Changes in Superficial Blood Distribution in Thigh Muscle During LBNP Assessed by NIRS

Tesshin Hachiya, Andrew P. Blaber, and Mitsuru Saito

Hachiya T, Blaber AP, Saito M. ***Changes in superficial blood distribution in thigh muscle during LBNP assessed by NIRS.*** **Aviat Space Environ Med 2004; 75:118–22.**

Introduction: The present study was designed to determine how superficial blood distributed in the lower limb muscle during graded lower body negative pressure (LBNP). ***Methods:*** Near-infrared spectroscopy (NIRS) was used to evaluate the blood volume change in the thigh muscles of seven volunteers during 35 min graded LBNP (rest, −10, −20, −30, −40, −50 mm Hg, and recovery). ***Results:*** Deoxygenated and total hemoglobin (Hb) increased in proportion to the magnitude of LBNP applied to the thigh muscles. Oxygenated Hb rose significantly at −10 mm Hg LBNP, although the increase leveled off during subsequent increments of LBNP. Systolic pressure significantly decreased from 120 mm Hg at rest, to a value of 108 at −50 mm Hg LBNP. In contrast, mean and diastolic pressures were well maintained during graded LBNP. The increased total and deoxygenated Hb might indicate that blood was held in venous space, and the magnitude of rise in blood volume corresponded to the change in LBNP. On the other hand, oxygenated Hb change seems to reflect mainly blood accumulated in arterial space by interacting between mechanical stretch induced by LBNP and vasoconstriction caused by augmented sympathetic nerve activity. ***Conclusion:*** From these results, blood distribution in thigh muscles was different and was affected by the strength of LBNP. The data assessing oxygenation sites of Hb were found to be useful as indices of estimating superficial blood pooling in the muscle during LBNP.

Keywords: orthostatic tolerance, heart rate, blood pooling, blood pressure.

DURING DAY-TO-DAY activities involving postural changes, even apparently healthy people may experience presyncope: exhibiting nausea, dizziness, or headache. Lower body negative pressure (LBNP) has often been used to simulate these orthostatic hemodynamic and cardiovascular changes in supine human subjects. Since venous vasculature is of high compliance (2,6), it is believed that during LBNP, blood is pooled in the venous vascular bed of the lower body due to a passive expansion of vessels (5). The diameter of capacitance vessels expands as negative pressure applied to the lower body is augmented despite the fact that LBNP activates sympathetic nerve activity that produces vasoconstriction (10,23). However, our current knowledge on the distribution of blood in the skeletal muscle tissue of the lower limbs during LBNP is limited.

In arterial vascular beds, microvessels and arterioles are known to be less distensible and do not hold much blood (19). However, the diameter of these vessels can be modulated by neural, humoral, and local metabolic stimuli (4,11,18,21). The interaction of mechanical stretch and neurally mediated constriction on the response of arterial microvessels during LBNP remains to be elucidated. We hypothesized that in capacitance vascular beds, the amount of blood accumulated would be proportional to changes in negative pressure; and, since arterioles would be less likely to expand passively, blood pooled in the arterial side would be unaffected by graded LBNP.

Recently, near-infrared spectroscopy (NIRS) was used to detect limb skeletal muscle oxygenation as well as superficial blood flow under different orthostatic stresses (1,16). Not only can this technique be used to determine tissue oxygenation, but it can also be used to investigate hemodynamic changes. By observing changes in oxygentated and deoxygenated hemoglobin (Hb) content, blood distribution in both arterial and venous vascular beds can be determined. The purpose of the present study was to use NIRS to determine characteristics of superficial arterial and venous blood distributions in the lower limbs during graded LBNP and to further elucidate vascular mechanisms associated with orthostatic stress.

METHODS

There were seven volunteers who participated as subjects for this experiment. All subjects were familiar with the laboratory testing. The experimental protocol was approved by Human Subjects Protecting Committee of the Toyota Technological Institute, Nagoya, Japan. Informed written consent was obtained from each subject before participating in this study.

