WISE 2005: responses of women to sublingual nitroglycerin before and after 56 days of 6° head-down bed rest

K. A. Zuj,1 H. Edgell,1 J. K. Shoemaker,2 M. A. Custaud,3 P. Arbeille,4 and R. L. Hughson1

1Faculty of Applied Health Sciences, University of Waterloo, Waterloo, Ontario; 2School of Kinesiology, University of Western Ontario, London, Ontario, Canada; 3Explorations Fonctionnelles Vasculaires, Centres Hospitaliers Universitaires d’Angers, Angers; and 4Unité Médecine et Physiologie Spatiale, Centres Hospitaliers Universitaires Trousseau, Tours, France

Submitted 6 April 2012; accepted in final form 24 May 2012

WISE 2005: responses of women to sublingual nitroglycerin before and after 56 days of 6° head-down bed rest. J Appl Physiol 113: 434–441, 2012. First published May 31, 2012; doi:10.1152/japplphysiol.00445.2012.—This study tested the hypothesis that cardiovascular effects of sublingual nitroglycerin (NG) would be exaggerated after 56 days of 6° head-down bed rest (HDBR) in women, and that an aerobic and resistive exercise countermeasure (EX, n = 8) would reduce the effect compared with HDBR without exercise (CON, n = 7). Middle cerebral artery maximal blood flow velocity (CBFV), cardiac stroke volume (SV), and superficial femoral artery blood flow (Doppler ultrasound) were recorded at baseline rest and for 5 min following 0.3 mg sublingual NG. Post-HDBR, NG caused greater increases in heart rate (HR) in CON compared with EX (+24.9 ± 7.7 and +18.8 ± 6.6 beats/min, respectively, P < 0.0001). The increase in HR combined with reductions in SV to maintain cardiac output. Systolic, mean, and pulse pressures were reduced 5–10 mmHg by NG, but total peripheral resistance was only slightly reduced at 3 min after NG. Reductions in CBFV of −12.5 ± 3.8 cm/s were seen after NG, but a reduction in the Doppler resistance index suggested dilation of the middle cerebral artery with no differences after HDBR. The femoral artery dilated with NG and blood flow was reduced ~50% with the appearance of large negative waves suggesting a marked increase in downstream resistance, but there were no effects of HDBR. In general, responses of women to NG were not altered by HDBR; the greater increase in HR in CON but not EX was probably a consequence of cardiovascular deconditioning. These results contrast with the hypothesis and a previous investigation of men after 90 days of head-down bed rest (HDBR) (34) and in the women reported in the present study after 60 days of HDBR (35). In peripheral vascular beds, a trend has been observed for greater flow-mediated dilation (FMD) responses after HDBR (5, 30) thought to be mediated by NO mechanisms (17). However, different responses have been observed with respect to the dilatory effects of sublingual nitroglycerin (NG), where two studies have shown no effect (7, 30) compared with enhanced dilation after 52 days of horizontal bed rest (5). It has been speculated that changes in NO-mediated mechanisms of blood flow regulation might contribute to orthostatic intolerance observed after spaceflight and spaceflight simulation (7, 18). A greater heart rate response to NG has been reported after 7 days of HDBR (7), but little information is available on the systemic and regional responses to this NO donor.

The present experiments were conducted within the Women’s International Simulation for Space Exploration (WISE)-2005 spaceflight analog study, which involved women who completed 56 days of continuous 6° HDBR with or without exercise countermeasures. We hypothesized that 56 days of HDBR would exaggerate cardiovascular responses to NG, reflecting poorer orthostatic tolerance following HDBR, and that the aerobic and resistive exercise countermeasure would attenuate these effects.

METHODS

WISE-2005. The WISE study was an international collaborative study between the Centre National d’Etudes Spatiales (CNES), European Space Agency (ESA), Canadian Space Agency (CSA), and the National Aeronautics and Space Administration (NASA). This study reports on 15 healthy women between the ages of 25 and 40 yr of age who completed 60 days of continuous 6° HDBR with the experiments for this study completed on day 56 of HDBR. The research was conducted in Toulouse, France, at the MEDES Space Medicine Research Facility of CNES, and approved by the Comité Consultatif de Protections des Personnes dans la Recherche Biomédicale, Midi-Pyrénées (France), Committee for the Protection of Human Subjects. 

