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AUTONOMIC FUNCTIONS ASSOCIATED WITH BLOOD PRESSURE REGULATION AND ORTHOSTATIC PERFORMANCE IN WOMEN

UNITED STATES ARMY MEDICAL
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Fort Detrick, Maryland 21702-5012

VICTOR A. CONVERTINO

AEROSPACE MEDICINE DIRECTORATE
CLINICAL SCIENCES DIVISION
PHYSIOLOGICAL RESEARCH BRANCH
2507 Kennedy Circle
Brooks Air Force Base, TX 78235-5117

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Victor A. Convertino

VICTOR A. CONVERTINO, Ph.D.
Project Scientist

Daniel L Va Syoc

DANIEL L. VAN SYOC, Lt Col, USAF, MC, CFS
Acting Chief, Clinical Sciences Division

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Victor A. Convertino

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Armstrong Laboratory (AFMC)
Aerospace Medicine Directorate
Clinical Sciences Division, Physiological Research Branch
2507 Kennedy Circle
Brooks AFB, TX 78235-5117

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Functions of baroreflex control of heart rate and vascular resistance, adrenoreceptor responsiveness, indices of baseline vagal and sympathetic tone, plasma volume, and venous compliance were compared in men and women to test the hypothesis that greater orthostatic intolerance in women would be associated with impairment of specific mechanisms of blood pressure regulation. Heart rate (HR), stroke volume (SV), cardiac output (Q), mean arterial blood pressure (MAP), forearm (FVR) and leg (LVR) vascular resistance, catecholamines (NE), and changes in leg volume (%LV) were measured during various protocols of lower body negative pressure (LBNP), carotid stimulation, and infusions of adrenoreceptor agonists in 10 females and 10 males matched for age and fitness. LBNP tolerance for women (797 ± 63 mmHg•min) was 35% lower (P = 0.0017) than for men. At presyncope, SV, Q, MAP and %LV were tolerance in females was associated with impairment of the heart rate response to carotid baroreceptor stimulation, lower baseline cardiac vagal activity, greater decline in Q and SV induced by LBNP, increased β1-adrenoreceptor responsiveness, greater vasoconstriction under equal LBNP, lower levels of NE at presyncope, and lower blood volume. Results support the hypothesis that women have significant deficiencies in mechanisms that underlie blood pressure regulation under orthostatic challenge. These findings should be considered in selection and training of women for military combat, especially in combat missions requiring high-G aerial maneuvers.

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FOREWORD

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Introduction

Adequate maintenance of arterial blood pressure during orthostatic challenges in combat environments is critical to successful combat performance. Impaired cardiovascular mechanisms that normally contribute to blood pressure regulation could increase the risk of failure for combat task performance by enhancing the potential for impaired or loss of consciousness. Recent data reported from several investigations provide evidence that females have lower tolerance to various orthostatic challenges compared to males. In one study (Hordinsky et al. 1981) observed tolerance to lower body negative pressure (LBNP) to be as much as 15% lower in women than men. In another study (Montgomery et al 1977), 6 men and 4 women were exposed to three tests consisting of 20, 40, and 60 mmHg LBNP for 5 min each. Although this experiment was not designed to determine orthostatic tolerance of these subjects, it was reported that all men completed all LBNP tests while women completed only 2 of their 12 tests. Women have also predicted lower tolerance to passive +3Gz acceleration (Ludwig et al 1987). In more recent experiments designed to elicit tolerance, orthostatic performance was 22%-61% lower in women than men (Hogan et al 1995; White et al 1996).

Orthostatic compromise may include increased venous compliance of the lower extremities (Hoffler 1977; Luft et al 1976), reduced blood volume (Convertino 1987, 1995; Ludwig & Convertino 1994), impaired baroreflex function (Convertino 1991, 1995; Convertino et al 1990, 1991, Cowley et al 1973; Engelke et al 1994, 1995, 1996; Fritsch et al 1992), and decreased cardiac filling pressure and left ventricular end-diastolic volume, with consequent lowering of stroke volume and cardiac output (Levine 1993). It is reasonable to suspect that differences in orthostatic tolerance between men and women are associated with differences in some or all of these mechanisms. When measured at the same absolute orthostatic challenge (e.g., 50 mmHg LBNP), women demonstrate greater heart rate (Frey et al 1986; Hudson et al 1987; Montgomery et al 1977), less increase in systemic vascular resistance (Frey & Hoffler 1988), and less blood pooling in the legs (Frey & Hoffler 1988) with greater blood pooling in the pelvic region (White & Montgomery 1996). These observations may indicate that women have compromised cardiovascular functions compared to those of men and have lead to hypotheses that women may respond to orthostatic challenges with vagal withdrawal while men may respond with greater sympathetic stimulation to the peripheral vasculature (Frey et al 1986, 1988). However, we are unaware of any investigations that have elucidated differences in various cardiovascular characteristics and autonomic functions between men and women that are associated with differences in orthostatic performance. Such information could prove critical to design of life-support equipment and training procedures that enhance combat readiness in ground, naval, and aerial military personnel.

Functions of carotid and aortic baroreflex control of heart rate, cardiopulmonary baroreflex control of vascular resistance, adrenoreceptor responsiveness, neuroendocrine responsiveness, baseline vagal and sympathetic tone, and venous compliance were compared in men and women to test the hypothesis that greater orthostatic intolerance in women would be associated with impairment of specific mechanisms of blood pressure regulation.

Methods

Subjects. Ten women and ten men matched for age volunteered to participate as subjects for this investigation after all procedures and risks associated with the experiments were explained and their voluntary written informed consent to participate in the study was obtained as required by AFR 169-3. All procedures were approved by the Institutional Review Board at Brooks Air Force Base, TX. The physical characteristics of the groups are presented in Table 1. All subjects were nonsmokers and normotensive, and their selection into the study was based on results of a screening evaluation comprised of a detailed medical history, physical examination, blood chemistry analysis, urinalysis, and resting and treadmill electrocardiogram to assure

absence of cardiovascular disease. To minimize potential variability in responses associated with menstrual cycles, female subjects were tested during the follicular phase of their menstrual cycle (days 3-10 with day one the first day of menses). All women completed an initial urine pregnancy test within 24 hours of test participation to assure that they were not pregnant during the experimental procedures. Individuals with a history of hyperthyroidism or taking prescription drugs were excluded and subjects refrained from taking medication at the time of the experiments, with the exception of oral contraceptives. Because of the potential effect of vascular volume and baroreflex function, subjects were asked to refrain from exercise and stimulants such as caffeine and other non-prescription drugs 48 hours prior to testing. During an orientation period that preceded the experiments, subjects were made familiar with the laboratory, the protocol, and procedures.

Experimental protocol. The experimental protocol consisted of 3 days of tests and measurements of physical and physiological functions. On day 1, subjects underwent the following tests: 1) cardiopulmonary baroreflex control of peripheral vascular resistance; 2) aortic baroreflex control of heart rate; 3) adrenergic receptor responsiveness; and 4) measurement of plasma volume. On day 2, subjects underwent measurements for: 1) heart rate variability; 2) carotid baroreflex control of heart rate; 3) heart rate and blood pressure responses to a Valsalva maneuver; 4) measurement of leg volume and compliance; and 5) tolerance to lower body negative pressure (LBNP). On the third test day, subjects underwent tests for measurement of their maximal oxygen uptake (VO_2max) and estimated body composition. All tests conducted on days 1 and 2 were always separated from VO_2max tests by a minimum of 48 hours. All measurements were conducted at the same time of day and in the same sequence.

Maximal oxygen uptake (VO_2max). A graded treadmill protocol was used to elicit VO_2max. The exercise protocol began with the subject walking at a speed of 2.0 miles per hour (mph) and 0% grade for 1 min followed by 3.0 mph for 2 min. At constant grade (0%), the treadmill speed was increased by 1 mph each 30 sec until the subject indicated that he/she had reached a comfortable running speed. At this point in the test, speed was maintained constant and the grade of the treadmill was increased by 2% every minute until the subject reached volitional exhaustion. Subjects breathed through a low-resistance valve, and the volume and composition of expired gas was collected and analyzed on a Beckman model H metabolic measurement cart for the fractions of mixed expired oxygen and carbon dioxide. Since body fat contributes significantly to the calculated difference in VO_2max between males and females (Drinkwater 1984), we expressed VO_2max as a function of estimated lean body mass ($ml \cdot kg^{-1}$ LBM$\cdot min^{-1}$) for the purpose of matching the fitness level of our men and women (Cureton 1981).

Estimation of body composition. Skinfolds were taken at five sites for the females (thigh, illium, abdomen, triceps, scapula) and six sites for the males (chest, thigh, illium, abdomen, triceps, scapula). The sum of the skinfold measurements for each gender was used to estimate percentage of body fat according to the formula of Pollock et al (1980).

Lower body negative pressure (LBNP). Orthostatic tolerance was determined while the subject was in the supine posture by progressively reducing pressure around the lower body relative to ambient pressure. The LBNP protocol consisted of a 2-min baseline period followed by decompression to -15 and -30 mmHg for 10 min each. Further 10-mmHg reductions in pressure were added every 3 min until test termination. The duration of the test was determined by: a) completion of 3 min at -100 mmHg, b) onset of presyncopal symptoms including a drop in systolic blood pressure $\geq$ 15 mmHg and or sudden bradycardia $\geq$15 beats, c) progressive reduction in systolic blood pressure to $\leq$ 80 mmHg, and d) onset of symptoms such as nausea, sweating, grey-out, or dizziness. A cumulative stress index for LBNP tolerance was

derived by summing the products of negative pressure in mmHg and time in minutes during each pressure stage (Luft et al 1976).

Hemodynamic measurements. Baseline systolic and diastolic arterial blood pressures were measured non-invasively from the left arm with a Collins automated sphygmomanometer blood pressure measurement device. In addition, beat-by-beat continuous measurement of arterial blood pressure was monitored during Valsalva maneuvers, tests for adrenoreceptor responsiveness, and LBNP exposures using finger photoplethysmographic techniques (Finapres, Ohmeda Inc.) with the hand held at the level of the mid-sternum. Total finger arterial volume under the blood pressure finger cuff was maintained constant by modulating cuff pressure in parallel with intraarterial pressure using an electropneumatic servo feedback system and measured with an infrared plethysmograph. Blood pressure measurements obtained by auscultation were used to verify readings obtained from the Finapres. Mean arterial pressure (MAP) was calculated by dividing the sum of systolic pressure and twice diastolic pressure by three. Four silver tape electrodes, two around the neck and two around the thorax, were attached to a Minnesota Impedance Cardiograph (Model 304B) for non-invasive rheographic determination of stroke volume during rest and LBNP (Convertino et al 1994). Continuous heart rate (HR) was recorded during all tests using a four-lead electrocardiogram. Cardiac output (Q) during rest and LBNP was calculated as the product of heart rate and stroke volume. Total systemic peripheral resistance (TPR) was calculated by dividing MAP by Q. Changes in leg volume during each LBNP stage were measured with a strain gauge placed around the point of maximum girth of the left calf. Percent changes in calf volume (ml/100ml) were calculated from circumference changes.

