation is to provide the supply of oxygen and various nutrients and to remove waste products of muscle metabolism. Acute systemic whole-body hypoxia leads to increased diameters of arterioles and venules due to the action of adenosine mediated by nitric oxide (NO), which is one of the endothelium-dependent relaxing factors released by the vascular endothelium and mediates vasodilation. Muscle in chronically hypoxic rats had larger

diameters of capillaries and higher capillary density, which helped maintain oxygen supply to muscle fibers despite low values of oxygen in arterial blood (Hudlicka 2011). Also, vascular endothelial growth factor (VEGF) plays a vital role in the growth and differentiation of vascular cells as well as lymphatic endothelial cells in hypoxia condition (Bloor 2005, Vital *et al.* 2014, Xie *et al.* 2014, Boos *et al.* 2018).

Some studies have found the augment of cutaneous blood flow in hypoxia, but data in trained athletes and effect of prolonged hypoxia accommodation are sparse. Therefore, the purpose of this study was to conduct a controlled study of trained rowers training in hypoxia to examine whether four-week hypoxia training could improve the cutaneous microcirculation and the underlying mechanisms. We hypothesized that hypoxia training could improve the rowers' cutaneous microcirculation by vasodilation and angiogenesis.

Materials and methods

Subjects

Twenty male rowers who had no altitude or hypoxia training experience were recruited from the Shanghai Rowing Team, and divided into either the HHL group (HHL, n = 12) or the normoxia training group (NOM, n = 12, but four rowers quit because of injuries). Participant characteristics are presented in Table 1. The study protocol was approved by the Ethics Commission of Shanghai University of Sport. All participants provided written informed consent before participating. This study is in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

Training program

The participants used the HHL/NOM program for four weeks. HHL group slept daily

and trained six days per week in normobaric hypoxia (2500 m-3000 m). Additionally, they did sprint training three times per week at sea level. The hypoxic condition was achieved by TOSMA (Berlin, Germany). NOM group slept and trained daily at sea level. The training volume and intensity of the two groups were similar (see Table 2).

Experimental protocol

Forearm and leg cutaneous blood flow (CBF) was monitored using a laser Doppler flowmeter (PeriFlux6000, Perimed, Sweden) at room temperature (22°C) with the subject in a lying position. We tested the forearm and leg blood flow and also the blood flow when exposed to localized heating to 44°C for 3 min. Microvascular reactivity (MVR) was evaluated from the maximal post-occlusive reactive hyperemia (PORH) following 3-min forearm ischemia produced by cuff inflation (200 mm Hg). Similar procedures have been used by other investigators (Lenasi and Strucl 2004, Shatilo *et al.* 2008, Tew *et al.* 2010). Blood pressure was measured by brachial auscultation. SPO₂ and heart rate were measured by a handheld pulse oximeter (NONIN, 9500, USA). The blood flow was measured four times: baseline, at the end of the first week, the third week, and post-HHL.

Measurement of VO_{2peak} and maximum power output

VO_{2peak} tests were conducted before and after the intervention using an incremental rowing ergometer (Concept 2 Model D, USA) protocol via a portable metabolic analyzer (COSMED K5, Italy). Participants started the protocol with 4-minute stages till exhaustion. The test began with each participant exercising at a 500 m split time of 1 min 56 s. Thereafter, the split time was decreased by 4 s each stage until the participant reached volitional exhaustion. The 500m split time of each stage has been calculate to

watt in table 3. When the participant reached exhaustion, the max power output (MPO) was recorded.

Measurement of P₄

Subjects performed an incremental test of 5 stages, each of 4-minute duration on rowing ergometer (Concept 2 Model D, USA). The 4-minute stages were separated by 30-second breaks for blood sampling to test blood lactic acid (EKF c-line, Germany). The power at blood lactic acid of 4 mmol/l(P₄) is calculate by quadratic polynomial (Microsoft Excel, USA) (Pardono *et al.* 2008, Gregg *et al.* 2010). The polynomial trend line was used because of the high correlation coefficient of determination (r-squared value) between the data and trend line.

Measurement of blood serum

Ten milliliter of blood was collected four times, before HHL, at the second week, at the fourth week, and post HHL. Serum samples were stored at –80°C for later analysis. Hypoxia-inducible factor (HIF), NO, and VEGF were determined with ELISA kits (Wksu-BIO, China) on a Multiskan FC (Thermo, USA). To ensure accuracy, all blood parameters for ELISA used duplicate tubes. The average intra-assay CV (%) is 3.8% and average inter-assay CV (%) is 6.2%. All results were calculated using the Skant software (Windows, 5.0).