Address for reprint requests and other correspondence: R. L. Hughson, Faculty of Applied Health Sciences, Univ. of Waterloo, Waterloo, ON N2L3G1, Canada (e-mail: hughson@uwaterloo.ca).
at Johnson Space Center, and local ethics committees including the Office of Research Ethics, University of Waterloo. All study protocols were in accordance with the Declaration of Helsinki. Each subject signed an informed consent form and was aware of her right to withdraw from the study without prejudice.

Countermeasures and exercise schedule. Subjects were randomly assigned to either the control (CON) or exercise (EX) group after first stratifying them according to maximal oxygen uptake. Participants assigned to either the control (CON) or exercise (EX) group after first withdraw from the study without prejudice.

Participants were recreationally active with an average height of 164.3 ± 6.2 cm and weight of 58.2 ± 5.0 kg. Maximal oxygen uptake data have been previously presented (19, 37) with averages of 38.9 ± 6.8 and 37.9 ± 4.0 ml·min⁻¹·kg⁻¹ for CON and EX, respectively. One participant in the CON group did not complete the NG testing post-HDBR and was excluded from all analysis. The CON group (n = 7) did not receive any countermeasure during the 56 days of bed rest, while the subjects in the EX group (n = 8) performed two types of exercise. Three to four times per week, the EX group exercised for 40 min between 40 and 80% of their peak oxygen uptakes on a treadmill inside a lower-body negative pressure (LBNP) chamber with LBNP applied to provide a ground reaction force of 1–1.2 body weight (9). Treadmill exercise was followed by 10 min of static LBNP. Every third day of the bed rest period, the EX group completed flywheel resistance exercise (33).

Physiological measures. Heart rate (HR) was determined using a standard three-lead electrocardiogram. Finger photoplethysmography (Finometer, Finapres Medical, Amsterdam, The Netherlands) was used for the determination of arterial pressure. Mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP) were determined from the recorded pressure wave forms. Data were recorded on a PowerLab (ADInstruments, Colorado Springs, CO) data collection system.

Aortic blood velocity was determined using a 2-MHz probe oriented to determine the velocity of blood at the aortic root (Multigon, New York, NY). Ultrasound imaging (Acuson 128XP, Paris, France) was used to assess the cross-sectional area of the aortic root, which was then combined with velocity measures to determine cardiac stroke volume (SV). Cardiac output (Q) was determined as the product of SV and HR. Total peripheral resistance (TPR) was calculated as TPR = MAP/Q.

Superficial femoral artery blood velocity was determined using a 4-MHz pulsed Doppler probe (CardioLab, ESA-CNES device). Echo Doppler ultrasound (Acuson 128XP, Paris, France) was used to provide images of the superficial femoral artery for the determination of vessel cross-sectional area and the calculation of femoral blood flow. Blood flow data were normalized to 100 ml of lower limb lean tissue calculated from magnetic resonance imaging (S. Trappe and T. Trappe, personal communication). Femoral vascular conductance was calculated as FC = femoral flow/MAP. Vascular conductance was calculated for this study since the calculation of vascular resistance (MAP/femoral flow) had a skewed distribution with some extremely high values as, in some individuals, femoral blood flow was reduced to near zero with NG simulation.

Echo Doppler imaging of the portal vein (Acuson 128XP) was used for the assessment of portal vein cross-sectional area. Due to technical issues, the data from only eight of the subjects were analyzed for pre-HDBR responses.

Transcranial Doppler ultrasound was performed for the assessment of cerebral blood flow velocity (CBFV). A 2-MHz pulsed Doppler probe (CardioLab, ESA-CNES device) was positioned over the temporal window allowing for the insonation of the middle cerebral artery (MCA). Throughout the test, the probe was held in place using a head band. The outer envelope of the Doppler spectrum was recorded and CBFV was determined as the mean of the outer envelope over a cardiac cycle. Two indexes of cerebral vascular resistance, CVRi and RI, were calculated from the waveform. CVRi was determined from CVRi = MAP/CBFV, whereas RI was calculated from RI = (CBFVsys − CBFVdia)/CBFVsys where CBFVsys and CBFVdia are the maximum and minimum points of the wave, respectively, during a cardiac cycle.