Measurement

Heart rate (HR) signals were collected using a two-lead electrocardiogram from the chest. Arterial blood

From the Laboratory of Applied Physiology, Toyota Technological Institute, Nagoya, Japan (M. Saito), and the School of Kinesiology, Faculty of Applied Science, Simon Fraser University, Burnaby, British Columbia, Canada (T. Hachiya, A.P. Blaber).

This manuscript was received for review in April 2003. It was revised in May 2003. It was accepted for publication in June 2003.

Address reprint requests to: Mitsuru Saito, Laboratory of Applied Physiology, Toyota Technical Institute, 2-12 Hisakata, Tempaku-ku, Nagoya Japan 468-8511.

pressure (BP) was obtained every minute by a non-invasive automated blood pressure monitor (Colin BP203, Tokyo, Japan) from the right arm of the subjects. Mean arterial pressure was calculated as one-third of pulse pressure plus diastolic pressure. EMG electrodes were set as close as possible to the NIRS probes on the right thigh to monitor muscle activity throughout the experiment.

A sensor (Omegawave, BOM-L1, Tokyo, Japan) for Hb oxygenation was placed on the right vastus lateralis muscle. NIRS probes were placed parallel to each other 2 cm apart on the skin surface of vastus lateralis. This allowed for a 3-cm detection depth. The basic principle of NIRS is based on the ability of near infrared light (700–900 nm) to pass through tissues such as skin, muscles, and bone. The detailed mechanisms are described elsewhere (7,13). Briefly, laser light is diffused into the tissues and reflected light is sensed by a silicon photodetector. Both oxygenated and deoxygenated forms of Hb absorb the laser light at 810 nm, whereas absorption at 780 nm is primarily by the deoxygenated Hb. Total Hb is estimated by adding both deoxygenated and oxygenated forms of Hb. Since skeletal muscle tissue contains myoglobin (Mb) and the absorption of the wavelength of Mb is similar to that of Hb, the oxygenated states of two molecules are not separable. As Hb and Mb are oxygenated, the absorption at 780 nm decreases, but increases at 810 nm. Thus total Hb contains oxygenated Hb, deoxygenated Hb, and oxygenated Mb (Total Hb = oxyHb + deoxyHb + oxyMb). Although oxygenated Mb volume is modulated by different conditions such as metabolic rate or oxygen supply, during LBNP oxygenated Mb remains unchanged during rest because oxygen demand in muscle tissues is kept constant (3). In the present study, the change in total Hb was evaluated by the sum of both oxygenated and deoxygenated Hb during LBNP.

NIRS detects changes in oxygenation of muscle tissue intracellular sites such as arterioles, capillaries, and venules. Large vessels greater than 1 mm in diameter, such as resistance arterial vessels, are not considered to be sites monitored for oxygenation due to the low magnitude of appearance of photons caused by vessel wall thickness (13). We investigated relative changes in Hb from the control level, which reflect the changes in blood volume (3,22). Although we did not attempt to quantify absolute blood volume changes from the Hb data, these NIRS measurements allowed us to observe relative changes in superficial blood volume during graded LBNP.

Protocol

All subjects abstained from caffeinated or alcoholic beverages at least 12 h prior to the experiments. All tests were performed in the afternoon at least 2 h after a light lunch. Subjects were placed in the supine position with their legs extended into a wooden box with an airtight seal at the level of the iliac crest. Pressure control of LBNP was carried out manually via a vacuum with a voltage controller. Pressure was monitored via a pressure transducer attached to the inside of the chamber.

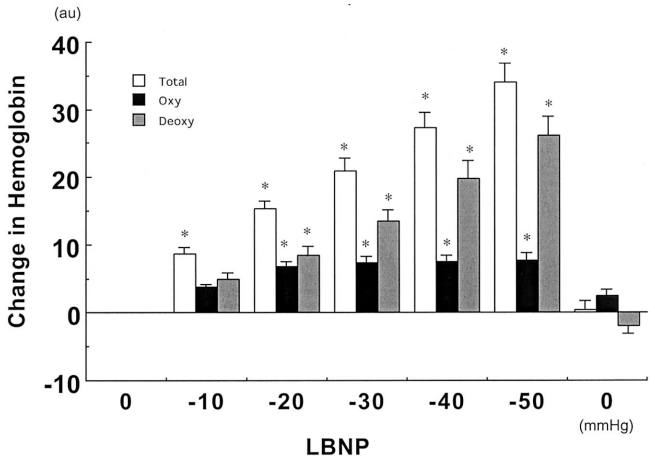

Fig. 1. Total, oxygenated, and deoxygenated Hb changes of thigh muscle assessed by near-infrared spectroscopy in seven subjects during graded LBNP. The results expressed as mean and vertical bars indicate the SE. * $p < 0.05$ vs. control. The values in each parameter during graded LBNP were calculated as relative changes.