Forearm and leg blood flows were measured by venous occlusion plethysmography using a dual loop mercury-in-silastic strain gauge placed around the left forearm or calf at the point of maximal circumference. Venous outflow from the forearm or calf was prevented by the placement of a cuff around the brachium just above the elbow or around the thigh just above the knee using an occlusion pressure of +40 mmHg for the arm and +60 mmHg for the leg. Arterial occlusion to reduce blood flow to the hand or foot was applied by a wrist or an ankle cuff inflated at a pressure of +250 mmHg. Following wrist or ankle cuff inflation for 1 min, venous occlusion was initiated for 10 s followed by its release for 10 s for six sequential occlusions. The relative change (percent) in strain gauge length over 10 s was quantified as a volume of blood per unit time, i.e., flow. Ten-second occlusions were repeated during the final 2 min of drug infusion at each stage of the adrenoreceptor tests (leg) and LBNP tests (forearm), and the average of the six measurements represented the flow for that test condition. An index of forearm or leg vascular resistance was calculated by dividing mean arterial pressure by average flow during the final 2 min of each test condition and expressed as peripheral resistance units (pru in mmHg•min•100 ml•ml^{-1}).

Subjects laid in the right lateral decubitus position and a 20-gauge catheter was inserted into an antecubital vein of the dependent right arm for measurement of estimated central venous pressure (CVP, Gauer & Seiker 1956). With the right arm being suspended, the valves in the veins become incompetent resulting in an unimpeded column of blood. Under these conditions, the pressure in the large vein of the right arm reflect CVP when the pressure transducer is centered at heart level (Gauer & Seiker 1956). The catheter was then cleared with isotonic saline solution and connected to a Baxter Uniflow™ model 43-260 pressure transducer for measurement of venous pressure. Prior to connection to the catheter, the transducer was positioned at the level of the mid-sternum with a ruler and level, and was calibrated with a known 20 mmHg pressure introduced from a digital manometer (Omega).

Leg volume and compliance. A series of 5 circumference measurements placed 5 cm apart on each thigh and calf was performed on each subject. The total geometric volume of each thigh and calf segment was estimated by calculating the volume of each sequential segment from its

mid-circumference value and length, and summing the values of all segments. This procedure assumes that each segment approximates the shape of a cylinder. Total leg volume was calculated as the sum of all segments of both legs. Compliance of both legs was measured during supine rest using a Whitney strain gauge placed at the point of greatest calf circumference. Following 30 min of supine control, the left leg was slightly elevated (~4 in) at the ankle and an occlusion cuff placed just above the knee was inflated to 30 mmHg for 180 s. Leg compliance was calculated by dividing the volume change (ml/100ml) at a plateau (i.e., point at which venous pressure equals cuff pressure) by the cuff pressure and expressed as Δvol%/ΔmmHg. The value for leg compliance was multiplied by 100 for convenience.

Heart rate variability. Each subject underwent collection of ECG data during 5 min in which the respiratory rate was controlled by the subject breathing at a constant rate of 15 breaths per min using a metronome. An index of cardiac vagal activity was assessed by calculating the standard deviation of R-R intervals (Crandall et al 1994).

Measurement of carotid-cardiac baroreflex. A silastic neck chamber device covering the area of the carotid arteries was utilized to elicit carotid baroreceptor stimulus-cardiac reflex response relationships. The stimulus profile consisted of raising neck chamber to 40 mmHg for five heart beats, followed by successive 15-mmHg R-wave triggered decrements to -65 mmHg. This produced a series of stair-stepped neck pressure reductions that were superimposed on seven successive carotid arterial pulses. To avoid respiration-related variations of cardiac vagal outflow (Eckberg 1983), neck pressure changes were applied only during held mid-expiration. A test session consisted of five successful applications of the neck pressure sequences. Each sequence lasted approximately 15 sec, and each test session lasted 15 min. Individual trials were discarded if the subject breathed during the stimulus sequence, or if the neck chamber failed to seal adequately. Neck chamber pressures and R-R intervals for the five acceptable sequences were averaged for each test session. Systolic pressure was measured via auscultation before and after each neck chamber test session and carotid pressure was calculated as systolic pressure minus neck chamber pressure applied during the heart beat; this calculation assumes complete transfer of pressure in the cuff to the carotid arteries and does not alter the comparisons of baroreflex parameters across time (Kasting et al 1987). A stimulus-response relationship of the baroreflex was derived by plotting R-R intervals at each pressure step against respective carotid distending pressure. From the average of each 5-trial sequence of responses, baroreflex relationships were reduced to the following parameters for statistical comparisons: 1) maximum slope to provide an index of reflex sensitivity; 2) position of operational point ([control R-R - minimum R-R]/range x 100%) to provide information about the position from which the baseline heart rate functions on the stimulus-response relationship; and 3) the estimated carotid pressure at maximum slope, i.e., point halfway between the pressures bracketing the maximum slope, to identify the point of maximal buffering. To determine the segment with the steepest slope, least squares linear regression analysis was applied to every set of three consecutive points on the response relationship.

Measurement of aortic-cardiac baroreflex. Subjects were instrumented for beat-to-beat measurements of heart rate, arterial pressures, and estimated CVP, and placed in the LBNP chamber. Following instrumentation, subjects rested quietly for 15 minutes, after which three minutes of baseline data were obtained. The aortic-cardiac baroreflex was assessed using a technique previously described (Shi et al 1993). The protocol was initiated with a steady-state infusion of phenylephrine (PE) into an antecubital vein of the arm opposite to that used for estimating CVP, with a goal of increasing mean arterial pressure (MAP) by 15 mmHg. PE was used because of its known baroreflex-mediated heart rate effect on aortic baroreceptors (Shi et al 1993). Every two to three minutes, the infusion rate was increased (ranging from 30 to 90 $\mu g \cdot min^{-1}$) until the desired elevation of MAP was attained. Once MAP reached its new steady-state level, the infusion rate was maintained constant throughout the remaining procedures.

After three minutes of data collection at the desired PE-induced hypertension, LBNP was applied (ranging from 5 to 20 mmHg) until estimated CVP was returned to pre-PE infusion levels. One minute of data were recorded once CVP had returned to baseline. Neck pressure equal to 1.4 times the increase in MAP was then applied to the anterior two-thirds of the neck with the intention of returning mean carotid sinus transmural pressure to pre-PE infusion values. This level of neck pressure was chosen to estimate complete transmission of pressure through the tissue of the neck (Ludbrook et al 1977). One minute of data were recorded during application of neck pressure. Clamping of CVP and carotid pressure at baseline levels by application of LBNP and neck pressure, respectively, was designed to remove PE-induced loading of cardiopulmonary and carotid baroreceptors, thereby isolating the influence of the aortic baroreceptors. Responsiveness of the aortic baroreflex control of heart rate was calculated as the ratio of the difference in HR to MAP (ΔHR/ΔMAP) between pre-PE infusion and post-PE infusion with LBNP and neck pressure. At the completion of the protocol, the subject was monitored until blood pressure returned to pre-testing baseline.

Measurement of cardiopulmonary baroreflex control of forearm vascular resistance. Subjects laid in the LBNP device in the right decubitus position for measurements of estimated CVP and the left arm was raised at heart level and used for simultansous forearm blood flow measurements. The LBNP protocol began with a 2-min baseline rest period with the LBNP pressure at 0 mmHg followed by continuous decompression at 5, 10, 15, and 20 mmHg every 2 min. The LBNP protocol was designed to selectively elicit the vascular constriction response caused by unloading the cardiopulmonary baroreceptors. Heart rate, forearm blood flow, and CVP were measured continuously throughout the LBNP test. Because forearm blood flow was measured continuously during LBNP, blood pressure measurement during this protocol was limited to the baseline and final stage of -20 mmHg after the final forearm blood flow measurement was completed.

Measurement of baroreflex responses to Valsalva maneuver. Each subject underwent a Valsalva maneuver that consisted of 15 s normal breathing to establish baseline, 15 s of Valsalva strain at 30 mmHg expiratory pressure, and 30 s post-strain. A small leak in the system prevented the subject from maintaining the expiratory pressure by occluding the glottis. Subjects were instructed to remain quiet and still during both the baseline and post-strain collection periods. Following baseline collection, the subject was asked to give a ready signal at the end of a normal inspiration. At this point, the subject was instructed to begin blowing into a mouthpiece connected to a calibrated pressure transducer (Propper Analog Manometer). After 15 s at 30 mmHg expiratory pressure, the subject was instructed to release pressure and breathe normally after the mouthpiece was removed. An aneroid guage positioned in front of the subject provided feedback on the expiratory pressure. Heart rate and blood pressure responses from three trials were averaged in a phase-by-phase manner for baseline, phase I, early phase II, and late phase II according to the technique described by Luster et al (1996). For phase I, ΔMAP was used in the analyses as an index of vascular volume (Stegemann et al, 1988). For late phase II, ΔMAP was used in the analyses as a marker for sensitivity of baroreflex-mediated control of peripheral vascular resistance (Sandroni et al 1991). The ratio ΔHR/ΔMAP was used in the analyses for early phase II because of its usefulness in describing integrated cardiac baroreflex responsiveness (Sandroni et al 1991; Stegemann et al, 1988).

Measurements of adrenoreceptor responsiveness. Following baseline measurements of heart rate, blood pressure, and leg blood flow, three graded infusions of α- and β-adrenoreceptor agonists were performed with isotonic saline as a vehicle. Each infusion interval was 9 min in duration to establish steady-state and allow adequate time for all measurements. The protocol and dosages of adrenoreceptor agonists were determined by laboratory experience to produce safe but significant physiological responses (Convertino et al

1997). The total volume infused was less than 50 ml. A recovery period of at least 25 min was allowed between the two agonist infusion protocols to allow hemodynamic measurements to return to pre-infusion baseline levels. During both infusion protocols, constant monitoring of beat-to-beat blood pressure and heart rate was performed and leg blood flows were measured at each infusion level.

α_1-adrenoreceptor responsiveness. Graded infusion of the α1-adrenoreceptor agonist phenylephrine (PE) was used to assess the responsiveness of these vascular receptors. PE was infused at three graded constant rates of 0.25, 0.50, and 1.00 μg/kg/min. An elevation of systolic blood pressure of 20 mmHg above or reflex reduction of heart rate 20 bpm below resting baseline were pre-determined end points for test termination. No tests were terminated using these criteria. The response of α1-adrenoreceptors was assessed by relating the PE dose with the reduction in leg vascular resistance. The relationships between PE doses and leg vascular resistance were linear, and the slopes describing these relationships were used to represent an index of α_1–adrenoreceptor responsiveness.

β-adrenoreceptor responsiveness. After heart rate and blood pressure had been allowed to return to baseline levels following PE infusions, infusions of isoproterenol (ISO) were used to assess the responsiveness of β_1- and β_2-adrenoreceptors. ISO was infused at three graded constant rates of 0.005, 0.01, and 0.02 μg/kg/min. An elevation of heart rate by 35 beats per minute (bpm) above resting baseline was the pre-determined end point for test termination. The test for one of the female subjects was terminated during the final infusion rate using this criteria. Linear regression relationships were then constructed relating the increase in heart rate and the decrease in leg vascular resistance to the dose of isoproterenol. The slopes describing the linear stimulus-response relationship between the dose of ISO and heart rate and leg vascular resistance provided a measure of the systemic responsiveness of β_1- and β_2-adrenoreceptors, respectively.

Plasma measurements. A 30-ml antecubital venous blood sample was taken without stasis before and immediately following termination of the LBNP test to determine the response of norepinephrine (NE) and epinephrine (E) to orthostasis. Immediately following each withdrawal, whole blood was taken from the syringe and transferred to a chilled tube containing sodium EDTA. Microhematocrit and hemoglobin (Coulter S+4 system) were measured in triplicate using ~1 ml of the EDTA-treated whole blood. The remaining whole blood was centrifuged at 2000 g for 20 min at 4°C. Immediately after centrifugation, the plasma was aliquoted and stored frozen until assays were performed.

