INVESTIGATING THE USE OF RESISTANCE BREATHING FOR THE DETECTION OF ACUTE HYPOVOLEMIA

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PRACTICUM REPORT

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ABSTRACT

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Introduction: Standard vital signs (e.g., heart rate and blood pressure) lack sensitivity and specificity to detect blood volume status following hemorrhage. Inspiratory resistance breathing has therapeutic potential to increase blood pressure and cardiac output following blood loss. We investigated the potential utility of resistance breathing as a novel method to detect volume loss. We hypothesized that resistance breathing would elicit greater increases in absolute and breath-to-breath amplitude of stroke volume and arterial pressure under hypovolemic vs. normovolemic conditions.

Methods: Data were retrospectively analyzed from 23 healthy human subjects aged 23-40 years. Subjects underwent lower body negative pressure (LBNP) protocols to simulate hemorrhage with and without resistance breathing (via an impedance threshold device, ITD). Continuous arterial pressure and stroke volume were measured via finger photoplethysmography. Comparisons of absolute and changes in the breath-to-breath amplitude of arterial pressure and stroke volume were made under 4 conditions: 1) normovolemia; 2) normovolemia + resistance breathing; 3) hypovolemia, and; 4) hypovolemia + resistance breathing. The sensitivity and specificity of breath-to-breath arterial pressure and stroke volume amplitude responses in distinguishing between normovolemia and hypovolemia were assessed via area under the curve (AUC) of receiver operating characteristic (ROC) curves. **Results:** With resistance breathing the amplitude of systolic arterial pressure ($P=0.007$), diastolic arterial pressure ($P<0.001$), and mean arterial pressure ($P<0.001$) increased during hypovolemia vs. normovolemia, and the amplitude of stroke volume decreased ($P=0.002$). In distinguishing between normovolemia and hypovolemia, the ROC

AUC were >0.86 for breath-by-breath mean, maximum and minimum stroke volume responses, and 0.77 for the amplitude response. The ROC AUC for mean arterial pressure amplitude was 0.88, and 0.64, 0.54, and 0.72 for the mean, maximum and minimum responses.

Conclusions: The dynamic responses of arterial pressure and stroke volume with resistance breathing during hypovolemia show promise as a diagnostic tool for detection of hypovolemia in humans.

ACKNOWLEDGEMENTS

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Off campus, I am so grateful for my cousin, Ben, and his family for allowing me to live in their home for the past two years while I completed my studies. Finally, I would like to thank my loving parents for the endless support they have provided me.

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CHAPTER I. BACKGROUND AND LITERATURE REVIEW

Epidemiology of Hemorrhage

There are approximately 50,000 trauma-related deaths each year in the US, and many of these deaths occur in young people (18-44 years of age), accounting for nearly 2 million years of life lost (2). Hemorrhage is a major cause of these traumatic deaths around the world (39%) (19), and about 50% (1) of traumatic deaths occur within 24 hours following the injury. Hemorrhage can occur following traumatic injury such as car accidents, gun shot and knife wounds; however, some surgical procedures, blood clotting disorders, ruptured ulcers, and childbirth also pose a risk of death from hemorrhage and create a need for rapid detection and treatment of the blood loss.

Hemorrhage Detection – Standard Vital Signs

In emergency medicine, the term "the golden hour" highlights the urgency for rapid treatment of traumatic injury due to increased morbidity and mortality when patients do not reach definitive care within 60 minutes of their injury (17). This time period is therefore critical for rapid and accurate detection of the physiological condition of the patient, such as assessment of their blood volume status. In the pre-hospital and emergency room settings, current measurements used to detect hypovolemia secondary to hemorrhage include intermittent arterial pressure, pulse oximetry, pulse character, respiration rate, and heart rate. Despite their wide use, however, these traditional vital signs exhibit limited sensitivity and/or specificity for the early and rapid detection of this life-threatening condition. In a retrospective study where vital signs from patients who required transport to a level 1 trauma facility were assessed, there were no differences in systolic, diastolic and mean arterial pressures between patients who lived or eventually died, nor were there differences in peripheral oxygen saturation or heart rate that would indicate the severity of their

volume status (8). Decreasing arterial pressure is a primary clinical indicator of hypovolemia, but it is unreliable for tracking progressive volume loss due to compensatory mechanisms, such as the baroreflex, which initiates vasoconstriction and tachycardia to keep arterial pressure relatively stable. Finally, while heart rate does respond rapidly to central hypovolemia with a reflex tachycardia (22), the range of possible causes for this response, such as psychological stress, pain, or use of illicit substances, make it a non-specific and unreliable diagnostic tool for medical personnel.

Hemorrhage Detection – Novel Methods

Due to the importance of reliably determining a patient's volume status, recent efforts have been made to develop alternative monitoring approaches that exhibit higher sensitivity and specificity than standard vital signs. Some of the novel measures of hypovolemia include indices developed from traditional vital signs that can be obtained from equipment that is currently used in clinical settings. For example, pulse pressure is calculated by subtracting diastolic arterial pressure from systolic arterial pressure to track stroke volume (18), and increasing R-wave amplitude from the ECG is also strongly related to decreases in stroke volume (R^2=0.99) (15). Another proposed index derived from the ECG is the standard deviation in the R-R interval (RRISD). At rest, the predominate neural input to the heart is parasympathetic via the vagus nerve. Due to the reciprocal relationship between parasympathetic and sympathetic activity, increases in sympathetic activity will be mirrored by decreases in parasympathetic activity. Accordingly, the RRISD is interpreted as an index of vagal tone, as a decrease in RRISD is strongly correlated with an increase in sympathetic nerve activity (R2=0.96) (18). R-wave amplitude and RRISD rely on high quality ECG signals and feature detection algorithms, which are very sensitive to movement of the patient

and accurate anatomical placement of electrodes. Furthermore, assessment of pulse pressure relies on non-invasive, intermittent blood pressure measurements (vs. continuous monitoring), and can also be difficult to accurately capture if the patient is moving.