Timing of measurements

During one training cycle (1 week), the rowers trained from Monday to Saturday morning, and rest on Saturday afternoon and Sunday. So, the blood test was performed on Monday morning, namely after 1 day and a half rest. We tested blood flow all day on Sunday, because 20 subjects need a long time to complete the test.

Data analysis

Laser Doppler data were stored in a computer for offline analysis with signal-processing software (Perisoft for Windows 2.5.5; Perimed AB). Skin blood flow responses were defined as follows: (1) baseline, i.e., the arithmetic mean of the fifth and sixth minutes, perfusion unit (PU); (2) the lowest and highest blood flow of PORH; (3) plateau, i.e., the arithmetic mean of the last 2 min of heating at 44°C; and (4) skin blood flow data (recorded in arbitrary perfusion units; PU). The baseline perfusion unit was divided by the mean arterial pressure (in millimeters of mercury) to give cutaneous vascular conductance (CVC; in PU per millimeter of mercury) (Lawley *et al.* 2014).

Data were analyzed using SPSS 25.0 (IBM). The Kolmogorov–Smirnov test was used to establish normality of the data. The differences between HHL and NOM were analyzed using a general linear model for repeated measures (normally distributed values) or linear mixed model for repeated measures (abnormally distributed values). The interaction of group by time was evaluated to examine time effect in responses to the intervention. And different times in HHL/NOM were analyzed using a Wilcoxon signed-rank test for abnormally distributed data. Significance was accepted at $P < 0.05$, with a trend of significance at $0.05 < P < 0.1$. All normally distributed values are expressed as mean \pm standard deviation, and all abnormally distributed values are expressed as the interquartile range, median (P_{25} , P_{75}). For effect size, the partial eta squared statistic was calculated. and 0.01, 0.06, and 0.14 were interpreted as small, medium, and large effect sizes, respectively(Richardson 2011).

Results

In order to establish that HHL can improve participants' cutaneous microcirculation,

we tested the blood flow of the forearm and thigh and some blood indicators of vessel vasodilatation and angiogenesis.

As shown in Table 4, general linear model with repeated measurement variance analysis showed a significant time by group interaction on VO_{2peak} ($p = 0.017$, $\eta^2 = 0.136$), P_4 ($p = 0.157$, $\eta^2 = 0.115$), MPO ($p = 0.253$, $\eta^2 = 0.048$). So, the hypoxia intervention gave a large-medium effect on VO_{2peak} and P_4 . HHL group improved the VO_{2peak} significantly (5553.1 ± 457.1 vs. 6217.0 ± 463.6 ml/min), $p < 0.01$. However, the NOM group showed little change of VO_{2peak} (4984.9 ± 498.3 vs. 5134.8 ± 788.3 ml/min), $p = 0.677$. The HHL group improved the MPO significantly (342.9 ± 15.8 vs. 358.4 ± 18.5 watt, $p = 0.039$), but NOM showed little change of maximum power output (316.6 ± 23.5 vs. 322.4 ± 35.5 watt, $p = 0.52$). And also, P_4 appeared in a similar trend. The HHL group improved the P_4 significantly (262.8 ± 12.5 vs. 281.5 ± 12.8 watt), $p < 0.01$. P_4 of NOM group increased from 233.0 ± 25.9 to 241.9 ± 36.4 watt, $p = 0.157$.

As shown in Table 5, there was a statistically significant difference in SPO_2 and transcutaneous oxygen pressure ($TcPO_2$) between groups. This was identified as determined by repeated measure linear mixed models, $p < 0.01$. Two related samples in a non-parametric test showed that blood flow and concentration of moving blood cells (CMBC) in the forearm of HHL subjects increased significantly at the first week, but these parameters in the thigh and the NOM group did not increase. The lowest and highest blood flow of PORH both increased at the first week ($0.05 < p < 0.1$). At the third week, the HHL group's resting blood flow and CMBC of the forearm were lower

than in the first week but higher than pretraining. The velocity of blood flow decreased at the first week.

As shown in Table 6, erythropoietin (EPO) and HIF of HHL subjects increased at the second week ($p > 0.05$), and HIF of HHL subjects also increased at the fourth week ($0.05 < p < 0.1$). The NO of HHL subjects increased at the fourth week significantly, while endothelial NO synthase (eNOS) did not show significant changes. Prostaglandin I₂ (PGI₂) of HHL subjects also increased during HHL but not significantly. Nevertheless, this PGI₂ may help to increase the blood flow. Endothelin (ET) of HHL increased at the second week ($p > 0.05$). At the fourth week and post-training, VEGF in the HHL group had a higher value compared to the baseline ($0.05 < p < 0.1$), but VEGF receptor (VEGFR) in the HHL group showed little change during hypoxia training.