Plasma NE and E concentrations were measured by high performance liquid chromatography (Waters). An internal standard, 3,4-dihydroxybenzylamine (DHBA), was added to the plasma samples and pH was adjusted with a buffer. Aluminum oxide (alumina) was added to adsorb NE and E. After centrifugation, the plasma was discarded and the alumina was washed with a dilute buffer solution to remove unwanted plasma residue. An acidic solution was added to the alumina, mixed and centrifuged. The eluent was then injected onto a C^{18} reverse phase column using a Waters WISP 712 auto-injector. A Waters 460 electrochemical detector was used to determine the concentrations of NE and E in the samples. Assayed external standards were included with each run. The auto-injector and detector were interfaced with a Digital 380 computer using Waters software. The within assay coefficient of variation was 1.4%; between assay coefficient of variation was 3.8%.

Plasma volume was determined by a modified dilution technique (Greenleaf et al, 1979) using sterile solutions of Evans blue dye contained in 10-ml ampules (The New World Trading Corp., DeBary, FL). After each subject was stabilized in the supine position for 30 min, a pre-injection control blood sample was drawn followed by an intravenous injection of 12.5 mg of dye

diluted with 2.5 ml isotonic saline solution. One ml of plasma from a 10-min post-injection blood sample was passed through a wood-cellulose powder (Solka-Floc SW-40A) chromatographic column so that the dye could be absorbed. The absorbed dye was eluted from the column using a 1:1 water-acetone solution (pH = 7.0) and collected in a 10-ml volumetric flask. The post-injection solution was compared with 1-ml samples from a preinjection time (zero control) and a standard dye solution (1:50 dilution with distilled water), and all samples were read at 615 nm with a spectrophotometer. Total blood volume was calculated from the plasma volume and peripheral venous hematocrit measurements. Using these procedures in our laboratory, test-retest correlation coefficient for blood volume was 0.969 (N = 12) and the average changes were 82 ml (average $\%\Delta$ = 1.5%, N = 17), 75 ml (average $\%\Delta$ = 1.5%, N = 19), and 56 ml (average $\%\Delta$ = 1.1%, N = 23) when measurements were determined 4, 8, and 15 days apart, respectively (Greenleaf et al 1979), and was 0.881 (N = 7) with average change of 25 ml (average $\%\Delta$ = 0.7%) when measurements were determined 11 months apart (Convertino et al 1996). Total circulating plasma NE and E were calculated as the product of plasma volume and plasma NE and E concentrations as a index of baseline sympathetic activity and catecholamine release during LBNP (Goldstein et al 1995).

Statistical analysis. Descriptive statistics were performed for all variables. Results are presented as means ± 1 standard error. A one-way analysis of variance was performed for comparisons between the two groups of all variables and slopes of responses. A multivariate analysis of variance was used for group comparisons across LBNP levels.

Results

Subjects. A summary of the subject descriptive data for the two gender groups, together with the resultant F and P values from statistical analyses, is presented in Table 1. The female group had statistically lower ($P < 0.012$) height, weight, baseline systolic blood pressure, and VO_2max expressed per body weight, and higher ($P < 0.001$) baseline heart rate and estimated body fat. Age, maximal heart rate and VO_2max expressed per kg of lean body mass were not statistically distinguishable between the two groups.

Responses to LBNP. All subjects demonstrated intolerance to LBNP by the onset of presyncopal symptoms including a drop in mean blood pressure below 80 mmHg with subsequent bradycardia and various degrees of symptoms such as nausea, sweating, or dizziness. Cumulative LBNP index for the women (797 ± 63 mmHg•min) was 35% lower ($F(1,18) = 13.612$; $P = 0.0017$) than 1235 ± 101 mmHg•min for the men (Figure 1). At the LBNP level at which presyncope occurred, there were no distinguishable differences between females and males in heart rate, total peripheral resistance, and forearm vascular resistance. However, females demonstrated lower ($P \leq 0.035$) stroke volume, cardiac output, mean arterial pressure, and leg pooling than the males at the point of presyncope (Table 2). The females had greater elevations ($F(1,18) = 5.0946$, $P = 0.0367$) in thoracic impedance during LBNP at the point of presyncope (1.6 ± 0.3 ohms) compared to the males (0.7 ± 0.2 ohms).

Heart rate, stroke volume, Q, MAP, TPR and FVR responses to graded LBNP from 0 through -50 mmHg are illustrated in Figure 2. Both females and males demonstrated gradual reduction in stroke volume with a subsequent decrease in Q despite a compensatory elevation in heart rate. Mean arterial pressure showed little change in the face of lower Q as a result of increased peripheral vascular resistance. The rate, i.e., slope, of elevation in heart rate, and total peripheral and forearm vascular resistance and reduction in cardiac output were greater in the females than in the males (Table 3). Percent increase in leg volume during graded LBNP from 0 through -50 mmHg was greater ($F(1,14) = 13.0307$, $P = 0.0028$) in males compared to females while the elevation in thoracic impedance was less ($F(1,15) = 6.2196$, $P = 0.0241$) in males (Fig. 3).

Heart rate variability. The average standard deviation of R-R intervals during controlled breathing was less ($F(1,18) = 4.5459$, $P = 0.0470$) in women (42.9 ± 4.6 msec) compared to an average response of 64.2 ± 8.8 msec in the men.

Baroreflex Responses. The average stimulus-response relationships of the carotid-cardiac baroreflex for each group is presented in Figure 3. Females and males demonstrated similar operational points (37.9 ± 6.5% and 38.9 ± 7.8%, respectively; $F(1,18) = 0.0096$, $P = 0.9229$) and cartoid distending pressures at maximum slope (120 ± 9 mmHg and 124 ± 6 mmHg, respectively; $F(1,18) = 0.1261$, $P = 0.7266$) for the carotid baroreflex relationship. However, carotid-cardiac baroreflex responsiveness, i.e., maximum slope of the stimulus-response relationship, was lower ($F(1,18) = 6.4487$, $P = 0.0205$) in the women (2.65 ± 0.29 ms/mmHg) compared to that in the men (3.93 ± 0.41 ms/mmHg). Aortic-cardiac baroreflex sensitivity in the women (-0.92 ± 0.40 bpm/mmHg) could not be statistically distinguished ($F(1,18) = 0.2182$, $P = 0.6460$) from that in the men (-0.71 ± 0.20 bpm/mmHg).

Average stimulus-response characteristics of the cardiopulmonary baroreflex control of forearm vascular resistance for women and men are plotted in Figure 4. Differences in slopes ($\Delta FVR/\Delta CVP$) between the gender groups were compared by analyzing the least squares linear estimates generated by each subject. Average $\Delta FVR/\Delta CVP$ of the cardiopulmonary baroreflex response was 40% greater ($F(1,18) = 1.9863$, $P = 0.1758$) in the women (-4.1 ± 1.2 pru/mmHg) compared to the men (-2.6 ± 0.3 pru/mmHg).

Average responses of beat-to-beat heart rate and mean arterial pressure during phase I and early and late phase II of the Valsalva maneuver for female and male groups are plotted in Figure 5. Women demonstrated similar ($F(1,18) = 0.6759$, $P = 0.4218$) elevation in MAP (ΔMAP = 21.6 ± 1.9 mmHg) during phase I of the Valsalva maneuver compared to that of the men (ΔMAP = 19.5 ± 1.8 mmHg). A higher rise in MAP during late phase II observed in the women (ΔMAP = 17.5 ± 3.9 mmHg) compared to the men (ΔMAP = 10.8 ± 1.7 mmHg) could not be distinguished statistically ($F(1,18) = 2.4456$, $P = 0.1353$). However, the $\Delta HR/\Delta MAP$ during early phase II was greater ($F(1,18) = 5.1466$, $P = 0.0358$) in the men (-1.3 ± 0.2 bpm/mmHg) than in the women (-0.9 ± 0.2 bpm/mmHg) as a result of a greater tachycardic response in the males with the same hypotensive stimulus (Fig. 5).

Adrenoreceptor responsiveness. The average slope of the individual subject dose-response relationships between ISO and heart rate was greater ($F(1,18) = 4.6096$, $P = 0.0457$) in the females (1853 ± 194 beats/μg/kg/min) compared to an average response of 1323 ± 153 beats/μg/kg/min in the males. Figure 6 (upper panel) represents the regressions calculated from the mean (±SE) heart rates at each ISO level. In contrast, there were no statistical differences between females and males in the average slope of the individual subject dose-response relationships between ISO and leg vascular resistance (-868 ± 241 pru/μg/kg/min and -690 ± 294 pru/μg/kg/min, respectively; $F(1,18) = 0.2177$, $P = 0.6464$) or between PE and leg vascular resistance (15.8 ± 4.2 pru/μg/kg/min and 19.5 ± 4.7 pru/μg/kg/min, respectively; $F(1,18) = 0.3529$, $P = 0.5599$). The regressions calculated from the mean (±SE) leg vascular resistances at each ISO and PE level are presented in the lower panels of Figure 6.

Leg volume and compliance. Total leg volume of the women (12.3 ± 0.5 liters) and men (12.1 ± 0.5 liters) were similar ($F(1,18) = 0.1239$, $P = 0.7289$) while calf compliance of the men (6.2 ± 0.5 ml/mmHg) was higher ($F(1,18) = 2.6888$, $P = 0.1184$) than that of the women (5.0 ± 0.5 ml/mmHg).

Plasma volume and norepinephrine responses. Total circulating blood volume in females was 26% less than males as a result of smaller total circulating plasma and red cell volumes (Table 4). When blood volume was standardized for body weight, females (61.5 ± 3.2 ml/kg) remained lower ($F(1,16) = 4.0882$, $P = 0.0602$) compared to males (71.5 ± 3.8 ml/kg). primarily as a result of lower ($F(1,16) = 5.2232$, $P = 0.0363$) circulating red blood cell volume (25.1 ± 1.9 ml/kg for females vs. 31.0 ± 1.7 ml/kg for males).

Baseline plasma NE and E concentrations and total circulating contents were not statistically different between the gender groups (Table 4). Presyncopal LBNP induced an elevation ($F(1,13) = 48.7301$, $P < 0.0001$) of total circulating NE to 1625 ± 253 ng in females and 2214 ± 306 ng in males. The change in total circulating plasma NE from baseline to presyncopal LBNP in the females (834 ± 122 ng) was less ($F(1,12) = 4.3719$, $P = 0.0585$) than the elevation of 1345 ± 275 ng observed in the males. Presyncopal LBNP induced an elevation ($F(1,13) = 5.4107$, $P = 0368$) of total circulating E to 307 ± 130 ng in females and 262 ± 90 ng in males. The change in total circulating plasma E from baseline to presyncopal LBNP in the females (187 ± 115 ng) was not statistically discernible ($F(1,12) = 0.0128$, $P = 0.9120$) than the elevation of 205 ± 95 ng observed in the males.