Testing protocol. Data were collected 7 days before the start of HDBR (pre-HDBR) and on the 56th day of the bed rest period (post-HDBR), ~24 h after a treadmill in LBNP exercise session for the EX group. All measures were made in a supine posture. Physiological measures were recorded continuously for 2 min baseline period and 5 min after the sublingual administration of 0.3 mg of NG (Nitraspray, Proctor & Gamble Pharmaceuticals). Imaging of the portal vein was performed at baseline, 2, and 4 min after NG stimulation for 8 of the 15 participants, pre-HDBR. Diameter measures of the superficial femoral artery were taken at baseline and 5 min after NG.

Statistical analysis. The analysis of HDBR responses used a three-way repeated-measures analysis of variance with the main effects for this analysis being HDBR (pre, post), countermeasure group (EX, n = 8; CON, n = 7), and time after drug. Responses to NG were determined every minute after NG and compared with baseline values before NG stimulation. SAS 9.1.3 analysis software was used for all statistical analysis with significance set at P < 0.05. Values in the text are expressed as means ± SD.

RESULTS

Pre-HDBR response to NG. No differences were seen for any measured variable between EX and CON pre-HDBR (filled symbols in figures). MAP was slightly reduced the first minute, decreased significantly (~4.9 ± 3.0 mmHg, Fig. 1A) for the second minute, and remained lower for the remainder of the test. A decrease in SBP (~5.5 ± 4.5 mmHg, Fig. 1B) combined with a slight increase in DBP to produce a decrease in PP (~5.8 ± 4.9 mmHg, Fig. 1C). SBP recovered back toward baseline after 4 min, but the small, significant increase in DBP (1.7 ± 3.4 mmHg, Fig. 1D) served to maintain the decrease in PP.

HR pre-HDBR increased significantly in the first minute after NG (4.2 ± 4.2 beats/min, Fig. 2A) then increased further by 2 min (11.8 ± 3.9 beats/min) and remained elevated. SV decreased significantly by the second minute, and Q was elevated only at the third minute (Fig. 2, B and D, respectively). TPR was reduced at the third minute in response to NG (Fig. 2C). The average baseline diameter of the portal vein was 10.9 ± 1.7 mm. Diameter increased at 2 min to 11.9 ± 1.9 mm and remained dilated after 4 min with an average value of 12.0 ± 1.7 mm (n = 8, P < 0.05). CBFV decreased over the first 2 min of NG (~9.0 ± 2.3 cm/s, Fig. 3A) then remained lower throughout the observation period. Two calculated resistance indexes revealed opposite effects. CVRi increased at 2 min (0.30 ± 0.15 mmHg·cm⁻¹·s⁻¹, Fig. 3B) then remained elevated until the end of the test. However, the Doppler resistance index, RI, remained constant for the first 2 min and decreased significantly at 3 min after NG stimulation (~0.025 ± 0.042, Fig. 3C).

Femoral blood flow was reduced by 49.1 ± 22.1 ml·min⁻¹·100 ml tissue⁻¹ (Fig. 4A, P < 0.05). This resulted primarily though a large reduction in downstream vascular conductance (Fig. 4B, P < 0.05) as the diameter of the superficial femoral artery increased after NG (11.7 ± 6.6 mm and 13.9 ± 10.2 mm for EX and CON, respectively, Fig. 4C, P < 0.05). The pattern of the blood velocity waveform (Fig. 5) changed dramatically during NG with the forward flow followed immediately by a large negative component.

Effects of HDBR. After HDBR, resting MAP, SBP, and PP were lower in the CON group with no change in the EX group (Fig. 1). A significant HDBR × NG interaction effect (P <
(0.05) was detected for SBP and PP whereby both variables decreased more in post-HDBR compared with pre-HDBR.

The HR responses to NG after HDBR were characterized by a significant three-way interaction of HDBR × group × NG effect (Fig. 2). Further analysis of this interaction showed that baseline HR, measured before NG stimulation, was elevated in CON but not in the EX group. In response to NG, both groups had greater HR increases post-HDBR. Peak increases in HR pre-HDBR (13.7 ± 5.8 and 13.0 ± 5.2 beats/min for EX and CON, respectively) were significantly less than post-HDBR with a smaller HDBR increase for EX than CON (18.8 ± 6.6 and 24.9 ± 7.7 beats/min for EX and CON, respectively, Fig. 2). There were no HDBR effects for SV (Fig. 2B), Q (Fig. 2D), or TPR (Fig. 2C).