Discussion

The purpose of this study was to investigate whether four weeks of HHL can improve participants' microcirculation function, EPO levels, and red blood cell levels. The primary findings of this study are the following. (1) HHL improves blood flow and forearm TcPO₂. (2) Importantly, at the different stages of HHL training, the function of microvessels differs, suggesting that HHL can improve vasodilation and increase angiogenesis, but participants need a long time to experience enough hypoxia in HHL. (3) HHL increases rowers' NO and VEGF, which may be the mechanism of increased blood flow.

These results suggest that at the early stage of HHL, vasoconstriction may be dominant.

However, when rowers experience more and more hypoxia, vasodilation and angiogenesis may play a key role in their skin blood flow. NO, ET, PGI₂ and VEGF are all related to vascular function and angiogenesis.

The principal finding of our study is that the effect of 4 weeks of HHL on microvascular function has a timing effect. The effect of HHL on microcirculation may be dependent on two factors, hypoxia and training. There have been controversies about the change in the microvascular function (vasoconstriction and/or vasodilation) during hypoxia. Some reports have demonstrated in untrained healthy men that intermittent hypoxia training had significant positive effects on cutaneous perfusion and microvascular function (Shatilo *et al.* 2008). Paparde *et al.* demonstrated that acute hypoxia (PO₂ = 12%) increases forearm skin blood flow, but the reason for this may be that sympathetic stimulation to the heart results in an increased heart rate and more massive cardiac output. Heart rate variability suggests that there are significant increases in sympathetic responses in acute hypoxia. So, blood flow increases despite vasoconstriction (Paparde *et al.* 2015). Simmons *et al.* have suggested that increased adrenalin production does not inhibit the vasodilation of blood vessels in hypoxia (Simmons *et al.* 2007). This is consistent with the results of ET and HR in our study. Although ET increased at the second week, blood flow also increased, which may be due to increased eNOS and PGI₂. We found similar results from rowers after four weeks HHL training, but only in their forearms. This is because the thighs are the primary working muscle of rowers, and they may be affected by training status and fatigue. Also, PORH reserve capacity is an indication of endothelial function (Lenasi and Strucl 2004).

In this study, we found that HHL rowers increase their PORH reserve capacity, which means that their endothelial function is improved by hypoxia training. Montero *et al.* found that microvascular dilator function of endurance athletes was enhanced more than that of age-matched untrained healthy subjects (Montero *et al.* 2015). Maione *et al.* reported oxygen uptake kinetics during the constant load exercise sub-anaerobic threshold are highly sensitive to endothelial function in muscular microcirculation (Maione *et al.* 2015). Therefore, it is speculated that long-term endurance training may improve microvascular health. This is consistent with Boegli's study, as he found that improved microvascular health is induced by NO (Boegli *et al.* 2003). Bloor *et al.* have suggested that exercise and training can increase angiogenesis by VEGF, but the inducing factor may be HIF because exercise can also upregulate VEGF and HIF transcription (Bloor 2005). Gustafsson *et al.* took muscle biopsy samples from subjects after single leg movements and found VEGF mRNA expression was significantly raised and was related to HIF mRNA. So, exercise results in muscle ischemia and hypoxia, which induce HIF and increase VEGF expression (Gustafsson *et al.* 2002). Therefore, HIF may be the core factor in NO and VEGF expression during hypoxia and exercise. Nitric oxide, and VEGF play a significant role in the regulation of baseline, evoking blood flow during hypoxia (Takuwa *et al.* 2010, Shafiqhi *et al.* 2012). Viboolvorakul *et al.* found that eight weeks of exercise training improved microvascular changes and hypoperfusion closely associated with the upregulation of VEGF and eNOS (Viboolvorakul and Patumraj 2014). Asano *et al.* found the timing effect of serum VEGF at high altitude (1886 m), where VEGF decreased ten days after the start of

altitude training, but reached a peak 19 days later (46.0 ± 14.6 pg/ml) (Asano *et al.* 1998). Their result of VEGF is higher than that observed in our study, which may be because of the difference between altitude and simulated hypoxia (HHL). However, Morici *et al.* found that people living at sea level had lower plasma VEGF 3–4 days after arriving at 5050 m (Morici *et al.* 2013). It is generally accepted that long-term hypoxia exposure can increase the density of capillaries, which can lead to a maximum diffusion of oxygen. Of course, different hypoxia levels may also be essential for angiogenesis.