Discussion

The results of the present study confirm those of previous investigations (Hogan et al 1995; Hordinsky et al. 1981; Ludwig et al 1987, Montgomery et al 1977; White et al 1996) that women have significantly compromised capacity to regulate blood pressure and maintain orthostatic function compared to men. Since greater height predicts lower LBNP tolerance (Ludwig & Convertino 1994), the shorter females might be expected to have an advantage in tolerance over the males. Lower LBNP tolerance in females in light of their height advantage underscores important differences between genders in underlying cardiovascular mechanisms. This investigation expanded upon previous work by identifying specific characteristics of cardiovascular functions that supported the hypothesis that greater orthostatic intolerance in women was associated with impairment of specific mechanisms of blood pressure regulation. The unique finding of this study was that presyncopal predisposition in females was associated with impairment of the heart rate response to carotid baroreceptor stimulation, lower baseline cardiac vagal activity, greater decline in Q and stroke volume induced by LBNP, increased β_1-adrenoreceptor responsiveness, greater vasoconstriction under equal LBNP, lower levels of total circulating NE at pre-syncope, and lower blood volume.

Some investigations have suggested that orthostatic performance may be compromised in athletically-trained individuals with high VO_2max (Convertino 1987). To minimize this confounding factor, we applied the criteria of expressing VO_2max as the rate of systemic oxygen uptake used per weight of lean body mass (Cureton 1981; Drinkwater 1984). The similar VO_2max of the gender groups in the present investigation suggests that physical fitness was an unlikely explanation for differences in orthostatic tolerance between females and males.

As expected, LBNP elicited increased heart rate, peripheral resistance, leg volume, and catecholamines to levels comparable to those observed by others who employed similar techniques to test orthostatic responses (Blomqvist & Stone 1983; Convertino 1987, 1993; Frey et al 1986, 1988; Hordinsky et al 1981; Hudson et al 1987; Montgomery et al 1977; Sather et al 1986; White et al 1996). It has been suggested that tolerance to progressive LBNP can be partially explained by differences in pre-LBNP Q reserves and higher compensatory increases in peripheral resistance to a given orthostatic stress (Convertino et al 1994; Sather et al 1986). These notions were not adequate to explain differences in LBNP tolerance between the gender groups in the present study since equal pre-LBNP Q and greater increases in peripheral resistance to a given LBNP level did not protect against earlier onset of presyncope

in the females. Despite lower stroke volumes at baseline and during LBNP (Fig. 2), females were able to elicit greater tachycardic response at equal LBNP. However, this reflex cardiac response was inadequate to maintain Q in the women compared to the men. Thus, the results of the present study indicate that the rate of Q reduction may be a significant factor that contributed to earlier onset of hypotension in females in the present study.

Baroreceptor-mediated tachycardia provides a means to buffer transient changes in arterial blood pressure. Investigations using both human and animal models have demonstrated that carotid-cardiac baroreflex dysfunction is associated with less cardioacceleration and greater incidence of hypotension during orthostasis (Convertino 1991; Convertino et al 1990, 1991; Cowley et al 1973). A positive correlation has been reported between the magnitude of impairment of carotid-cardiac baroreflex function and incidence of syncope during passive standing (Convertino et al 1990). In the present study, lower LBNP tolerance was assocaited with attenuated carotid-cardiac baroreflex responsiveness in the female subjects compared to their male counterparts. The isolated carotid-cardiac baroreflex stimulus-response relationship in the women was shifted such that a 33% lower maximum slope existed in the region of hypotension. This lesser responsiveness in carotid-cardiac baroreflex function was further verified by a lower $\Delta HR/\Delta MAP$ ratio during the Valsalva maneuver in the females compared to the males. This attenuated reflex response may have partly contributed to the impaired capacity of the females to buffer against transient reductions in blood pressure during LBNP.

The observation in this and other (Montgomery et al 1977; White et al 1996) investigations that females demonstrated greater elevation in heart rate than males during graded LBNP has led to the hypothesis that vagal withdrawal rather than sympathetic activation may be a more important mechanism underlying blood pressure regulation in women (Frey & Hoffler 1988). This hypothesis is consistent with the observation that an attenuated response of the vagally-mediated carotid-cardiac baroreflex and total circulating NE at presyncope were associated with impaired orthostatic performance in female subjects compared to males in the present study. A lower baseline heart rate variability and attenuated carotid-cardiac baroreflex responsiveness in the females compared to the males of the present study might suggest that vagal withdrawal from low baseline cardiac vagal tone represents a possible underlying mechanism for limited cardioacceleration in women.

Although lower cardiac baroreflex responsiveness was associated with earlier onset of presyncope in women, heart rate at presyncope in the female subjects did not differ from that observed in the males. This was especially surprising since the women elicited less elevation of total circulating plasma norepinephrine than the men, suggesting that less sympathetic activation induced similar tachycardic response during LBNP in females. This difference in the sympathetic stimulus-heart rate response relationship between women and men could be explained by the up-regulation of cardiac β-adrenoreceptors observed in the women (Fig. 6).

The finding that heart rate at presyncope did not differ between women and men makes the interpretation regarding the contribution of vagally-mediated baroreflex control of heart rate to blood pressure regulation less clear. An alternative method of interpreting the difference in baroreflex responsiveness between the two gender groups and its importance in maintenance of arterial pressure during hypotension is to calculate the change in Q which would be expected to occur when a given reduction in blood pressure elicits a given reflex change in heart rate (Levine 1993). In the present study, when average stroke volume at presyncope was multiplied by the carotid baroreflex gain during a change in mean arterial pressure from 100 mmHg (baseline) to 80 mmHg (presyncope), the unit change in Q was 105% greater in the men compared to the women (4.8 bpm x 48 ml/beat = 230 ml/min vs. 3.2 bpm x 35 ml/beat = 112 ml/min, for men and women, respectively). Therefore, despite small differences in the magnitude of the cardioacceleratory baroreflex response between groups, these calculations

suggest that the baroreflex-mediated cardioacceleration in female subjects may have significantly compromised the capacity to provide adequate cardiac output during LBNP.

Earlier onset of presyncope in our female subjects was associated with lower stroke volume and cardiac output compared to males. The capacity to maintain Q and systemic arterial pressure during an orthostatic challenge can be influenced by impaired venous return as a result of blood pooled in the lower body. The notion that females in the present study had lower venous return and cardiac filling compared to the males was supported by greater increases in thoracic impedance and lower stroke volumes in the women. Based on this observation, greater leg compliance and fluid accumulation during LBNP might be predicted for the females. Consistent with previous investigations (Frey & Hoffler 1988; Hordinsky et al 1981; Montgomery et al 1977), males of the present study demonstrated an average leg compliance 24% greater than the females and experienced greater fluid accumulation in their legs during LBNP compared to the females, as evidenced by a larger percentage increase in calf circumference. These observations dismiss the possibility that LBNP tolerance could be explained by differences in the quantity of blood sequestered in the legs alone. However, it is possible that females have greater reduction in venous return and stroke volume with less blood pooling the legs by selectively sequestering the majority of blood in their abdominal region since pelvic blood pooling has been as much as six-fold greater in women compared to men at equal orthostatic challenge (White & Montgomery 1996).

The capacity to increase total systemic peripheral resistance represents an important mechanism for buffering against the development of hypotension during an orthostatic challenge. Some data suggest that vasoactive responses of vascular adrenergic receptors was lower in women than men since men showed significant dose-related vasoconstriction to phenylephrine and vasodilation to isoproterenol while women did not (Freedman et al 1987). However, we found similar vasoactive responses to phenylephrine and isoproterenol between females and males of the present study, suggesting that differences in vascular adrenoreceptor responsiveness did not contribute to lower orthostatic performance in females. It has also been suggested that females may be compromised in their capacity to vasoconstrict since increased TPR during LBNP was less in females compared to males (Frey & Hoffler 1988). In contrast, several responses observed in the present study indicate that the capacity to vasoconstrict is not compromised in females. Total peripheral resistance at the onset of presyncope was similar for females and males in the present study. In addition, vasoconstriction responses during non-presyncopal levels of LBNP were substantially greater in the females compared to the males (Fig. 2, Table 3). The latter observation was further supported by a 40% greater average response of increased forearm vascular resistance to cardiopulmonary baroreceptor stimulation in females compared to males (Fig. 4). A possible limitation for vasoconstriction in females based on the present data may be the degree to which women utilize their vasoconstrictive reserve (defined as the difference between peripheral resistance at baseline and presyncope) (Engelke et al 1996). Females and males in the present study should have had similar vasoconstrictive reserve since their TPR and FVR at baseline and presyncope were similar. Since the vasoconstrictive response to LBNP in this investigation occurred at a faster rate in females, lower LBNP tolerance in females was associated with a greater elicitation of their maximal vasoconstrictive reserve at lower LBNP. It has been suggested that just prior to the point at which an orthostatic challenge is sufficient to elicit hypotension, compensatory mechanisms are usually operating at maximal capacity (Rowell 1986). If this is true, orthostatic tolerance of females may be limited by the inability to recruit further vasoconstriction from mechanisms that have reached their maximal capacity.

Circulating blood volume has a profound effect on arterial pressure during orthostasis (Blomqvist & Stone 1983; Convertino 1987, 1995; Convertino et al 1996; Levine 1993; Ludwig et al 1987, 1994). Subjects with reduced vascular volumes exhibit subnormal filling pressures (Blomqvist & Stone 1983) and may be shifted to the steep portion of their Frank-

Starling curve where capacity to buffer orthostatic reductions in central blood volume is limited (Levine 1993). Earlier onset of presyncope in female subjects of the present study was associated with lower stroke volume and cardiac output compared to males. It is possible that lower relative circulating blood volume in the females contributed to impairment of cardiac filling and subsequent failure to maintain blood pressure at lower levels of LBNP.

Low blood volume in the females compared to the males of the present investigation could also have contributed to earlier saturation of vasoconstriction during LBNP since hypovolemia elicits greater vascular resistance for equal reductions in venous pressure (Thompson et al 1990). This notion is consistent with the observation that females in this study demonstrated substantially greater vasoconstriction at similar levels of LBNP compared to the males and a greater average response (slope) for increased forearm vascular resistance to cardiopulmonary baroreceptor stimulation (Fig. 2 and 4). These responses have been interpreted to represent a greater utilization of vasoconstrictive reserve such that maximal pressor response is attained at a lesser reduction in central blood volume (Thompson et al 1990). It is therefore reasonable that predisposition for earlier onset of presyncope in female subjects may partly result from a relative hypovolemia that compromised cardiac filling and the capacity to increase systemic resistance.

Total circulating plasma NE, an index of sympathetic activity (Goldstein et al 1995), is increased during orthostasis and higher elevations are associated with increased LBNP tolerance (Convertino 1993; Engelke et al 1996). In the present study, the increase in total circulating plasma NE from baseline to presyncope was greater in males compared to females while changes in total circulating E similar between the gender groups. Therefore, it appeared that neuronal release of catecholamines were attenuated in females which may limit compensatory elevations in cardiac contractility, heart rate and systemic peripheral resistance required to defend against the onset of hypotension and syncope.

In summary, LBNP tolerance was significantly lower in females than males who matched for age and aerobic fitness. At presyncope, heart rate and peripheral vascular resistance were similar in both groups. Presyncopal predisposition in females was associated with impairment of the heart rate response to carotid baroreceptor stimulation, lower baseline cardiac vagal (parasympathetic) activity, greater decline in $\dot{Q}$ and stroke volume induced by LBNP, increased β_1-adrenoreceptor responsiveness, greater vasoconstriction under equal LBNP, lower levels of total circulating NE at pre-syncope, and lower blood volume. The results of this investigation support the hypothesis that women have significant deficiencies in mechanisms that underlie blood pressure regulation under orthostatic challenge compared to men.