The Indexed Heart to Arm Time (iHAT) method is derived from combining elements of the ECG and pulse oximetry (SpO_2) waveforms (21). The iHAT method incorporates estimates of the reduction in preload and increases in heart rate by dividing the interval between the ECG R-wave peak and the subsequent SpO_2 wave peak by the R-R interval. Using this index and an experimental model of hemorrhage called lower body negative pressure (LBNP) to induce central hypovolemia (described in more detail subsequently), investigators found an increase in iHAT from ~34% at baseline to ~54% at a LBNP stage of -80 mmHg, presenting a ~20 ml/kg hemorrhage (10, 21). A drawback to the iHAT method is the requirement for baseline measures for each patient, which is possible during some conditions such as planned surgery or childbirth, for example, but is not feasible under most other conditions where hemorrhage occurs.

Other characteristics of plethysmographic waveforms from pulse oximeters have also been examined for estimating volume loss. Using LBNP, investigators recorded plethysmographic waveforms using finger, forehead and ear pulse oximetry devices to test the hypothesis that alterations in pulse oximeter waveform characteristics would track progressive reductions in central blood volume (14). When represented as a percentage of tolerance time to experimental central hypovolemia induced by LBNP, these investigators reported that pulse amplitude and area under the curve (AUC) decreased at 60% LBNP tolerance compared with baseline, while pulse width only decreased at 80% of LBNP tolerance (14). In comparison, stroke volume decreased at

40% of LBNP tolerance. While pulse oximetry waveform characteristics were altered with simulated volume loss, changes in these measurements only occurred relatively late in the progression of central hypovolemia. This lack of sensitivity could result in delayed detection of hypovolemia in the clinical setting. It is also important to acknowledge that the signal quality of pulse oximeters can be negatively impacted by improper placement, interference of the signal due to contaminants on the skin or nails (nail polish for finger probes, for example), or poor circulation to the fingers or ears following a hemorrhagic accident.

The Compensatory Reserve Index (CRI) is a novel approach to detect hemorrhage, which also includes a proprietary algorithm developed to evaluate changes in the shape of the waveform generated from the pulse-oximeter (4). Under normovolemic conditions, the ejected and reflected waveforms from the pulse oximeter appear merged together into a single peak. In contrast, with hypovolemia, the reflected waveform appears as an entirely separate peak displaced from the ejected waveform. These different waveform characteristics have been utilized to develop a machine-learning algorithm that can be used to detect volume status. The CRI has been examined during both LBNP and actual hemorrhage protocols in primates, and tracked volume status with equivalent or higher sensitivity and specificity (analyzed via Receiver Operating Characteristic (ROC) curves) than stroke volume in both conditions. From the LBNP protocol, the ROC AUC for the CRI was 0.94, compared to 0.92 for stroke volume, and from the hemorrhage protocol the ROC AUC for the CRI was 0.94, compared to 0.84 for stroke volume.

Resistance Breathing for Detection of Volume Status

While these novel hypovolemia detection methods have their strengths, there is still a need to investigate additional alternative techniques that could benefit clinicians treating hemorrhagic injuries. In this study, we propose that detection of blood volume loss could be accomplished through the innovative use of resistance breathing. With spontaneous inspiration, both intrathoracic and intracranial pressures become slightly negative which facilitates the "suction" of blood toward the heart and brain (16). These reductions in intrathoracic pressure generated by expansion of the thorax, increase venous return, and increase preload. When inhaling against resistance, the magnitude of these reductions in intrathoracic and intracranial pressures increases, subsequently further increasing venous return, stroke volume, and arterial pressure (6).

Commercially available devices have been developed to elicit consistent resistance to inhalation, with valves that open when intrathoracic pressure reaches a pre-determined threshold. These devices facilitate controlled reductions in intrathoracic pressure and air flow into the lung with inspiration, and do not provide any resistance to exhalation. Resistance breathing has been shown to yield therapeutic benefits in experimental and clinical models of central hypovolemia. For example, in an animal study where anesthetized and intubated pigs were bled 55% of their initial blood volume, the use of resistance breathing along with positive pressure ventilation for 90-min increased mean arterial pressure from ~35 mmHg to 55 mmHg (3). Furthermore, of the pigs that underwent the hemorrhage plus resistance breathing protocol, all nine survived past 24 hours of recovery compared to only one survivor in the untreated control group (23). In human subjects experiencing central hypovolemia with LBNP, both mean arterial pressure and stroke volume were higher when the subjects were breathing against resistance compared with the control condition (18). Finally, in a prospective observational study conducted by a large metropolitan fire

department, application of resistance breathing in symptomatic hypovolemic patients in the field, resulted in increases in systolic arterial pressure of about 20 mmHg compared with the control condition without resistance (22).

While resistance breathing has known therapeutic benefits for *treating* hypovolemia, it is unknown whether the hemodynamic changes induced by this treatment could also be used as a diagnostic tool for *detection* of hypovolemia. In this investigation, we examine the effects of resistance breathing on changes in stroke volume and arterial pressure with and without central hypovolemia to evaluate its diagnostic potential for hemorrhage detection.