The subjects underwent a 35-min LBNP trial that consisted of five 5-min steps of subatmospheric pressure from 0, −10, −20, −30, −40, and −50 mm Hg. This was preceded by a 5-min control recording and followed by 5 min of recovery. The data presented in each 5-min interval were averaged values obtained during the last 4 min of each LBNP level.

Statistics

Since only a small number of subjects participated, the Shaprio-Wilks normality test was performed, determining that the data were normally distributed. Therefore, data were analyzed with a one-way repeated measures analysis of variance. When the effects were significant, post-hoc analysis with Fisher's PLSD was applied. Differences were considered significant when $p < 0.05$. All values were reported as mean ± SE.

RESULTS

Total, Oxygenated, and Deoxygenated Hb Concentrations

The magnitude of the increase in total Hb was proportional to the degree of the negative pressure applied to the lower body (**Fig. 1**). At −10 mm Hg, oxygenated Hb increased statistically significantly ($p < 0.01$), but leveled off during subsequent increments of LBNP (Fig. 1). However, deoxygenated Hb increased in proportion to the strength of negative pressures above −10 mm Hg (Fig. 1).

Arterial Pressure and Heart Rate

Mean arterial and diastolic pressures were not altered during graded LBNP (**Fig. 2**). However, systolic pressure at −50 mm Hg decreased to 108 ± 4 mm Hg ($p < 0.01$) compared with the control resting value (123 ± 3 mm Hg). Also, pulse pressure declined by 50 mm Hg ($p < 0.01$). HR was 66 ± 3 bpm at supine rest without negative pressure and unchanged at −10 mm Hg (64 ±

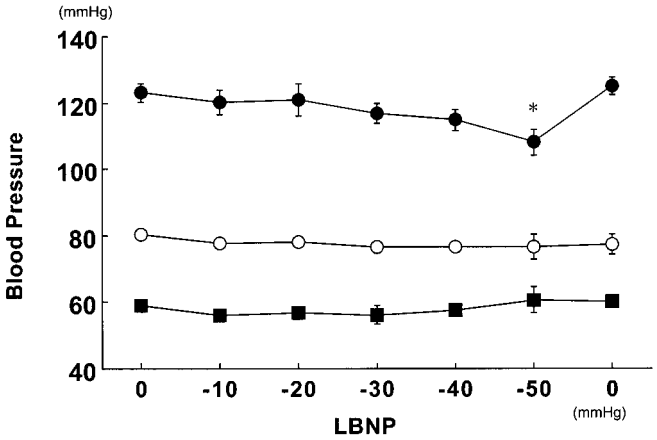

Fig. 2. Systolic, diastolic, and mean arterial pressure during graded LBNP. Each value is a mean ± SE * $p < 0.05$.

3 bpm). With increased LBNP, HR rose to 75 ± 4, and 80 ± 5 bpm at −40 and −50 mm Hg ($p < 0.01$, **Fig. 3**).

DISCUSSION

We used NIRS measurements of the changes in oxygenated, deoxygenated, and total Hb as indices of blood pooling to investigate the distribution of superficial blood in the lower limb muscles during LBNP. The increase in total Hb and, therefore, the pattern of superficial blood pooling to the lower limbs during LBNP, were similar to previous studies using strain gauge plethysmography (20) and impedance plethysmography (14). However, this study has provided an increased understanding of the distribution of blood volume within the vasculature of the lower limbs during LBNP. We observed dissociation in the trends of oxygenated and deoxygenated Hb during graded LBNP. Oxygenated Hb increased slightly but significantly at −10 mm Hg, and leveled off after −20 mm Hg LBNP. In contrast, after −10 mm Hg, deoxygenated Hb increased in parallel to total Hb, suggesting that the site and distribution of trapped blood in leg muscle tissue was affected by the level of LBNP.