Conclusions

The results of this study could have important implications for selection and training of high-performance aircraft pilots. The observation that the capacity to buffer against development of hypotension and poor orthostatic performance is significanly compromised in women compared to men should be carefully considered in the selection of females for aerial combat missions that require high Gz accelerations. Underlying mechanisms associated with lower orthostatic performance in females included attenuated carotid baroreflex control of heart rate, lower baseline cardiac vagal activity, greater decline in Q and stroke volume induced by LBNP, increased β_1-adrenoreceptor responsiveness, greater vasoconstriction under equal LBNP, increased levels of circulating NE at pre-syncope, and lower plasma volume. Whether crew selection, training or technological life support systems can be designed to enhance these physiological functions could be critical to the potential future of female pilots.

Another important finding of a recent investigation (Convertino et al, in press) was that women failed to demonstrate cardiovascular adaptation to high-G training compared to men. While high-G training increased stroke volume, cardiac output, and protected against orthostatic hypotension during the squat-stand test in men, it failed to alter any of these cardiovascular functions in women. This comparison of the influence of high-G training may be particularly important in that an absence of enhanced cardiovascular functions observed in females compared to males was associated with lower tracking performance during simulated air-to-air combat at high G in the women compared to the men. These results provided evidence that blood pressure regulation was associated with, and may be an important underlying mechanism for, cognitive task performance during high-G maneuvers. Perhaps as important is the possible implication that female pilots may have less physiological potential to adapt their cardiovascular functions during training to support optimal performance during aerial combat. If compromised mechanisms of blood pressure regulation observed in the females of the present study fail to adapt adequately to +Gz exposure, combat training and performance of female pilots may be significantly limited.

The results of the present study together with previous investigations (Frey & Hoffler 1988; Hordinsky et al 1981; White & Montgomery 1996) suggest that women accumulate less fluid in their legs and more fluid in their pelvic region under G-induced stress compared to men. These findings suggest that, in addition to the importance of well-fitted garments, the design of anti-G suits for female pilots should include greater counterpressure applied to the pelvic region with less required around the legs compared to men.

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References

1. Blomqvist, C.G., and H.L. Stone. Cardiovascular adjustments to gravitational stress. In *Handbook of Physiology: The Cardiovascular System. Peripheral Circulation and Organ Blood Flow.* edited by J.T. Shepherd and F.M. Abboud. Bethesda, MD: Am. Physiol. Soc., 1983, sect. 2, vol. 3, part 2, pp. 1025-1063.

2. Convertino, V.A. Aerobic fitness, endurance training, and orthostatic intolerance. *Exer. Sports Sci. Rev.* 15: 223-259, 1987.

3. Convertino, V.A. Carotid-cardiac baroreflex: relation with orthostatic hypotension following simulated microgravity and implications for development of countermeasures. Acta Astronautica 23:9-17, 1991.

4. Convertino, V.A. Endurance exercise training: conditions of enhanced hemodynamic responses and tolerance to LBNP. Med. Sci. Sports Exer. 25:705-712, 1993.

5. Convertino, V.A. Exercise and adaptation to microgravity. In: *Handbook of Physiology. Adaptation to the Environment.* Edited by M.J. Fregly and C.M. Blatteis. New York: Oxford University Press, 1995, pp. 815-843.

6. Convertino, V.A., W.C. Adams, J.D. Shea, C.A. Thompson, and G.W. Hoffler. Impairment of carotid-cardiac vagal baroreflex in wheelchair-dependent quadriplegics. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)* 260: R576-R580, 1991.

7. Convertino, V.A., D.F. Doerr, D.L. Eckberg, J.M. Fritsch, and J. Vernikos-Danellis. Head-down bed rest impairs vagal baroreflex responses and provokes orthostatic hypotension. *J. Appl. Physiol.* 68: 1458-1464, 1990.

8. Convertino, V.A., D.F. Doerr, D.A. Ludwig, and J. Vernikos. Effect of simulated microgravity on cardiopulmonary baroreflex control of forearm vascular resistance. *Am. J. Physiol. 266 (Regulatory Integrative Comp. Physiol.)*: R1962-1969, 1994.

9. Convertino, V.A., K. A. Engelke, D.A. Ludwig, and D.F. Doerr. Restoration of plasma volume induced by a single bout of maximal exercise following prolonged exposure to simulated microgravity. *Am. J. Physiol. 270(Regulatory Integrative Comp. Physiol.)*: R3-R10, 1996.

10. Convertino, V.A., J.L. Polet, K.A. Engelke, G.W. Hoffler, L.D. Lane, and C.G. Blomqvist. Evidence for increased β-adrenoreceptor responsiveness induced by 14 days of simulated microgravity in humans. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)* 273:R93-R99, 1997.

11. Convertino, V.A., L.D. Tripp, D.A. Ludwig, J. Duff, and T.L. Chellette. Female performance at high G: chronic adaptations of cardiovascular functions. *Aviat. Space Environ. Med.* (in press).

12. Crandall, C.G., K.A. Engelke, V.A. Convertino, and P.B. Raven. Aortic baroreflex control of heart rate following 15 days of simulated microgravity exposure. *J. Appl. Physiol.* 77:2134-2139, 1994.

13. Crandall, C.G., K.A. Engelke, J.A. Pawelczyk, P.B. Raven, and V.A. Convertino. Power spectral and time based analysis of heart rate variability following 15 days simulated microgravity exposure in humans. *Aviat. Space Environ. Med.* 65:1105-1109, 1994.

14. Cureton, K.J. Matching of male and female subjects using VO2max. *Res. Q. Exerc. Sport* 52:264-268, 1981.

15. Drinkwater, B.L. Women and exercise: physiological aspects. *Exerc. Sports Sci. Rev.* 12:21-51, 1984.

16. Cowley, A.W., Jr., J.F. Liard, and A.C. Guyton. Role of the baroreceptor reflex in daily control of arterial pressure and other variables in dogs. *Circ. Res.* 32:564-576, 1973.

17. Eckberg, D.L. Human sinus arrhythmia as an index of vagal cardiac outflow. *J. Appl. Physiol.* 54:961-966, 1983.

18. Engelke, K.A., D.F. Doerr, and V.A. Convertino. Effect of a single bout of exhaustive exercise on integrated baroreflex function following 16 days of simulated microgravity exposure. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)* 269:R614-R620, 1995.

19. Engelke, K.A., D.F. Doerr, C.G. Crandall, and V.A. Convertino. Application of acute maximal exercise to protect orthostatic tolerance after simulated microgravity. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)* 271:R837-R847, 1996.

20. Engelke, K.A., J.D. Shea, D.F. Doerr, and V.A. Convertino. Autonomic functions and orthostatic responses 24 hours after acute intense exercise in paraplegic subjects. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)* 266: R1189-R1196, 1994.

21. Freedman, R.R., S.C. Sabharwal, and N. Desai. Sex differences in peripheral vascular adrenergic receptors. *Circ. Res.* 61:581-585, 1987.

22. Frey, M.A.B., and G.W. Hoffler. Association of sex and age with responses to lower-body negative pressure. *J. Appl. Physiol.* 65:1752-1756, 1988.

23. Frey, M.A.B., K.L. Mathes, and G.W. Hoffler. Cardiovascular responses of women to lower body negative pressure. *Aviat. Space Environ. Med.* 57:531-538, 1986.

24. Gauer, O.H., and H.D. Seiker. The continuous recording of central venous pressure from an arm vein. *Circ. Res.* 4:74-78, 1956.

25. Goldstein, D.S., J. Vernikos, C. Holmes, and V.A. Convertino. Catecholaminergic effects of prolonged head-down bed rest. *J. Appl. Physiol.* 78:1023-1029, 1995.

26. Greenleaf, J.E., V.A. Convertino, and G.R. Mangseth. Plasma volume during stress in man: osmolality and red cell volume. *J. Appl. Physiol.* 47:1031-1038, 1979.

27. Hoffler, G.W. Cardiovascular studies of US space crews: an overview and perspective. In: *Cardiovascular Flow Dynamics and Measurements.* Edited by N.H.C. Hwang and N.A. Normann. University Park Press, Baltimore, MD, 1977, pp. 335-363.

28. Hogan, T.R., M. Cubitt, M.K. Ecken, and J.E. Davis. Effect of gender on orthostatic tolerance. *Med. Sci. Sports Exerc.* 27:S188, 1995 (abstract).

29. Hordinsky, J.R., U. Gebhardt, H.M. Wegmann, and G. Schafer. Cardiovascular and biochemical response to simulated space flight entry. *Aviat. Space Environ. Med.* 52:16-18, 1981.

30. Hudson, D.L., M.L. Smith, and P.B. Raven. Physical fitness and hemodynamic response of women to lower body negative pressure. *Med. Sci. Sports Med.* 19:375-381, 1987.

31. Kasting, G.A., D.L. Eckberg, J.M. Fritsch, and C.L. Birkett. Continuous resetting of the human carotid baroreceptor-cardiac reflex. *Am. J. Physiol.* 252:R732-R736, 1987.

32. Levine, B. Regulation of central blood volume and cardiac filling in endurance athletes. The Frank-Starling mechanism as a determinant of orthostatic tolerance. *Med. Sci. Sports Exerc.* 25:727-732, 1993.

33. Ludbrook, J., G. Mancia, A. Ferrari, and A. Zanchetti. Factors influencing the carotid baroreceptor response to pressure changes in a neck chamber. *Clin. Sci. Mol. Med.* 51:347s-349s, 1977.

34. Ludwig, D.A., V.A. Convertino, D.J. Goldwater, and H. Sandler. Logistic risk model for the unique effects of inherent aerobic capacity on +Gz tolerance before and after simulated weightlessness. *Aviat. Space Environ. Med.* 58:1057-1061, 1987.

35. Ludwig, D.A., and V.A. Convertino. Predicting orthostatic intolerance: physics or physiology? *Aviat. Space Environ. Med.* 65:404-411, 1994.

36.. Luft, U.C., L.G. Myhre, J.A. Loeppky, and M.D. Venters. *A Study of Factors Affecting Tolerance to Gravitational Stress Simulated by Lower Body Negative Pressure.* Research Report on Specialized Physiology Studies in Support of Manned Space Flight. Albuquerque, NM: Lovelace Found., 1976, 1-60. (NASA contract NAS9-14472).

37. Luster, E.A., N. Baumgartner, W.C. Adams, and V.A. Convertino. Effects of hypovolemia and posture on integrated baroreflex function. *Aviat. Space Environ. Med.* 67:308-313, 1996.

38. Montgomery, L.D., P.J. Kirk, P.A. Payne, R.L. Gerber, S.D. Newton, and B.A. Williams. Cardiovascular responses of men and women to lower body negative pressure. *Aviat. Space Environ. Med.* 48:138-145, 1977.

39. Pollock, M.L., D.H. Schmidt, and A.S. Jackson. Measurement of cardiorespiratory fitness and body composition in the clinical setting. Compr. Ther. 6:12-27, 1980.

40. Rowell, L.B. *Human Circulation: Regulation During Physical Stress.* New York: Oxford University Press, 1986, p. 157.

41. Sandroni, P., E.F. Benarroch, and P.A. Low. Pharmacological dissection of components of the Valsalva maneuver in adrenergic failure. *J. Appl. Physiol.* 71: 1563-1567, 1991.

42. Sather, T.M., D.J. Goldwater, L.D. Montgomery, and V.A. Convertino. Cardiovascular dynamics associated with tolerance to lower body negative pressure. *Aviat. Space Environ. Med.* 57:413-419, 1986.

43. Shi, X., J.M. Andresen, J.T. Potts, B.H. Foresman, S.A. Stern, and P.B. Raven. Aortic baroreflex control of heart rate during hypertensive stimuli: effects of fitness. *J. Appl. Physiol.* 73:1555-1562, 1993.