Hemorrhage Simulation via Lower Body Negative Pressure (LBNP)

To investigate our novel approach to volume status monitoring in human subjects, we must utilize an experimental method that will reliability induce central hypovolemia similar to actual hemorrhage. LBNP has been used for this purpose for many decades (13). LBNP is conducted by sealing human subjects inside a large vacuum chamber at the level of the iliac crest so that a relative negative pressure can be applied to the lower body. This stimulus sequesters blood volume to the lower extremities, away from the heart and the head, and away from the baroreceptors located in the aorta and carotid arteries, creating a state of central hypovolemia. Venous return subsequently decreases, which reduces stroke volume and cardiac output, and once the cardiovascular compensatory mechanisms have been overwhelmed, arterial pressure decreases and tissue perfusion is diminished (12). Often, LBNP is applied until subjects reach their individual tolerance, which is generally defined by attaining a pre-determined arterial pressure threshold (e.g., 80 mmHg systolic arterial pressure) or the onset of pre-syncopal symptoms (e.g., lightheaded, nausea,

dizziness). At these tolerance points, central blood volume has decreased by ~50%, indexed by the reduction stroke volume (12). A previous study from our laboratory (figure 1) demonstrated that the LBNP stimulus is reproducible in regards to the magnitude of decrease in stroke volume and cardiac output (12).

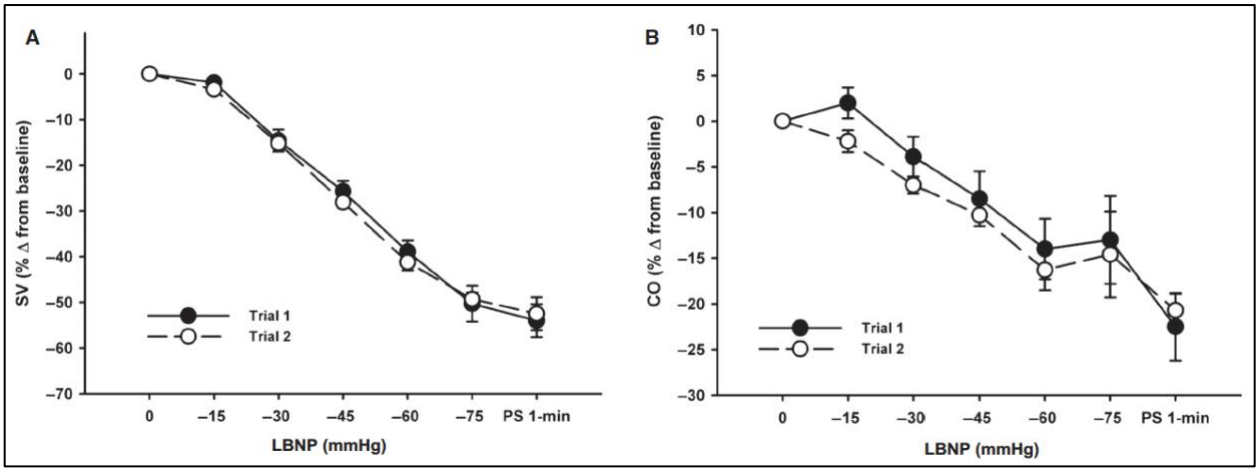

Figure 1 *Reproducibility of stroke volume and cardiac output responses to LBNP ramp protocol* (12)

When using LBNP to induce central hypovolemia, mean arterial pressure is stable even up to LBNP of -60 mmHg (7) representing a ~20% reduction in central blood volume (10). Similarly, peripheral oxygen saturation values do not change throughout LBNP (14), and respiration rates either do not change at all, or change only at the end of LBNP (5), even when subjects undergo maximal reductions in central blood volume, represented by the onset of presyncopal symptoms. These findings reinforce the lack of sensitivity in these vital signs for early and accurate detection of blood volume loss and reiterates the need for a novel volume status detection method.

Summary

With this knowledge in mind, this investigation focuses on answering the following questions:

1. When resistance breathing is in use, are the changes in absolute arterial pressure (systolic, diastolic, pulse, and mean) and stroke volume different during an experimental model of hemorrhage versus the normovolemic condition?

2. When resistance breathing is in use, are the changes in waveform characteristics of arterial pressure (systolic, diastolic, pulse, and mean) and stroke volume different during an experimental model of hemorrhage versus the normovolemic condition?

3. Can differences in these responses in arterial pressure and stroke volume with resistance breathing facilitate differentiation of the normovolemic and hypovolemic conditions?

Our **central hypothesis** is that, with the application of resistance breathing via an impedance threshold device (ITD), the differences in arterial pressure and stroke volume characteristics will allow for the sensitive and specific determination of a subject's volume status (normovolemia vs. hypovolemia).

If we demonstrate that inspiratory resistance breathing can reliably distinguish hypovolemia from normovolemia in this experimental model, further investigations examining the use of inspiratory resistance breathing as a diagnostic tool will be warranted. These investigations may include studies with blood loss protocols or investigations focusing on different hemodynamic variables using equipment more readily available in the clinical setting.

CHAPTER II. RESEARCH PROJECT

Specific Aims

Specific Aim 1: Under hypovolemic conditions, demonstrate that inspiratory resistance breathing elicits greater increases in absolute stroke volume and arterial pressure, and the amplitude of variation in stroke volume and arterial pressure compared with the normovolemic condition. This aim will be achieved by retrospective analysis of stroke volume and arterial pressure waveform data collected from human subjects breathing against resistance with and without central hypovolemia (induced by application of lower body negative pressure, LBNP). We **hypothesize** that 1) the increases in absolute stroke volume and arterial pressure will be greater when subjects breathe with inspiratory resistance during hypovolemia vs. normovolemia, and; 2) the amplitude of the variations in stroke volume and arterial pressure will be greater with resistance breathing during hypovolemia vs. normovolemia.