Our results are consistent with the hypothesis that hypoxia training improves rowers' cutaneous microcirculation, and the mechanism may be vasodilation and angiogenesis, related to NO and VEGF. However, the benefit has a timing effect, and changes in functionality and mechanisms are not synchronized. Based on NO and VEGF, vasodilatation and angiogenesis appear to persist until the end of hypoxia training.

In conclusion, in addition to the traditional research on the hematologic system in hypoxia training, we have found that four weeks of HHL training increases forearm blood flow and improves endothelial function. HHL increases rowers' NO and VEGF, which may be the mechanism of increased blood flow. The increased of CBF seems to be one mechanism for improving performance. However, more studies are needed to confirm this.

Limitations

The study documents a practical example of 4 weeks of HHL use by elite rowers and includes a control group. However, the number of control group presents a limitation, which is 4 rowers' quit because of injury. Although we tried to manage the difference

in training between HHL and NOM, training is one key factor influencing blood flow, NO, VEGF, and related markers. One of the limitations about the study is lack of hypoxia group (without training) to interpret the training factor. The other Limitation of the study is lack of traditional hematological data used to describe the course of adaptation to the hypoxic training in rowers. In the future, we will expand our research scope and observation in our coming studies.

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Author Contributions Statement

Zhijun Meng wrote the paper. Binghong Gao reviewed the paper. Zhijun Meng and Huan Gao did most of the experiments. Peng Ge, Tao Li and Yuxin Wang did some of works.

Conflict of Interest Statement

None declared.

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Table 1 Characteristics of study participants

Group	HHL	NOM
N	12	8
Age, years	18.7 ± 2.0	16.2 ± 1.3
Height, cm	185.9 ± 5.2	188.3 ± 6.2
Mass, kg, pre	77.8 ± 6.1	78.7 ± 13.4
Mass, kg, post	75.6 ± 6.3	77.9 ± 13.2
Body fat, %, pre	12.4 ± 3.1	19.1 ± 4.9
Body fat, %, post	10.6 ± 3.6	17.5 ± 5.2

Table 2 Hypoxia training (HHL) and normoxia training (NOM) program

Group	Sleeping	Training	Training	Objectives of Training
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		altitude (m)	altitude (m)	volume (km)	
Week 1	HHL	2500	2500	262	Adapt to hypoxia training, large training volume
	NOM	100	100	250	Endurance training to improve aerobic capacity
Week 2	HHL	3000	3000	260	Maintain training volume
	NOM	100	100	252	Maintain training volume
Week 3	HHL	3000	3000	245	Increase training intensity
	NOM	100	100	238	Increase training intensity
Week 4	HHL	3000	3000	190	Decrease training volume and prepare for test after intervention
	NOM	100	100	225	Decrease training volume and prepare for test after intervention

Table 3 Incremental rowing ergometer protocol

Stage	Time (min)	Power (Watt)	Stroke rate (per minute)
1	4	224	18
2	4	249	20
3	4	278	22
4	4	311	24
5	4	350	26
6	4	396	28

Table 4 The VO_{2peak} , MPO and P_4 of rowers

	HHL		NOM		IE	ES
	baseline	post	baseline	post		
VO_{2peak} (ml/min)	5553.1 ± 457.1	6217.0 ± 463.6*	4984.9 ± 498.3	5134.8 ± 788.3	0.017	0.136
MPO (watt)	342.9 ± 15.8	358.4 ± 18.6*	316.6 ± 23.5	322.4 ± 35.5	0.253	0.048
P_4 (watt)	262.8 ± 12.5	281.5 ± 12.8*	233.0 ± 25.9	241.9 ± 36.4	0.442	0.115