44. Shi, X., C.G. Crandall, J.T. Potts, B.H. Foresman, and P.B. Raven. A diminished aortic-cardiac reflex during hypotension in aerobically fit young men. *Med. Sci. Sports Exerc.* 25:1024-1030, 1993.

45. Stegemann, J., F.M. Baer, and U. Hoffman. The Valsalva maneuver as an indirect, non-invasive indicator of central blood volume shift. *Aviat. Space Environ. Med.* 59: 422-427, 1988.

46. Thompson, C.A., D.L. Tatro, D.A. Ludwig, and V.A. Convertino. Baroreflex responses to acute changes in blood volume in humans. *Am. J. Physiol.* (*Regulatory Integrative Comp. Physiol.*) 259: R792-R798, 1990.

47. White, D.D., R.W. Gotshall, and A. Tucker. Women have lower tolerance to lower body negative pressure than men. *J. Appl. Physiol.* 80:1138-1143, 1996.

48. White, D.D., and L.D. Montgomery. Pelvic blood pooling of men and women during lower body negative pressure. *Aviat. Space Environ. Med.* 67:555-559, 1996.

APPENDIX 1

Figures

Figure 1. Individual and average cumulative index for LBNP tolerance in females and males.

Figure 2. Hemodynamic responses to LBNP through -50 mmHg in females (closed circles, solid lines) and males (open circles, broken lines). Values are mean ± SE for N = 10. † indicates slopes of responses with $P \leq 0.05$.

Figure 3. Percent changes in leg volume and thoracic impedance to LBNP through -50 mmHg in females (closed circles, solid lines) and males (open circles, broken lines). Values are mean ± SE for N = 10. † indicates responses across LBNP with $P \leq 0.03$.

Figure 4. Illustration of the carotid baroreceptor stimulus-cardiac response relationship for female (closed circles, solid lines) and male (open circles, broken lines) groups, shown as the change (Δ) in R-R interval from baseline (0) as a function of carotid distending pressure. Symbols represent mean (circles) ± 1 SE (lines) for the two groups.

Figure 5. Cardiopulmonary baroreflex stimulus-response relationship between forearm vascular resistance and estimated central venous pressure (PVP) in females (closed circles and solid lines) and males (open circles and broken lines). The linear equation for the mean female response is $y = -3.62x + 70.7$ ($r^2 = 0.978$) and for the mean male response is $y = -2.46x + 57.8$ ($r^2 = 0.933$). Symbols are means ± SE (N = 10 in each group).

Figure 6. Schematic generated from the average responses of 10 female (solid lines) and 10 male (broken lines) subjects illustrating mean arterial pressure (top panel) and heart rate (bottom panel) during phase I (A), early phase II (B), and late phase II (C) of a 15-s Valsalva maneuver at an expiratory pressure of 30 mmHg.

Figure 7. Dose-response relationships between ISO and heart rate (upper panel), between ISO and leg vascular resistance (middle panel), and between PE and leg vascular resistance (lower panel) for females (closed circles) and males (open circles). Linear regressions are calculated from mean values. For ISO vs. heart rate, the linear equation for females is $y = 1880x + 61.8$ ($r^2 = 0.995$) and for males is $y = 1303x + 52.6$ ($r^2 = 0.993$). For ISO vs. leg vascular resistance, the linear equation for females is $y = -865x + 48.5$ ($r^2 = 0.957$) and for males is $y = -690x + 51.7$ ($r^2 = 0.876$). For PE vs. leg vascular resistance, the linear equation for females is $y = 16.0x + 47.6$ ($r^2 = 0.903$) and for males is $y = 19.5x + 45.4$ ($r^2 = 0.948$).

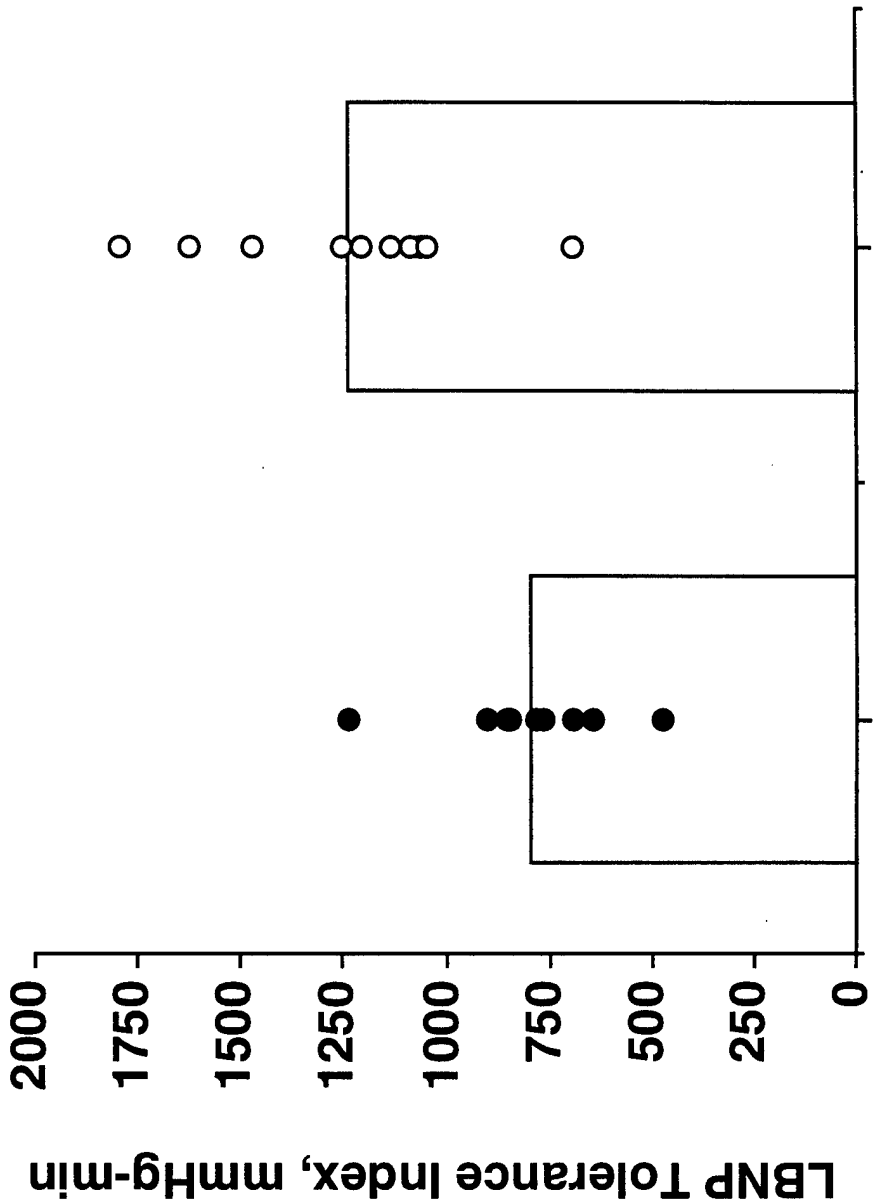

Figure 1. Individual and average cumulative index of LBNP tolerance in females and males.

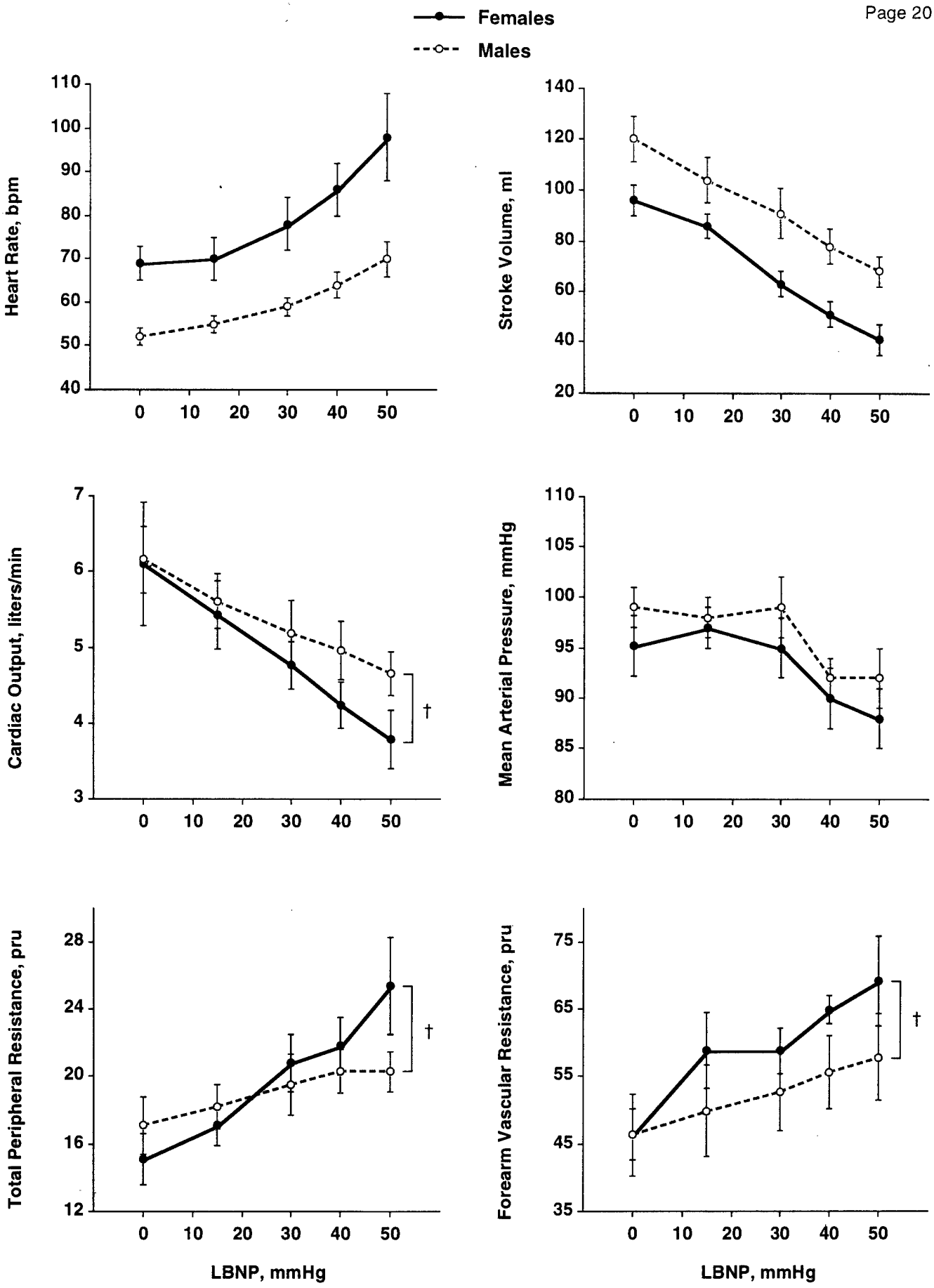

Figure 2. Hemodynamic responses to LBNP through -50 mmHg in females and males.