Specific Aim 2: **Demonstrate that inspiratory resistance breathing can be used to distinguish between normovolemia and hypovolemia with high sensitivity and specificity when compared with traditional vital signs.** This aim will be achieved by conducting ROC curve analysis on the arterial pressure and stroke volume responses to resistance breathing (from Specific Aim 1), in addition to responses of the standard vital signs of heart rate, arterial pressure, and respiration rate during hypovolemia without resistance breathing. We **hypothesize** that the ROC AUC for the arterial pressure and stroke volume responses to resistance breathing will be greater than the ROC AUC for the standard vital sign data, indicating higher sensitivity and specificity of resistance breathing for detection of hypovolemia.

Significance and Innovation

This project is significant because we are investigating a technique that we anticipate will improve the ability of medical personnel to quickly and accurately determine the volume status of patients who have suffered hemorrhagic injuries, particularly in the pre-hospital and emergency room trauma settings. Current monitoring technologies lack the specificity and sensitivity required for early, rapid, and accurate detection of volume loss. The findings from this study will demonstrate whether our novel approach of hemorrhage detection (resistance breathing) is sensitive and specific when compared with standard vital signs. This is an important area of investigation as nearly two million years of life are lost every year to traumatic accidents (2), and of those traumatic deaths, 39% are the result of hemorrhage (19).

This proposal is innovative for three primary reasons:

1) We will safely simulate hemorrhage in an experimental setting in healthy human subjects through the use of LBNP. LBNP has been validated against actual blood loss in studies conducted in both humans and animals. In a baboon study where each animal underwent both LBNP and an actual hemorrhage protocol, stages of LBNP were matched to specific percentages of blood volume lost based on central venous pressure and pulse pressure [see data in Table 1 (10)].

Table 1 *Blood volume (BV) removal with the matching LBNP stages from a baboon study* (10)

	$n = 14$	$n = 14$	$n = 14$	$n = 12$
BV, %	6.25	12.5	18.75	24.5 ± 0.8
BV, ml	136 ± 13	271 ± 26	408 ± 39	529 ± 58
BV, ml/kg	4.5 ± 0.1	9.1 ± 0.2	13.6 ± 0.4	17.8 ± 0.6
LBNP, mmHg	−22 ± 6	−41 ± 7	−54 ± 10	−71 ± 7

Data are means ± SD. Blood volume (BV) loss values are represented as percent, absolute, and relative measurements. Lower body negative pressure (LBNP) levels that matched the BV loss are shown for each level of hemorrhage.

Additionally, in a study where human subjects underwent both blood loss and LBNP protocols, similar responses in mean arterial pressure, stroke volume and heart rate were observed between the two conditions (11). A safety feature of LBNP is that it is a controlled experimental procedure that allows for immediate termination of the stimulus when subjects reach pre-determined threshold criteria, making it a relatively low risk procedure to investigate physiological responses to central hypovolemia in human subjects.

2) We propose a novel diagnostic application of resistance breathing, which has previously been shown to have therapeutic benefit. While resistance breathing has been shown to be protect stroke volume and arterial pressure in animal and human experimental studies of central hypovolemia, and in patients experiencing hypotension, the application of this intervention for diagnostic purposes has not yet been investigated.

3) We will compare the sensitivity and specificity of hemodynamic responses to resistance breathing with traditional vital signs for the determination of volume status. Should this novel approach for detecting volume status be successful in the current investigation, future studies can be conducted to apply resistance breathing in animal studies and clinical studies for detection of actual hemorrhage.

Materials and Methods

Subjects: To address both specific aims, data from healthy young (18-45 y) male and female human subjects (n=23) were utilized in this retrospective analysis. Data from two independent studies were used for a power analysis to determine if 23 subjects was sufficient to address our aims. In these studies, a ~10% increase in stroke volume was observed with resistance breathing during normovolemia (10), and a ~17% increase in stroke volume was observed with use of resistance breathing during hypovolemia (22). Based on these data, 23 subjects are adequate to address our aims with a power $(1-\beta) > 0.95$ and α of 0.05. Each subject had clinically unremarkable standing and seated 12-lead ECGs, resting systolic arterial pressure <140 mmHg, resting diastolic arterial pressure <90 mmHg, and no signs or history of cardiovascular or cerebrovascular abnormalities. Subjects were not included in the study if they used tobacco/nicotine products, prescription or non-prescription drugs, or herbal supplements known to alter autonomic function unless approved prior to the study. Female subjects were tested in the early follicular phase of their menstrual cycle (days 1-4 by self-report) to reduce the potential effects of high estrogen and progesterone on vascular activity. Female subjects using hormonal contraceptives were tested during the sugar pill or no pill phase. All female subjects completed a urine pregnancy test to ensure they were not pregnant before being approved to participate in the study, and immediately prior to every experiment. Our study physician reviewed and approved all screening documents and medical information before each subject's experimental session.

Methods: For the purposes of this investigation, each subject completed 4 visits to the laboratory. In the 24 hours prior to each session, subjects were asked to refrain from exercise, caffeine and prescription and non-prescription medications to reduce the potential effects on cardiovascular

responses. **DAY 1** was a familiarization session to acquaint the subjects with the procedures and equipment that were to be used during the following 3 experimental sessions outlined in figure 2.