Blood Pooling During LBNP Stress

It has been recognized that blood is gradually pooled in capacitance vessels in the lower body as negative pressure applied to the lower limb is enhanced (5,19). Total Hb, which was determined with NIRS sensors situated on the vastus lateralis, increased during graded LBNP. These results are in accordance with a study conducted by Nishiyasu et al. (16) who found that total Hb increased in the thigh muscles during −25 mm Hg and −50 mm Hg LBNP. Since total Hb determined by NIRS has been shown to reflect the volume of blood in muscle tissues (3,22), these results demonstrated that blood was held in the lower limbs during graded LBNP. Furthermore, the magnitude of the rise in blood pooled was proportional to given changes in pressures applied to the lower limbs.

In the present study, oxygenated Hb increased significantly at −10 mm Hg and leveled off above −20 mm Hg LBNP. We suggest that changes in oxygenated Hb might predominantly reflect superficial blood pooled in arterial vessel space in muscle tissues (1).

Mancini et al. (13) observed an increase in oxygenated Hb during administration of nitroprusside, a vasodilator, and a decrease in oxygenated Hb with the administration of a high dose of norepinephrine, a vasoconstrictor. Although muscle tissue oxygenation detected by NIRS contains both oxygenated Hb and Mb, it was unlikely that the Mb oxygenated state was modulated significantly (3). Throughout the experiment, no detectable change in EMG was observed from the area monitored by NIRS and Mb oxygenation could be reasonably expected to be unaltered in relaxing muscle. These results, therefore, imply that the change in oxygenated Hb up to −20 mm Hg LBNP reflected predominantly increased blood in the arterial space.

It might also be argued that oxygenated Hb was pooled not only in arterioles but also in venous microvessels. However, if the oxygenated Hb had been held in venous space, it should have increased in parallel to the change in deoxygenated Hb. The increase in oxygenated Hb at −10 mm Hg LBNP may be related to arteriole stretch due to negative pressure applied to the lower body despite the location of arterioles under the cutaneous level. During further increases in negative pressures, the steady state in oxygenated Hb observed might reflect competition of mechanical stretch with augmented vasoconstriction. It has been well documented that muscle vasoconstrictor nerve activity increases during LBNP (10,23).

Nishiyasu et al. (16) observed a decrease in oxygenated Hb during −25 and −50 mm Hg LBNP that implied the possibility of augmented muscle sympathetic nerve activity. Although we did not observe this decrease in oxygenated Hb, one of our subjects exhibited the same trend as shown in their study. One reason for the discrepancy might be the result of different protocols used: in this study, a graded increase in LBNP was used, whereas Nishiyasu et al. applied −50 mm Hg first, followed by 2 min of −25 mm Hg. Taken together, these studies indicate that mechanical stretch and reflex vasoconstriction of arterioles during graded LBNP de-

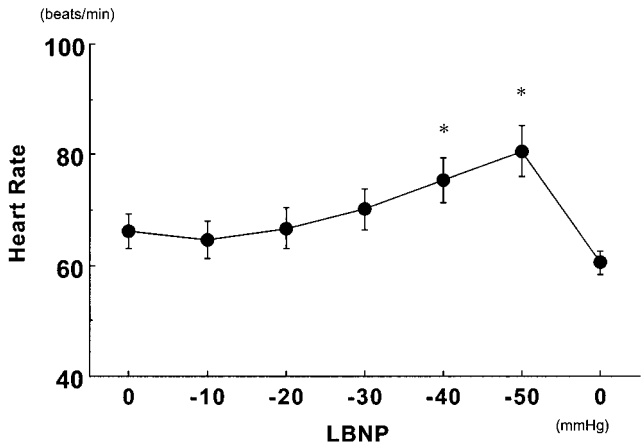

Fig. 3. HR response in seven subjects during graded LBNP. The results expressed as mean and vertical bars indicate the SE. * $p < 0.05$.

termined the magnitude of blood pooling in arterial space.