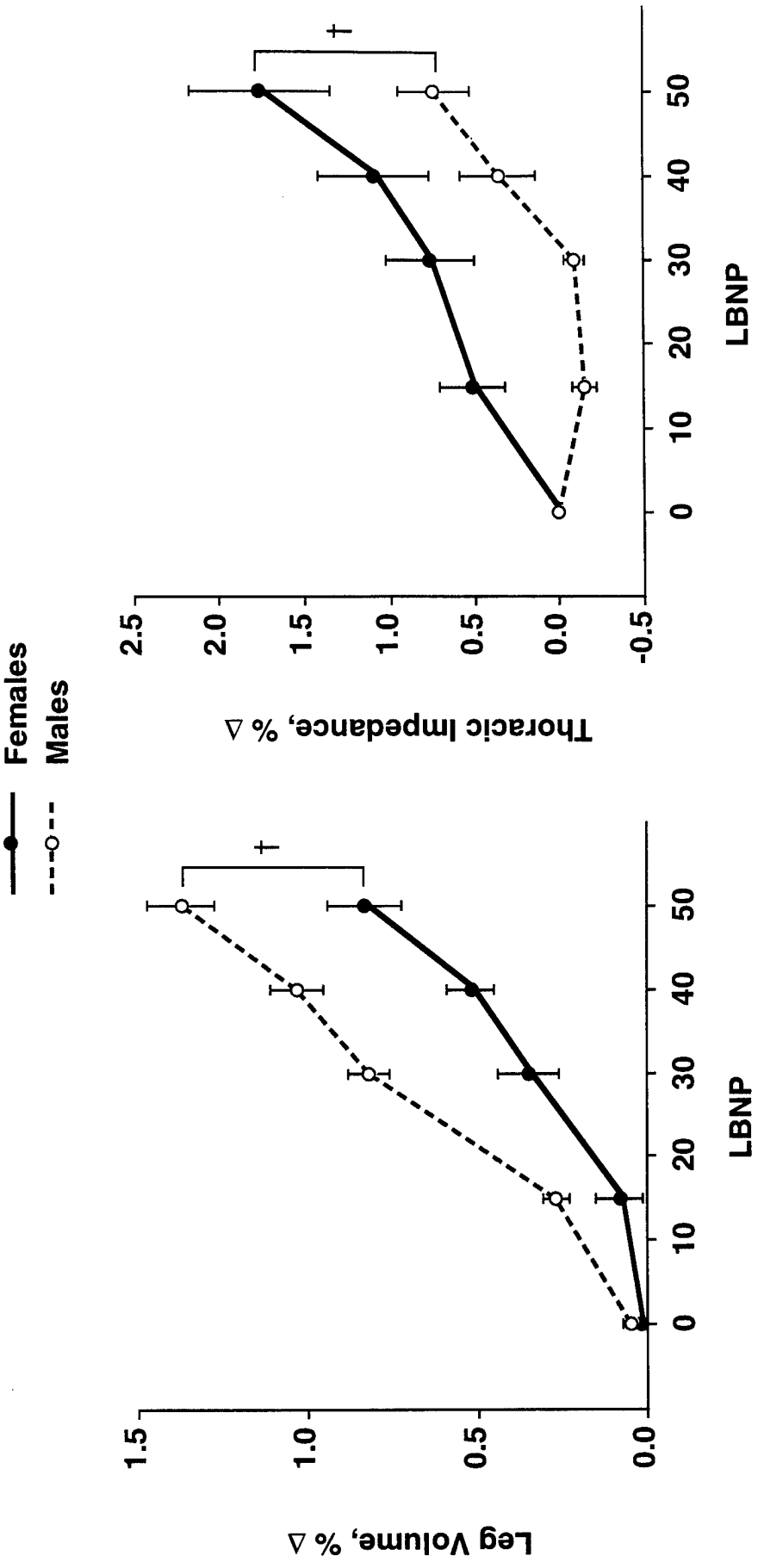

Figure 3. Percent changes in leg volume and thoracic impedance to LBNP through -50 mmHg in females and males.

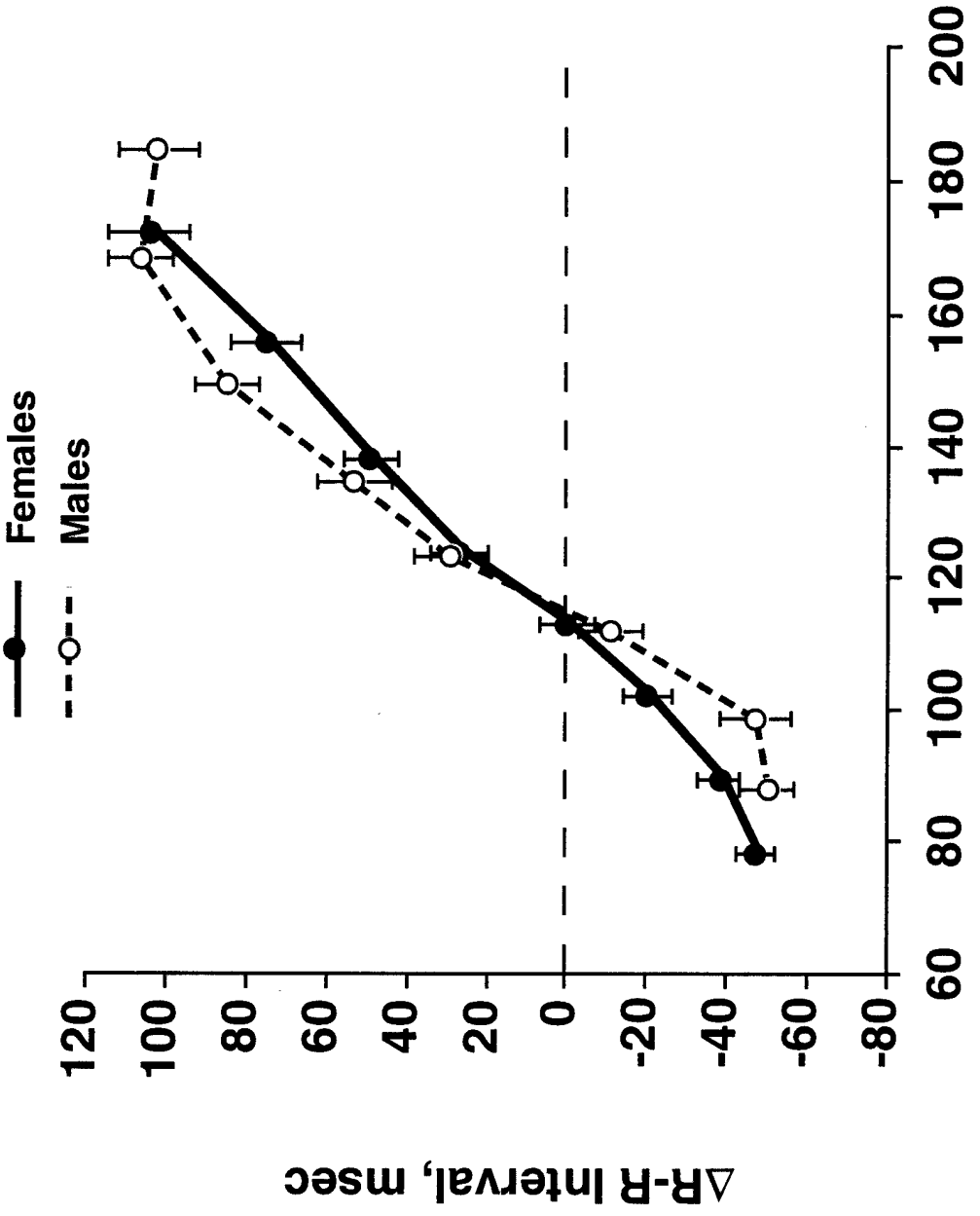

Figure 4. Illustration of the carotid baroreceptor stimulus-cardiac response relationship for female and male groups.

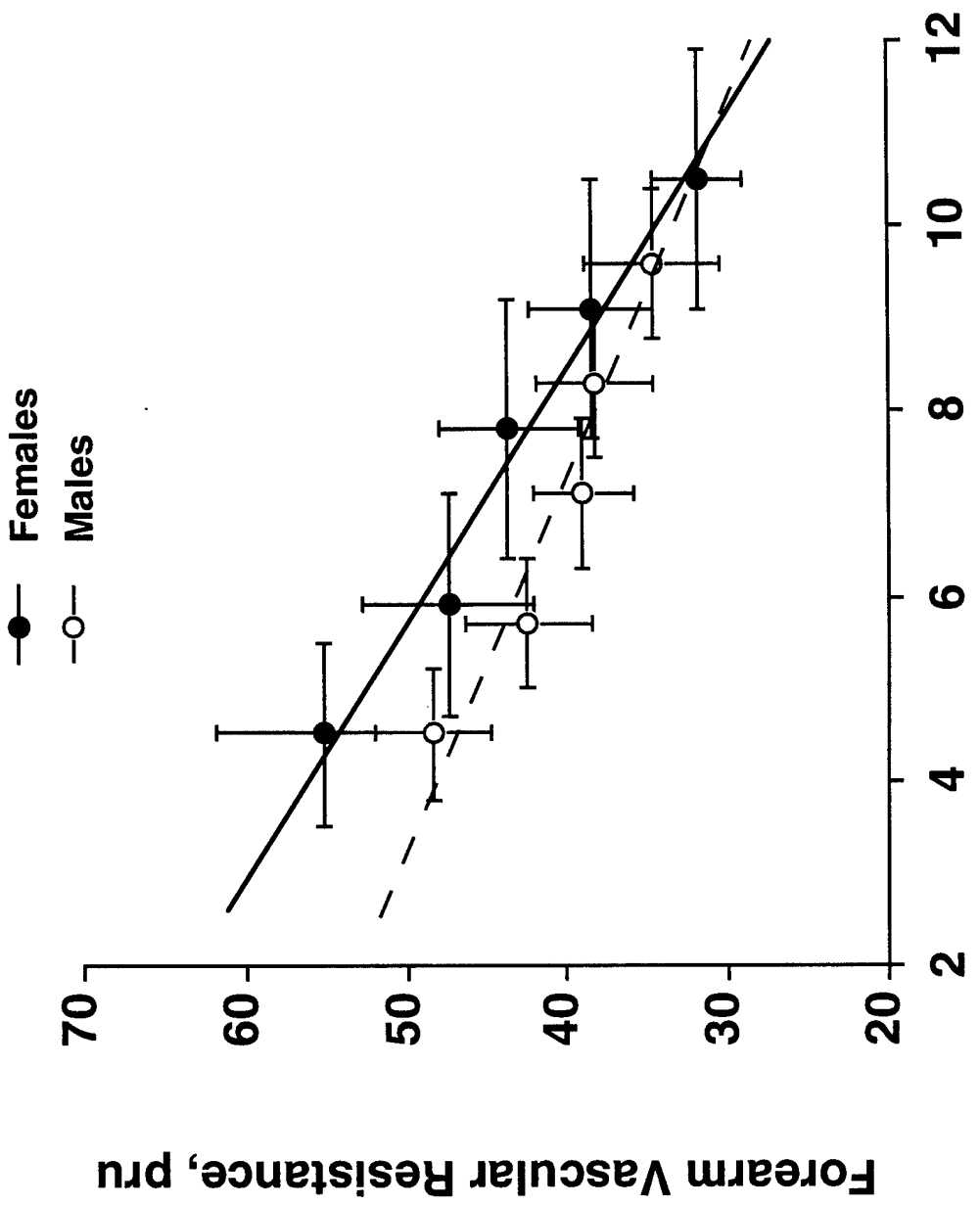

Figure 5. Cardiopulmonary baroreflex stimulus-response relationship between forearm vascular resistance and estimated central venous pressure in females and males.