* $p < 0.05$ versus baseline; # $0.05 < p < 0.1$ versus baseline

MPO: maximum power output. IE: time by group interaction effect. ES: effect size

Table 5 Characteristics of microcirculation-related parameters

	HHL				NOM				IE	
	Baseline	week 1	week 3	Post-HHL	Baseline	week 1	week 3	Post-NOM	<i>p</i> value	
	Heart rate (beats/min)	61.3 ± 10.9	58.0 ± 6.6	54.9 ± 5.7#	55.5 ± 3.9#	69.5 ± 7.6	60.0 ± 8.0	62.4 ± 8.5*	57.0 ± 4.4*	0.012
	SPO ₂ (%)	98.2 ± 0.6	95.5 ± 1.3*	94.8 ± 1.1*	98.7 ± 0.7#	99.3 ± 0.9	100.0 ± 0.0	97.9 ± 0.4*	98.9 ± 0.5	0.001
Forearm	Resting (PU)	8.9 (7.0, 12.8)	13.0 (8.0, 15.0) *	9.3 (7.7, 10.5)	8.8 (7.4, 10.8)	7.6 (6.6, 11.4)	8.4 (6.8,10.0)	8.0 (7.1, 15.0)	11.0 (6.5, 18.0)	0.278
	CMBC	112.0 (75.3, 142.0)	151.0 (105.0, 159.0) *	124.5 (83.5, 145.8)	98.5 (83.8, 108.5)	121.5 (73.3, 166.8)	87.0 (78.0,96.0.)	118.0 (84.0, 136.0)	108.0 (77.0, 113.0)	0.002
	Velocity	9.2 (7.5, 12.0)	9.1 (6.1, 11.0)	8.2 (7.6, 10.3)	9.1 (7.9, 10.7)	8.0 (6.8, 9.2)	10.1 (9.1,11.0)	9.5 (7.6, 9.8)	9.6 (7.9, 18.0)	0.499
	TcPO ₂	81.0 (68.3, 91.3)	71.0 (66.0, 74.0) *	68.0 (59.8, 75.0) #	96.5 (80.8, 110.0) *	91.2 (78.9, 104.3)	92.0 (87.0,97.0)	80.0 (74.0, 84.0)	79.0 (76.0, 95.0)	0.001
	Heat (PU)	113.9 (92.5, 118.0)	112.0 (76.0, 212)	110.5 (86.3, 120.5)	117.5 (104.3, 144.8)	128.5 (92.5, 189.0)	107.5 (83.0,132)	105.0 (80.0, 125.0)	120.0 (85.0, 133.0)	0.001
	Heat reserve	12.0 (9.6, 16.3)	11.0 (7.8, 13.6)	11.9 (9.8, 14.1)	14.0 (13.0, 15.8)	15.9 (12.9, 18.6)	12.7 (12.2, 13.2)	11.4 (10.2, 15.8)	11.5 (8.2, 12.6)	0.416
	PORH (lowest PU)	2.9 (2.4, 3.3)	3.2 (2.5, 3.9) #	2.6 (2.4, 3.0)	2.8 (2.5, 4.0)	2.8 (2.6, 3.7)	3.3 (2.6,3.9)	3.0 (2.6, 3.5)	3.2 (2.9, 3.4)	0.355
	PORH (highest PU)	46.0 (36.3, 52.5)	53.0 (37.0, 71.0) #	44.5 (40.0, 57.8)	48.0 (41.0, 58.3)	42.0 (34.0, 61.0)	34.5 (29.0,40.0)	37.0 (33.0, 59.0)	49.0 (23.0, 64.0)	0.090
	PORH reserve	4.9 (3.9, 5.7)	4.8 (4.1, 5.2)	5.2 (3.7, 6.3)	5.2 (4.1, 6.9)	5.5 (4.1, 6.8)	4.1 (4.0,4.3)	4.6 (3.3, 6.0)	3.5 (3.2, 4.7)	0.200
	CVC (PU/mmHg)	0.10 (0.08, 0.16)	0.13 (0.09, 0.16)	0.10 (0.08, 0.12)	0.09 (0.08, 0.13)	0.09 (0.08, 0.13)	0.10 (0.08,0.11)	0.09 (0.08, 0.16)	0.12 (0.06, 0.21)	0.656
Thigh	Resting (PU)	8.0 (6.1, 10.5)	9.5 (6.0, 12.0)	9.8 (7.0, 16.0)	6.8 (5.9, 8.6)	8.2 (6.3, 9.4)	6.4 (4.5,8.2)	7.8 (7.1, 12)	5.7 (4.3, 12)	0.003
	CMBC	82.5 (62.8, 133.3)	106.0 (84.0, 140.0)	109.5 (97.5, 154.3)	71.0 (61.5, 95.5)	104.5 (56.3, 108.0)	109.0 (46.0,172)	94.0 (85.0, 124.0)	87.0 (50.0, 126.0)	0.028
	Velocity	9.3 (7.3, 11.4)	8.2 (7.7, 9.5)	9.9 (8.0, 11.8)	8.6 (7.9, 12.1)	9.1 (8.0, 13.3)	8.8 (6.6,11.0)	10.0 (7.9, 12.0)	9.1 (7.4, 12.0)	0.990
	Heat (PU)	89.0 (58.5, 114.5)	86.0 (84.0, 122.0)	89.5 (79.0, 109.8)	84.5 (68.3, 111.8)	107.0 (67.0, 121.5)	157.5 (81.0,234)	103.0 (71.0, 153.0)	99.0 (75.0, 130.0)	0.708
	Heat reserve	11.3 (7.5, 14.1)	9.9 (8.3, 15.0)	8.7 (7.0, 13.2)	11.8 (8.8, 16.8)	13.9 (9.5, 15.3)	23.3 (18.0,28.5)	13.6 (7.9, 15.5)	15.3 (14.2, 21.1)	0.021
	CVC (PU/mmHg)	0.09 (0.07, 0.11)	0.09 (0.07, 0.14)	0.11 (0.08, 0.14)	0.08 (0.06, 0.09)	0.09 (0.07, 0.13)	0.07 (0.05,0.09)	0.08 (0.08, 0.14)	0.05 (0.04, 0.13)	0.012