It was found that deoxygenated and total Hb increased in proportion to changes in negative pressures. We assumed that total and deoxygenated Hb reflected blood accumulated mainly in venous space since arterial oxygen saturation would not be expected to change under normal resting conditions. These results demonstrated that the distribution of blood pooling in the lower limb muscles during graded LBNP differentiated both superficial vascular beds, arterial and venous, in response to changes in negative pressure applied to the lower body.

It has been well documented that blood pooling occurs in capacitance vessels in the lower legs during orthostatic stress. In this study, deoxygenated Hb increased proportionally as negative pressures are enhanced. This tendency was considerably similar for all subjects; however, oxygenated Hb, thought to be pooled in arterial compartment, increased slightly but significantly at −10 mm Hg, and leveled off above −20 mm Hg. The individual variations in the pooling trend of oxygenated Hb were found to be relatively large, which may indicate that examining mechanisms for this diverse blood pooling response in the arterial compartment might provide clues to the occurrence of orthostatic intolerance.

Although non-invasive measurements of any kind should be viewed with caution, NIRS provides information that would otherwise be difficult to obtain. According to Mancini et al. (13), NIRS measures tissue oxygenation states by photon penetration of tissues, including muscle and microvasculature, for a 2 to 4 cm depth. Thus, NIRS measurements provide information on relative changes in superficial blood volume, not quantitative values, and reflect fluctuations in blood volume for only limited small areas. However, we demonstrated that total Hb increased in response to enhanced negative pressure to the lower limbs. Edward et al. (3) compared strain gauge plethysmography with NIRS, finding that the two measurements were consistent. The pattern of the increased total Hb has been found to be similar to blood volume changes during exercise and graded LBNP (8,13,14).

Cardiovascular Response

HR increased significantly during −40 to −50 mm Hg (Fig. 3). This is in accordance with data obtained from the studies conducted by Halliwill (5) and Khan (12) as well as the early research done by Johnson et al. (9). Neither mean arterial pressure nor diastolic pressure changed during graded LBNP, whereas systolic pressure declined at −50 mm Hg. During 60 or 70° head-up tilt, which is equivalent to −40 mm Hg LBNP (17), systolic pressure gradually decreases with elapsed tilt time despite no further change in degree of the tilt angles (15). The magnitude of LBNP, −50 mm Hg, may not have been the only cause of the reduction in systolic pressure since the subjects were already exposed to negative pressure for 15 min.

Compared with systolic pressure responses, mean arterial pressure was well preserved during graded LBNP, while blood volume increased gradually in the lower limb during graded LBNP. This would imply that reflex vasoconstriction was the most essential mechanism for maintaining arterial BP, although HR increased to prevent the further reduction in cardiac output to compensate for the reduction in venous return. Our results show that oxygenated Hb, as an index of arterial blood level, was constant during graded LBNP and indicate that neural vasoconstriction seemed to be enhanced to counteract mechanical stretch of vessels, and to raise peripheral vascular resistance, such that a fall in mean arterial pressure was prevented.

CONCLUSIONS

We examined the pattern of relative changes in oxygenated, deoxygenated, and total Hb to investigate superficial blood pooling in the thigh muscle during LBNP. It was demonstrated that blood was held in the lower body, since there was a relative increase in Hb with LBNP. These results indicate that NIRS measurements could be useful to assess the trends of blood trapped in the lower limb muscles. More importantly, the superficial blood pooling in the lower legs, evaluated by deoxygenated Hb changes, increased proportionally to the applied pressure during graded LBNP; but in arterioles, the superficial blood pooling, assessed by oxygenated Hb, leveled off above −20 mm Hg LBNP. The separated measurements of both oxygenated and deoxygenated Hb might enable us to distinguish the tendency of blood distribution between the arterial and venous compartments during orthostatic stress. The examination of the superficial blood pooling pattern in both arterial and venous vasculatures during graded LBNP provided further elucidation of the possible mechanisms involved with orthostatic intolerance.

ACKNOWLEDGMENTS

We wish to thank our subjects for their cooperation. This work was supported by funds from the Toyota High-Tech Research Grant Program, and Grant-in-Aid for Scientific Research in Japan Society.