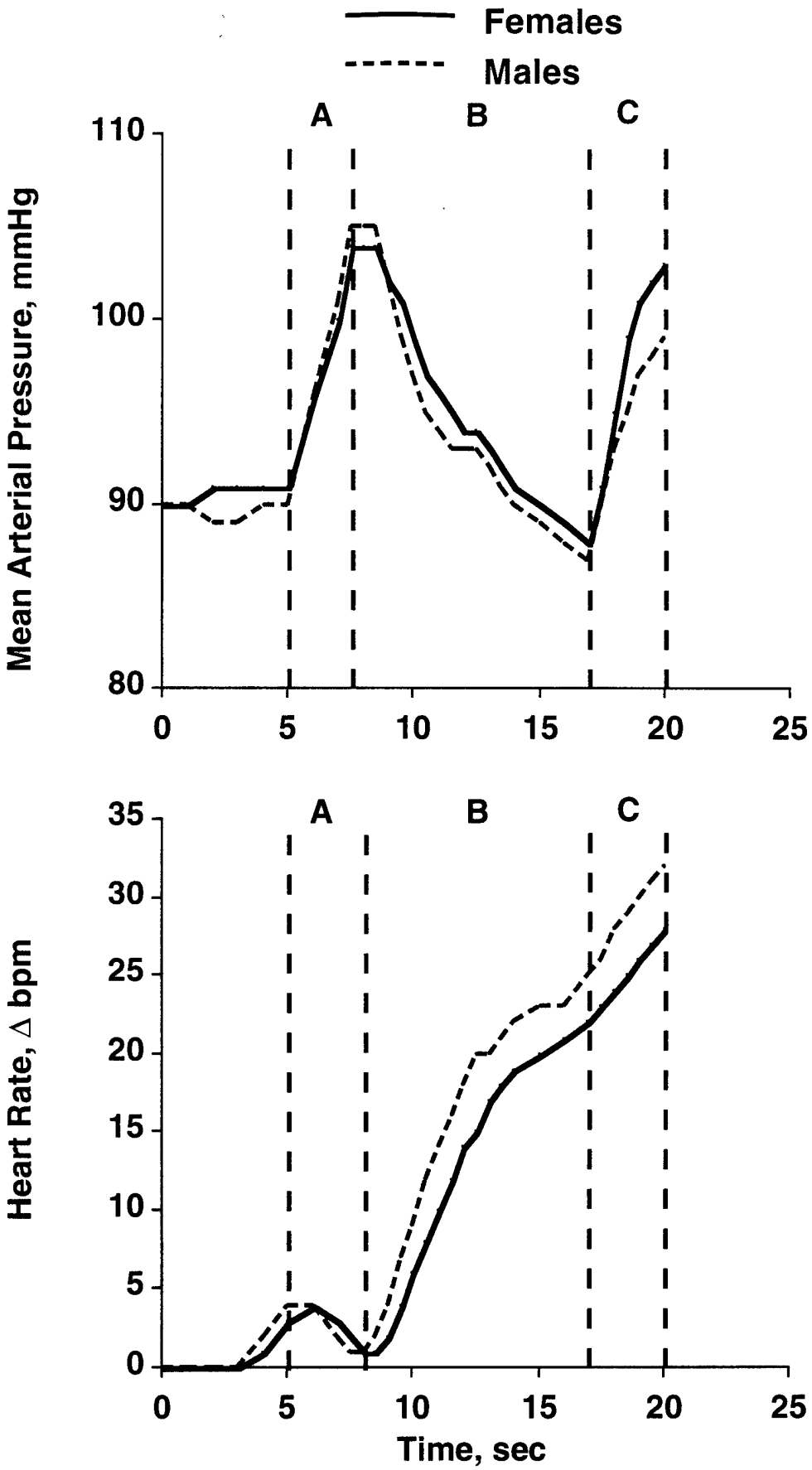

Figure 6. Schematic generated from the average of female and male subjects illustrating mean arterial pressure and heart rate during phase I (A), early phase II (B), and late phase II (C) of a Valsalva maneuver.

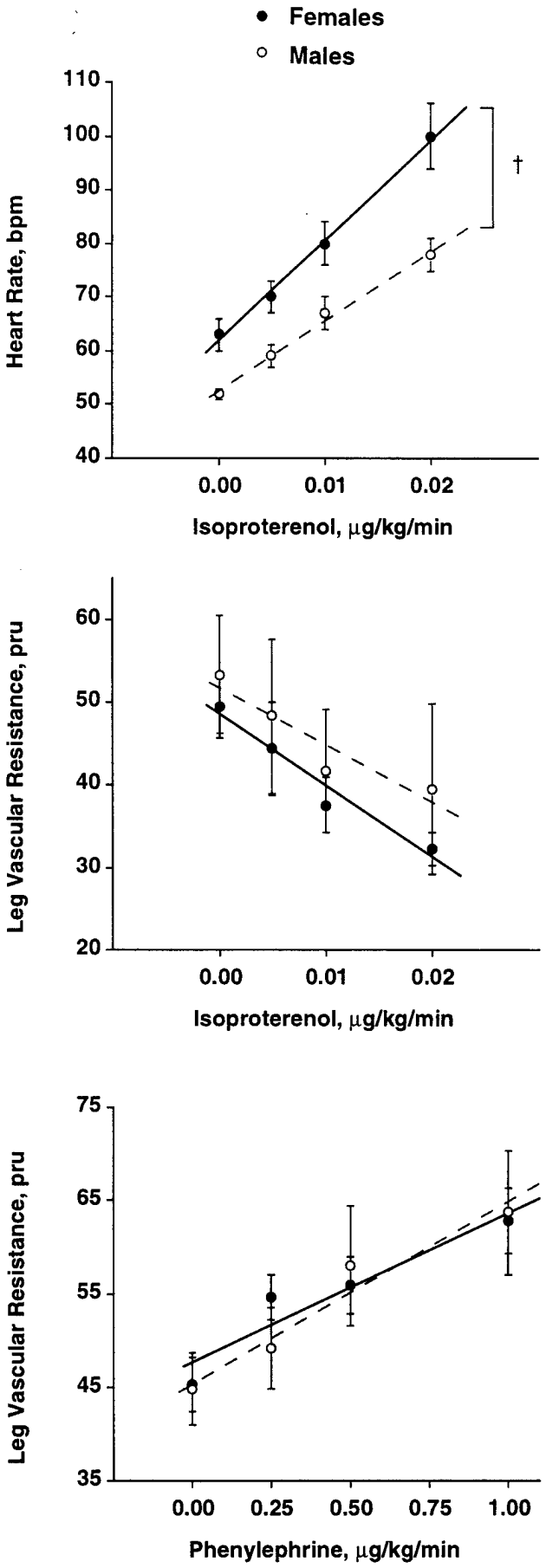

Figure 7. Dose-response relationships between ISO and heart rate, between ISO and leg vascular resistance, and between PE and leg vascular resistance for females and males.

APPENDIX 2

Tables

Table 1. Subject descriptive data.

Table 2. Hemodynamic responses at presyncope level of LBNP.

Table 3. Hemodynamic responses (slopes) to graded LBNP.

Table 4. Baseline blood volume and catecholamine data.

Table 1. Subject descriptive data.

Variable	Men N = 10	Women N = 10	F	P
Age, yr	38 ±2	36 ±1	1.8999	0.1860
Height, cm	175 ±2	164 ±2	13.6792	0.0018
Weight, kg	78.9 ±2.5	65.4 ±2.8	11.2462	0.0038
Estimated body fat, %	14.7 ±1.0	26.4 ±2.0	26.6683	<0.0001
Rest SBP, mmHg	122 ±2	113 ±2	7.9287	0.0119
Rest DBP, mmHg	74 ±2	70 ±2	2.2910	0.1485
Rest heart rate, bpm	52 ±2	64 ±3	9.5447	0.0067
Max heart rate, bpm	182 ±3	187 ±4	0.7966	0.3862
VO_2max, $ml \cdot kg^{-1} \cdot min^{-1}$	47.5 ±2.0	38.2 ±1.5	11.6113	0.0039
VO_2max, $ml \cdot kg^{-1}$ LBM$\cdot min^{-1}$	55.3 ±2.0	53.1 ±2.3	0.4735	0.5019

Values are mean ± 1 standard error. SBP, systolic blood pressure; DBP diastolic blood pressure; VO_2max, maximal oxygen uptake

Table 2. Hemodynamic responses at presyncope level of LBNP.

Variable	Men N = 10	Women N = 10	F	P
Heart rate, bpm	100 ± 7	99 ± 7	0.0028	0.9580
Stroke volume, ml	48 ± 5	35 ± 3	5.1972	0.0350
Cardiac output, liters/min	4.64 ± 0.44	3.42 ± 0.28	5.5339	0.0302
Mean arterial pressure, mmHg	99.5 ± 2.5	88.7 ± 2.6	9.0305	0.0076
Total peripheral resistance, pru	24.3 ± 1.8	25.7 ± 2.3	0.2069	0.6546
Forearm vascular resistance, pru	72.4 ± 8.2	67.2 ± 4.8	0.3053	0.5874
Leg volume change, ml/100ml	1.96 ± 0.19	0.82 ± 0.13	25.0996	<0.0001

Values are mean ± 1 standard error. bpm, beats per minute; pru, peripheral resistance units

Table 3. Hemodynamic responses (slopes) to graded LBNP.

Variable	Men N = 10	Women N = 10	F	P
Heart Rate Slope, bpm/mmHg	0.37 ±0.05	0.54 ±0.08	3.4106	0.0813
Stroke Volume Slope, ml/mmHg	-1.06 ±0.10	-1.21 ±0.14	0.7905	0.3857
Cardiac Output Slope, l/min	-0.03 ±0.01	-0.06 ±0.01	5.5384	0.0302
MAP Slope, mmHg/mmHg	-0.15 ±0.03	-0.17 ±0.05	0.1144	0.7391
TPR Slope, pru/mmHg	0.18 ±0.10	0.42 ±0.05	4.7474	0.0429
FVR Slope, pru/mmHg	0.07 ±0.02	0.16 ±0.03	7.7491	0.0123

Values are mean ± 1 standard error. bpm, beats per minute; pru, peripheral resistance units

Table 4. Baseline blood volume and catecholamine data.

Variable	Men N = 9	Women N = 9	F	P
Total Blood Volume, ml	5544 ±257	4116 ±152	22.8321	0.0002
Plasma Volume, ml	3139 ±155	2624 ±121	6.8838	0.0184
Red Blood Cell Volume, ml	2405 ±118	1621 ±94	26.8458	<0.0001
Hct, percent	43.4 ±0.8	39.2 ±1.1	9.4012	0.0074
Baseline Norepinephrine, pg/ml	277 ±27	302 ±37	0.2982	0.5926
Total Norepinephrine, ng	864 ±108	791 ±118	0.2076	0.6556
Baseline Epinephrine, pg/ml	29 ±7	40 ±25	0.1938	0.6657
Total Epinephrine, ng	95 ±27	120 ±76	0.0922	0.7658

Values are mean ± 1 standard error. pg, picograms; ng, nanograms

APPENDIX 3

Final Report

Bibliography:

Publications: Convertino, V.A. Gender differences in autonomic functions associated with blood pressure regulation. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)* (submitted for publication).

Meeting Abstract: Convertino, V.A. Autonomic functions associated with blood pressure regulation and orthostatic performance in women. Submitted for presentation at the 1998 Annual Meeting of the Aerospace Medical Association.

List of Personnel Receiving Pay From this Effort:

Principal Investigator
Contractor Engineer
Contractor Electronic Technicians (2)