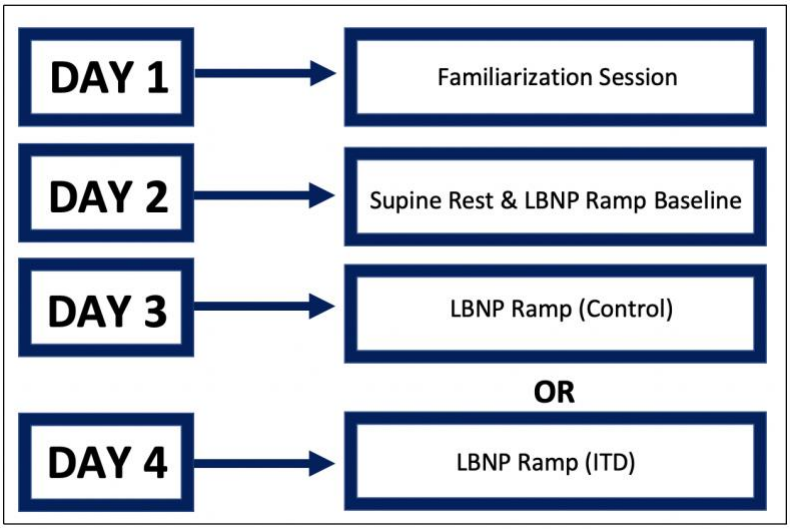

Figure 2 *Overview of the experimental protocol*

For each experimental session (**DAYS 2-4**), subjects were assisted into the LBNP chamber (VUV Analytics Inc., Austin, TX) and the following equipment was attached as shown in figure 3:

- A continuous lead II electrocardiogram (ECG; shielded leads, cable and amplifier, AD Instruments, Bella Vista, NSW, Australia) provided a cardiac rhythm from which the R-R interval was used to determine the subject's heart rate.
- A finger photoplethysmograph (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands) to non-invasively provide continuous arterial pressure recordings.
    - The arterial pressure waveform from the Finometer was analyzed by a proprietary algorithm to obtain beat-to-beat stroke volume.
- Respiration rate was measured with a face mask and tubing attached to an end-tidal gas analyzer (ML206 Gas Analyzer, AD Instruments, Bella Vista, NSW Australia).

Respiration rate was calculated based on the respiratory excursions observed on the end-tidal CO_2 waveform.

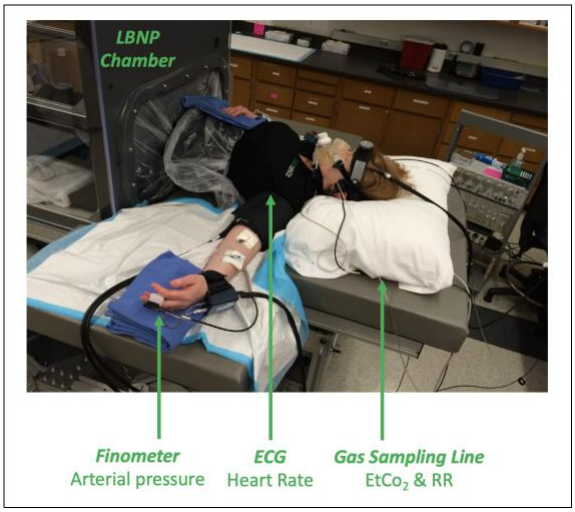

Figure 3 *Subject instrumented and sealed in the lower body negative pressure (LBNP) chamber. $EtCO_2$, end-tidal CO_2; RR, respiratory rate.*

During **DAY 2**, the subjects underwent both a **supine rest** and a **baseline LBNP** protocol. For the **supine rest** protocol, the subject was secured into the LBNP chamber with the seal at the level of their iliac crest. Following instrumentation and a 5-min stabilization period, subjects breathed for 5-min at a rate of 12 breaths/min with an impedance threshold device (ITD; ResQGARD ITD 7, Advanced Circulatory Systems Inc., Roseville, MN) with a cracking pressure of 7 cm.H_2O attached to the facemask (figure 5), or without an ITD (randomized, cross-over design). Following a 5-min rest period, the subjects completed the second breathing protocol (figure 4).

Start	5-min	5-min	5-min	5-min
Instrumentation	Stabilization	ITD OR No-ITD	Rest	ITD OR No-ITD

Figure 4 *Timeline for supine resting protocol*

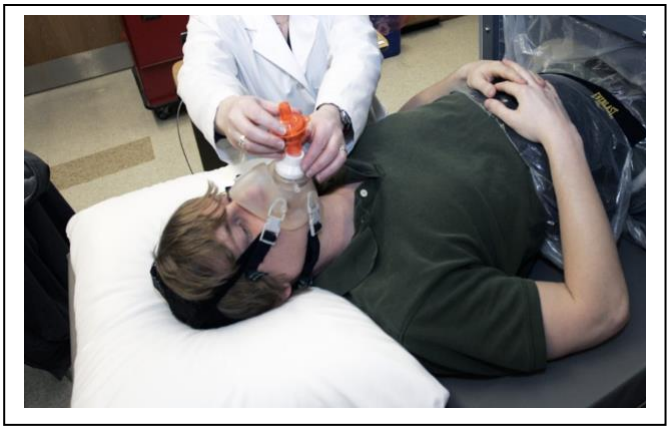

Figure 5 *Subject breathing through an impedance threshold device (ITD) attached to the facemask*

Next, the **baseline LBNP** protocol was used to determine LBNP tolerance of each subject, and to determine when to apply the ITD in subsequent visits.