REFERENCES

1. Binzoni T, Quaresima V, Ferrari M, et al. Human calf microvascular compliance measured by near-infrared spectroscopy. J Appl Physiol 2000; 88:369–72.
2. Convertino VA, Doerr DF, Flores JF, et al. Leg size and muscle functions associated with leg compliance. J Appl Physiol 1988; 64:1017–21.
3. Edward AD, Richardson C, van der Zee P, et al. Measurement of hemoglobin flow and blood flow by near-infrared spectroscopy. J Appl Physiol 1993; 75:1884–9.
4. Evans JM, Leonelli FM, Ziegler MG, et al. Epinephrine, vasodilation, and hemoconcentration in syncopal, healthy men and women. Auton Neurosci 2001; 93(1–2): 79–90.
5. Halliwill JR, Lawler LA, Eickhoff NM, et al. Reflex responses to regional venous pooling during lower body negative pressure in humans. J Appl Physiol 1998; 84:454–8.
6. Halliwill JR, Minson CT, Joyner MJ. Measurement of limb venous compliance in humans: technical considerations and physiological findings. J Appl Physiol 1999; 87:1555–63.
7. Hampson NB, Piantadosi CA. Near infrared monitoring of human skeletal muscle oxygenation during forearm ischemia. J Appl Physiol 1988; 64:2449–57.

8. Homma S, Hideo E, Ogasawara S, Kagaya A. Near-infrared estimation of O2 supply and consumption in forearm muscles working at varying intensity. J Appl Physiol 1996; 80:1279–84.
9. Johnson JM, Rowell LB, Niederberger M, Eisman MM. Human splanchnic and forearm vasoconstrictor responses to reductions of right arterial and aortic pressure. Cir Res 1973; 34:515–24.
10. Joyner MJ, Shepherd JT, Seals DR. Sustained increases in sympathetic outflow during prolonged lower body negative pressure in humans. J Appl Physiol 1990; 68:1004–9.
11. Joyner MJ, Halliwill JR. Topical Review: Sympathetic vasodilation in human limbs. J Physiol 2000; 526:471–80.
12. Kahn MH, Sinoway LI, MacLean DA. Effects of graded LBNP on MSNA and interstitial norepinephrine. Am J Physiol 2002; 283:2038–44.
13. Mancini DM, Bolinger L, Li H, et al. Validation of near-infrared spectroscopy in humans. J Appl Physiol 1994; 77:2740–7.
14. Montgomery LD, Kirk PK, Payne PA, et al. Cardiovascular responses of men and women to lower body negative pressure. Aviat Space Environ Med 1977; 48:138–45.
15. Morillo CA, Ellenbogen KA, Pava LF. Pathophysiologic basis for vasodepressor syncope. Cardiol Clin 1997; 15:233–49.
16. Nishiyasu T, Tan N, Kondo N, et al. Near-infrared monitoring of tissue oxygenation during application of body pressure at rest and during dynamical exercise in humans. Acta Physiol Scand 1999; 166:123–30.
17. Olsen H, Vernersson E, Lanne T. Cardiovascular response to acute hypovolemia in relation to age. Implications for orthostasis and hemorrhage. Am J Physiol Heart Circ Physiol 2000; 278:222–32.
18. Reed AS, Tschakovsky ME, Minson CT, et al. Skeletal muscle vasodilation during sympathoexcitation is not neurally mediated in humans. J Physiol 2000; 525:253–62.
19. Rowell LB. Human cardiovascular control. New York: Oxford University Press; 1993:3–36.
20. Stewart JM. Pooling in chronic orthostatic intolerance. Circulation 2002; 105:2274–81.
21. Tschakovsky ME, Sujirattanawimol K, Ruble SB, et al. Is sympathetic neural vasoconstriction blunted in the vascular bed of exercising human muscle? J Physiol 2002; 541:623–35.
22. Van Beekvelt MCP, Colier WNJM, Wevers RA, Van Engelen BGM. Performance of near-infrared spectroscopy in measuring local O2 consumption and blood flow in skeletal muscle. J Appl Physiol 2001; 90:511–9.
23. Victor RG, Leimbach Jr WN. Effects of lower body negative pressure on sympathetic discharge to leg muscle in humans. J Apple Physiol 1987; 63:2558–62.