After HDBR, CBFV and CVRi responses to NG were not statistically different from pre-HDBR (Fig. 3). There was a significant reduction in RI post-HDBR in both the EX and CON groups (HDBR main effect), but there was no difference in the response to NG. Baseline femoral blood flow, conductance, and diameter were not significantly different post-HDBR, and the responses to NG, including the change in diameter (13.9 ± 13.6% and 18.6 ± 17.2% for EX and CON, respectively), were also not changed (Fig. 4).

**DISCUSSION**

Sublingual NG was used in the present study to investigate the overall cardiovascular response to an exogenous source of NO and to determine if the responses were altered after 56 days of HDBR. The pre-HDBR responses to NG were consistent with previous research with an increase in heart rate (7), reduction in cardiac stroke volume (39), and lower arterial systolic, mean, and pulse pressure (22, 38, 39). A novel observation was the almost 50% reduction in femoral blood flow that reflected a marked reduction in downstream vascular conductance as demonstrated by the large reverse (negative) component, shown in Fig. 5, of the Doppler ultrasound trace (2). Mean cerebral blood flow velocity was reduced in response to NG, but the reductions in the Doppler resistance index suggested that brain blood flow might have increased or at least remained constant as observed previously using other imaging techniques (8, 11, 12, 42).

This is the first study to explore the potential effects of an exercise countermeasure on the hemodynamic responses to NG in women after HDBR. Consistent with our hypothesis and in agreement with a shorter duration HDBR study of men (7), there was a greater increase in HR with NG stimulation that was attenuated by the exercise countermeasure. Conversely, post-HDBR there was a greater reduction in both systolic blood pressure and arterial pulse pressure that was not affected by the exercise countermeasure. No effects of HDBR were seen for any of the cerebrovascular indicators examined in this study. Similarly, there were no differences in femoral artery dilation responses to NG, which was consistent with results from two studies (7, 30) but contrasted with the results from the Berlin Bed Rest Study in men (5). Overall, these data revealed only small changes in the cardiovascular responses to NG after HDBR, which tend to contrast with previously reported data from both human males and rodent models.
Overall responses to NG. This study provided a comprehensive view of the cardiovascular response of young, healthy, women to sublingual NG both before and after 56 days of HDBR. Previous work has focused on regional responses to NG in men. NG is an NO donor acting primarily on vascular smooth muscle (10, 21), but potentially affecting the endothelium (26). The major clinical application of NG has been to alleviate the effects of angina by reducing the preload on the heart through venodilation and by the direct action of dilating coronary arteries (1). In research settings, NG is used extensively in the study of vasodilatory properties of conduit arteries as a method for eliciting maximal vasodilation independent of the endothelium (5, 7, 18). In the present study, NG was used to determine hemodynamic responses to NO both before and after HDBR.

There was evidence in the present study from the decline in SV that the cardiac preload was reduced by NG. The decline in SV was consistent with data from hypertensive men (39) but in contrast to that study Q˙ was elevated at 3 min, potentially as a function of the increase in HR resulting from baroreflex compensation for the decline in SBP and MAP. The different responses could reflect a sex difference in the effect of NG or could simply reflect the contrast between hypertensive patients and the healthy subjects of the present study.

The peripheral vascular responses to NG suggested the dilation of conduit arteries but also a marked peripheral vasoconstriction as seen by the dilation of the superficial femoral artery, but reduction in superficial femoral blood flow. Despite this local effect, the overall change in TPR was very small, only significantly reduced at 3 min when Q˙ was elevated, suggesting that other vascular beds, possibly the splanchnic (see below), had directionally opposite changes in vascular resistance than the peripheral vasculature. Similar increases in vascular resistance in the limbs have been previously reported in studies examining blood flow control in the dog (6, 28) and are probably resultant from an initial elevation in muscle sympathetic nerve activity with NG (27). To the best of our knowledge, this is the first study to demonstrate a marked reduction in femoral vascular conductance in response to NG in women.