Upon completion of the supine rest protocol, the subject entered a 5-min recovery period before 5-min of baseline measurements were made. A ramp LBNP protocol was used where negative pressure was applied at a rate of 3 mmHg/min using the pre-programmed LBNP chamber control system. The chamber pressure was immediately released after 1-min at -99 mmHg, or with the onset of one or more of the following criteria: 1) a precipitous fall in systolic arterial pressure (SAP) of >15 mmHg; 2) progressive reduction in SAP to below 80 mmHg; 3) sudden bradycardia; or 4) subject voluntary termination due to the onset of pre-syncopal symptoms such as dizziness, nausea, sweating, gray-out or general discomfort. Upon the release of chamber pressure, pre-syncopal symptoms usually resolved within 30-60 s, but the subjects remained in the chamber for a 10-min recovery period before the instrumentation was removed. Subjects were then assisted out of the chamber and monitored by research personnel for 10-15 min before they were allowed to leave the laboratory.

During the **DAY 3** and **DAY 4** visits, the subjects either underwent the **control LBNP** protocol or the **LBNP + ITD** protocol. The **control LBNP** protocol was identical to the **baseline LBNP** protocol described above, but it was designed to limit the order effect that may have occurred due to the intervention always following the control condition.

For the **LBNP + ITD** protocol, subjects experienced the same LBNP protocol at 3 mmHg/min of chamber pressure. The ITD was placed on the facemask either 5-min prior to the pre-syncopal point determined during the baseline protocol, or if the subject experienced a 30% reduction in stroke volume from baseline (which was calculated during the first 5-min of baseline of this protocol), whichever event came first. After application of the ITD, subjects were instructed to breathe at a depth and rate most comfortable for them for the remainder of the protocol, which ended when the criteria described for the baseline protocol were reached.

Data Analysis: Continuous waveform data for ECG, arterial pressure, stroke volume, and $etCO_2$ were collected at a frequency of 1000 Hz (PowerLab and LabChart, AD Instruments, Bella Vista, NSW, Australia). The data were analyzed via LabChart for assessment of absolute changes in stroke volume and arterial pressures (systolic, diastolic, mean, and pulse pressure), and the amplitude of variations in stroke volume and arterial pressures (Specific Aim 1).

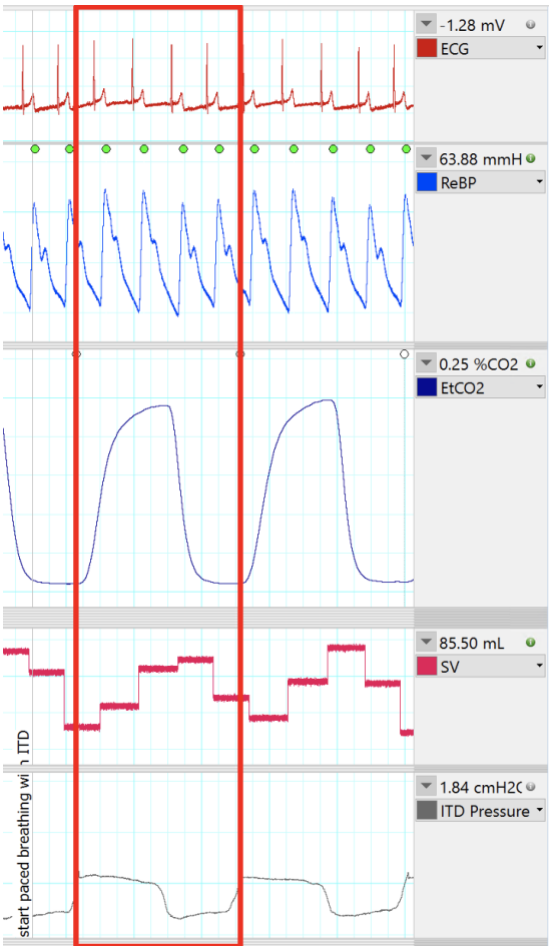

Figure 6 *Example of recordings from LabChart showing hemodynamic measures observed across two full respiratory cycles with one cycle enclosed within the red box. ReBP, reconstructed blood pressure; $EtCO_2$, end-tidal CO_2; SV, stroke volume; ITD, inspiratory threshold device.*

Waveform data from LabChart (figure 6) were analyzed over multiple respiratory cycles. In the $EtCO_2$ channel from the screen capture above, the lowest $EtCO_2$ values were automatically marked (white circles) and the time between each of these markers represents one complete respiratory cycle. With the respiratory cycle determined, LabChart was used to calculate the mean, maximum, minimum, and amplitude values from the reconstructed arterial pressure and stroke volume signals for each breath (as seen in figure 7). This breath-by-breath analysis was conducted for a 2-minute period following ITD application (or at the corresponding time in the no-ITD protocols), and mean values were calculated for each waveform characteristic.