* *p* < 0.05 versus baseline; # 0.05 < *p* < 0.1 versus baseline.

Heat reserve: (heat PU)/(resting PU); PORH reserve: PORH (highest PU)/(resting PU); IE: time by group interaction effect.

Table 6 Characteristics of HIF, NO, and VEGF-related parameters

	HHL				NOM				Interaction effect <i>p</i> value
	Baseline	week 2	week 4	Post-HHL	Baseline	week 2	week 4	Post-NOM	
EPO	10.4 (8.8, 13.1)	12.7 (10.1, 13.5)	11.1 (10.1, 13.0)	6.5 (4.9, 9.9) *	9.0 (6.3, 10.1)	9.1 (5.9, 12.6)	8.7 (6.4, 10.7)	8.6 (5.1, 10.0)	0.008
HIF (pg/ml)	27.0 (19.8, 66.4)	27.7 (15.4, 75.5)	31.1 (25.4, 66.2) #	30.0 (26.6, 53.5)	42.6 (29.8, 135.0)	43.1 (27.5, 84.5)	48.4 (37.3, 123.0)	68.1 (35.2, 201.6)	0.075
NO (μmol/l)	0.05 (0.04, 0.15)	0.06 (0.04, 0.19)	0.08 (0.06, 0.14) *	0.08 (0.05, 0.13)	0.10 (0.06, 0.21)	0.09 (0.05, 0.19)	0.10 (0.08, 0.29)	0.11 (0.06, 0.32)	0.247
eNOS (IU/ml)	2.2 (1.5, 6.5)	2.5 (1.5, 8.6)	3.3 (1.7, 6.3)	3.0 (1.9, 6.5)	4.4 (2.5, 8.7)	4.3 (1.9, 8.3)	3.8 (2.6, 12.6)	6.1 (2.5, 24.6)	0.078
ET (pg/ml)	10.2 (6.9, 29.3)	12.9 (6.8, 46.3)	10.4 (8.0, 20.5)	9.8 (6.3, 17.7)	16.8 (10.2, 39.8)	18.8 (9.0, 38.6)	15.9 (10.4, 37.0) *	12.7 (7.8, 36.7)	0.101
PGI2 (mIU/ml)	8.9 (6.1, 22.6)	9.5 (5.0, 38.0)	9.9 (7.0, 22.6)	9.2 (6.2, 20.1)	15.7 (9.1, 39.7)	18.9 (8.6, 50.8)	20.8 (14.2, 55.0)	15.6 (11.3, 46.5)	0.364
VEGF (pg/ml)	13.5 (8.9, 39.2)	14.3 (8.6, 43.6)	17.6 (10.7, 45.5) #	17.0 (11.6, 42.0) #	15.3 (11.8, 39.0)	18.8 (11.2, 34.5)	28.7 (18.4, 81.3)	26.5 (13.9, 85.8)	0.124
VEGFR (ng/ml)	1.6 (0.9, 3.1)	1.6 (0.7, 4.0)	1.7 (1.0, 3.3)	1.7 (1.0, 2.6)	1.5 (1.0, 6.0)	2.3 (1.5, 4.4)	2.3 (1.6, 6.1)	2.2 (1.1, 6.2)	0.494

* $p < 0.05$ versus baseline; # $0.05 < p < 0.1$ versus baseline.