The overall maintenance of TPR with clear indications of increased local vasoconstriction in the legs suggests that other vascular beds must have had reduction in resistance. The increase in portal vein diameter was expected due to the venodilator actions of NG (1). Given a positive relationship between portal vein diameter and blood flow (4) it is possible that portal vein flow increased with NG as found previously in humans (32) and pigs (25), potentially due to a reduction in splanchnic vascular resistance thereby offsetting the observed increase in peripheral vascular resistance and allowing for the maintenance of TPR; however, an incomplete data set with no measures of velocity suggests caution in interpreting the impact on portal vein blood flow.

There were marked reductions in CBFV after sublingual NG. While it is often assumed that the cross-sectional area of the middle cerebral artery does not change during an experi-
mental manipulation and that observed changes in velocity are proportional to changes in volume flow, this does not appear to be correct under the current conditions as MRI angiography has confirmed the dilation of the MCA with NG (20). This study provides further support for the dilation of the MCA with NG in that the Doppler resistance index (RI) indicated a reduction in resistance in contrast to the calculated CVRi, which increased. The reduced RI coincident with lower MAP after NG might suggest relatively unchanged cerebral blood flow, which is consistent with observations from positron-emission tomography (PET) scanning techniques following NG stimulation (8, 42).

Effects of NG after HDBR. Long-duration HDBR as used in the present study is associated with a general cardiovascular deconditioning (29). The present study was designed with an exercise countermeasure (EX) intervention to contrast with the control (CON) group that simply rested in bed throughout the entire 56 days. The EX group showed preservation of baseline values of HR and SV in the post-HDBR baseline condition while there was a significant increase in HR in the CON group.

Fig. 3. Responses of cerebral blood flow velocity (CBFV) (A), cerebrovascular resistance index (CVRi) (B), and resistance index (RI) (C) to NG administered at time = 0 before and after HDBR (symbols as in Fig. 1). The assessment of NG effects are shown as differences from time = 0 (*P < 0.05). No HDBR effects were found with either CBFV or CVRi; however, post-HDBR, RI was significantly reduced (#P < 0.05). CON, n = 6; EX, n = 7.

Fig. 4. Responses of superficial femoral artery flow (A), conductance (B), and diameter (C) in response to NG pre-HDBR (solid bars) and post-HDBR (hatched bars) for EX (gray) and CON (white). All variables showed a significant effect of NG (*P < 0.05). No HDBR effects were found for femoral flow, conductance, or diameter. CON, n = 5; EX, n = 6.
Post-HDBR, SV for the CON group was reduced by 16% compared with pre-HDBR values but this was not significant as technical problems limited the sample size. However, other data sets for different experiments conducted on these same women found significant reductions in SV in the CON group (16, 19) as well as evidence of reduced left ventricular volume and mass in the CON group and a significant increase in mass for the EX group (15). In combination with these results, reductions in total blood volume of −9% for CON and −4% for EX (19) probably contributed to the elevation in HR required to maintain cardiac output in the CON group as observed in this study.

There were no differences in the diameter of the superficial femoral artery, blood flow, or vascular conductance as a function of HDBR. Also, there were no changes in the response to sublingual NG in either the CON or the EX groups with HDBR. Previous research with men found reduction in leg artery diameter after 52 days horizontal bed rest (5) and after 60 days HDBR with or without resistance or vibration exercise countermeasures (40). It is possible that the women in the present study were not as physically active prior to HDBR as the men so there was less difference between pre- and post-HDBR values, but technical difficulties reduced the sample size to 5 so the negative findings in the women should be viewed with caution. In the present study, the EX group showed no differences in pre- and post-HDBR baseline diameter values, suggesting that the exercise countermeasures, which included treadmill running, were sufficient to maintain vascular properties, while resistance or vibration exercise in men was not sufficient to maintain femoral artery diameter (40).

The findings of no difference in response to NG after HDBR in the women of the present study contrast with data from the study of men in horizontal bed rest (5), but are similar to the findings for the tibial (30) and brachial (7) arteries of men after HDBR. In another investigation of the same subjects in the present study, Demiot et al. (14) showed that microvascular dilation of skin vessels to sodium nitroprusside was not affected by HDBR, suggesting that peripheral vascular responses to NO from donor molecules was not altered by HDBR in women. Although there are no data for potential changes in the NOS expression of the conduit arteries studied in the present study, muscle biopsy samples from these same women revealed that the exercise countermeasures were associated with an increased number of capillaries and greater total eNOS (35).