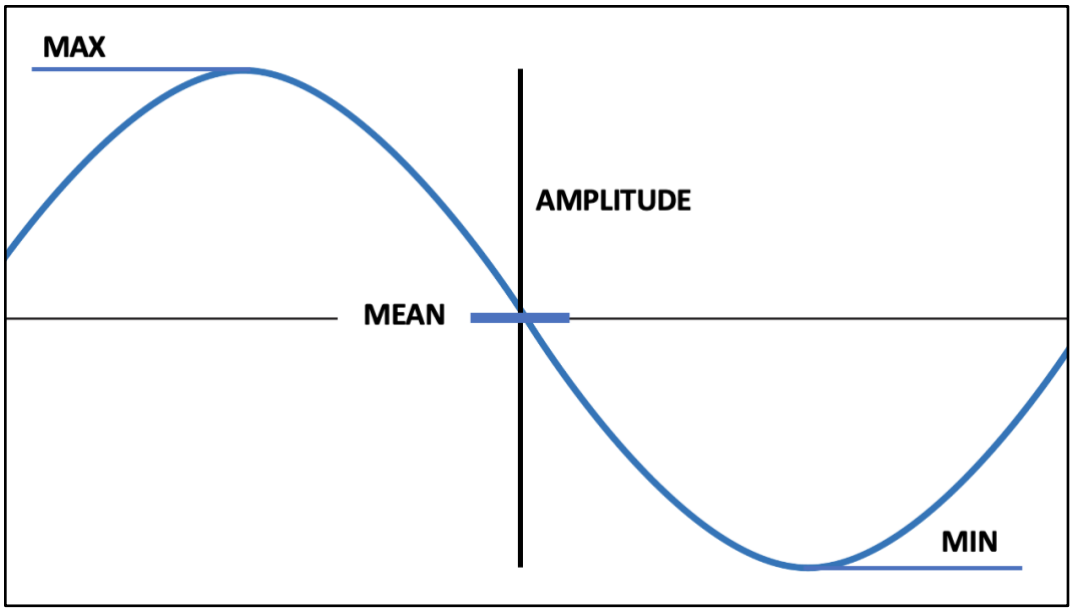

Figure 7 *Representation of a hemodynamic waveform across a single breath. The mean, maximum (max), minimum (min), and amplitude of each waveform was recorded over a 2-min window.*

For comparison of the effects of resistance breathing on stroke volume and arterial pressure during the **normovolemic** condition, measurements from the DAY 2 **supine rest** protocol were analyzed. Specifically, the measurements recorded during the 5-min paced breathing segments either with or without the ITD were compared (see figure 3).

For comparison of the effects of the resistance breathing on stroke volume and arterial pressure during the hypovolemic condition, measurements from the DAY 3 and DAY 4 protocols were compared. Specifically, the measurements recorded within the first 2-min after the application of the ITD in the **LBNP + ITD** protocol. The exact same time period was used to compare measurements for each individual subject during the **control LBNP** protocol. Because the time of ITD application was different for each subject (as determined during their baseline LBNP protocol)

it was important to use the same timeframe within each subject to determine the effects of resistance breathing on cardiovascular responses.

Statistical Analysis: All data are presented as means ± standard deviation, and actual P-values are presented for all comparisons. To address Specific Aim 1, a two-way ANOVA with repeated measures, and paired t-tests or Wilcoxon Signed Rank tests were conducted in SigmaPlot (Version 11.0, Systat Software Inc., San Jose, CA). For the ANOVA, the first factor was whether the subject was normovolemic (supine rest) or hypovolemic (LBNP), while the second factor was whether or not the subject was breathing through the ITD. This analysis allowed for comparison of absolute responses in stroke volume and arterial pressure that occurred with application of the ITD during both the normovolemic and hypovolemic conditions. Holm post-hoc tests were conducted to reduce Type I errors (false positive) that can occur with multiple tests.

Data were tested for normality to determine whether Wilcoxon Signed Rank tests (non-parametric) or paired t-tests (parametric) were appropriate to compare the absolute change in (Δ) systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), stroke volume, and pulse pressure between the normovolemic and hypovolemic conditions (see figure 8).

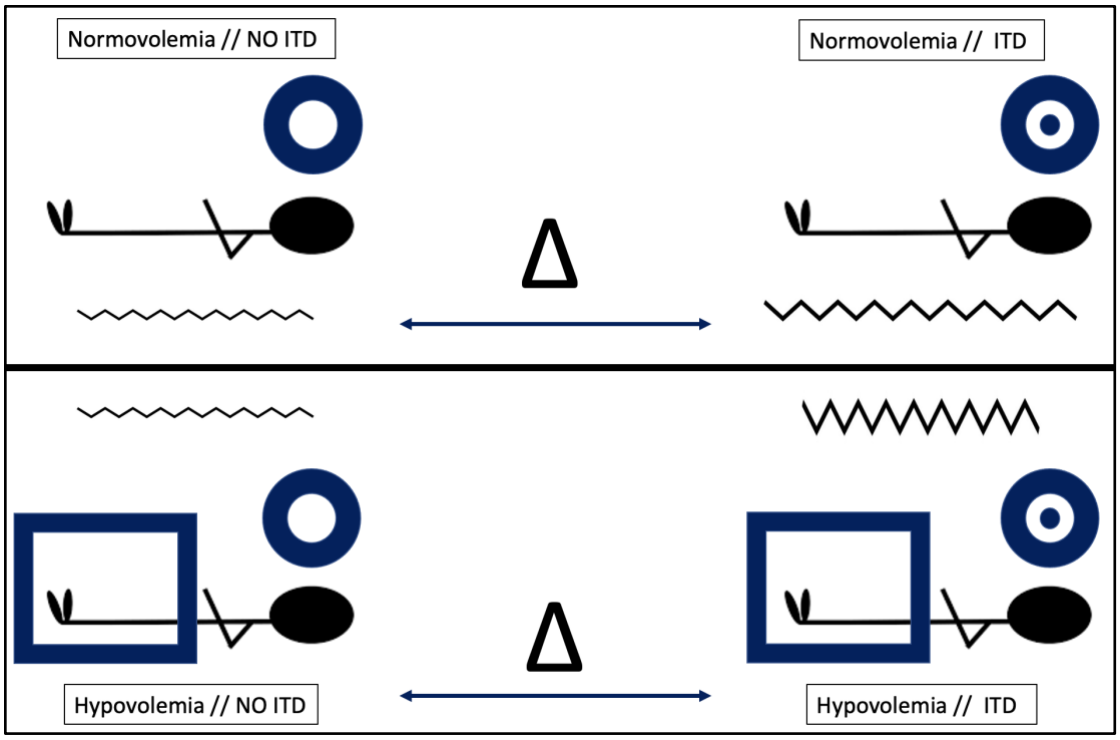

Figure 8 *Schematic of the conditions compared when evaluating the changes in arterial pressure and stroke volume during normovolemia and hypovolemia. ITD, impedance threshold device.*

Paired t-tests or Wilcoxon Signed Rank tests were also used to compare the breath-by-breath amplitude and maximum of SAP, DAP, MAP, and stroke volume between the normovolemic and hypovolemic conditions with resistance breathing (figure 9).