No changes in the relative density of eNOS were reported but sarcolemmal-associated NOS1 was reduced in the CON and increased in the EX women.

No HDBR effect was observed for CBFV or CVRi for either baseline values or in responses to NG, but there was a significant baseline reduction in RI post-HDBR. The interpretation of the differences between CVRi and RI response to NG could suggest a dilation of the MCA, but interpretation of HDBR effects remain uncertain. Unchanged CBFV has been previously reported with HDBR and spaceflight (3, 29, 47) and has been taken to reflect no changes in cerebral blood flow as indicated by measures of common carotid artery flow in cosmonauts (3). However, the common carotid artery supplies both cerebral and extracranial vascular beds and therefore might not reflect changes in cerebral blood flow. Animal models of microgravity exposure have shown vascular smooth muscle hypertrophy of cerebral blood vessels (23, 43, 45, 46) and a smaller luminal cross-sectional area (45) that is associated with reduced cerebral blood flow (43, 44). The different cerebrovascular response to head-down position between animals and humans might be due to the application of quantitative methods in animals compared with indirect indicators of brain blood flow in humans. These differences could potentially be resolved by application of quantitative ultrasound techniques to assess blood flow through the internal carotid artery.

Limitations. The WISE bed rest study was conducted as a large, multi-investigator study of the effects of long-duration HDBR and the potential benefits of exercise countermeasures. Technical issues related to the acquisition of the Doppler signals for cardiac stroke volume and leg blood flow reduced the sample size, and it was impossible to repeat the measurements. Therefore, while the statistical analysis did not find significant bed rest effects on cardiac SV and femoral artery blood flow, it is possible that the main effects would have been significant bed rest effects on cardiac SV and femoral artery blood flow, it is possible that the main effects would have been significant bed rest effects on cardiac SV and femoral artery blood flow, it is possible that the main effects would have been significant bed rest effects on cardiac SV and femoral artery blood flow, it is possible that the main effects would have been significant bed rest effects on cardiac SV and femoral artery blood flow, it is possible that the main effects would have been significant bed rest effects on cardiac SV and femoral artery blood flow, it is possible that the main effects would have been significant bed rest effects on cardiac SV and femoral artery blood flow, it is possible that the main effects would have been significant bed rest effects on cardiac SV and femoral artery blood flow, it is possible that the main effects would have been significant bed rest effects on cardiac SV and femoral artery blood flow, it is possible that the main effects would have been significant bed rest effects on cardiac SV and femoral artery blood flow.
contrary to previous work in men, no changes were seen in resting superficial femoral diameter post-HDBR, and no differences were seen in the femoral vascular response to NG, which included a large reduction in femoral vascular conductance. These data indicate that the primary impact of HDBR on the women of this study was on cardiovascular deconditioning and that there were no changes in the response to NG as an exogenous source of NO.

ACKNOWLEDGMENTS
The study WISE-2005 was sponsored by the European Space Agency (ESA), the National Aeronautics and Space Administration of the USA (NASA), the Canadian Space Agency (CSA), and the French “Centre National d’Études Spatiales” (CNES), which has been the “Promoteur” of the study according to French law. The study was performed by MEDES, Institute for Space Physiology and Medicine, in Toulouse, France. The authors are grateful for the outstanding contributions of the 24 WISE women who committed themselves to the success of this study. The authors thank the MEDES staff for excellent support throughout.

GRANTS
This research was supported by contract 9F007–033004/001/ST from the Canadian Space Agency to R. L. Hughson and J. K. Shoemaker, and CNES Grant no. 4900000367, and in part by the Natural Sciences and Engineering Research Council (RGPIN6473–07). H. Edgell was supported by a Graduate Scholarship from the Natural Sciences and Engineering Research Council and by the President’s Scholarship of the University of Waterloo. K. A. Zuj was supported by a Graduate Scholarship from the Canadian Institutes of Health Research and by the President’s Scholarship of the University of Waterloo.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES

J Appl Physiol • doi:10.1152/japplphysiol.00445.2012 • www.jappl.org