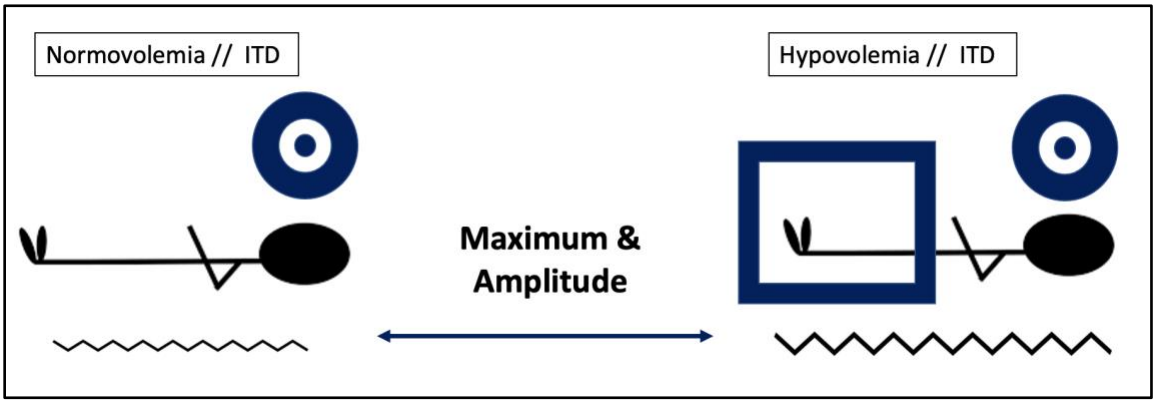

Figure 9 *Schematic of the conditions compared when evaluating the changes in arterial pressure and stroke volume with resistance breathing during normovolemia and hypovolemia. ITD, impedance threshold device.*

To address **Specific Aim 2**, ROC curves were created using SigmaPlot software to assess the effectiveness of using resistance breathing to detect volume status (normovolemia vs. hypovolemia). By comparing true positives (y-axis) and false positives (x-axis), the ROC analysis generates curves as shown below in figure 10. The green highlighted curve is interpreted as a method that is more sensitive and specific because it reaches closer to the top left corner of the graph where more true positives are recorded with fewer false positives; subsequently, the AUC is greater for this curve compared with the other curves. ROC AUCs were determined for heart rate, respiration rate and systolic mean arterial pressure during the normovolemia without ITD condition and the hypovolemia without ITD condition to evaluate traditional vital sign methods for detecting hypovolemia. ROC AUCs were then determined for the mean, amplitude, maximum, and minimum of SAP, DAP, MAP, and stroke volume and for mean pulse pressure by comparing the normovolemia with ITD condition to the hypovolemia with ITD condition.

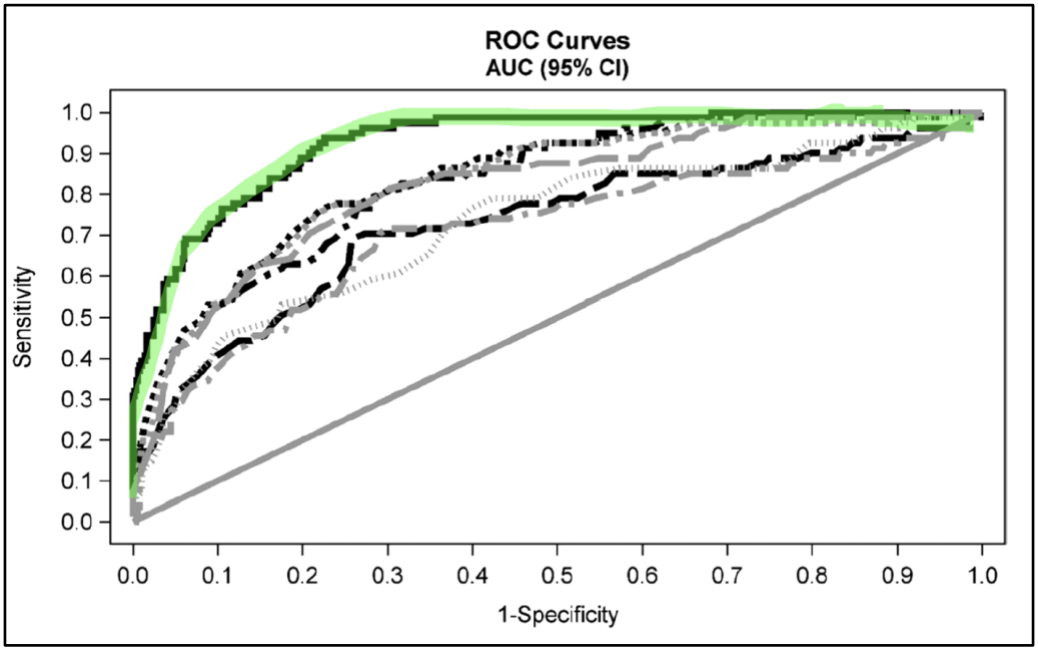

Figure 10 *Example of receiver-operating characteristic (ROC) curves. The measurement with a higher ROC area under the curve (AUC) is considered to be more sensitive and specific (figure adapted from reference (20)).*

Results

Subjects: Data from twenty-three subjects were included in the final statistical analysis for this investigation (15 male, 8 female; age 27.4 ± 4.4 y; height, 170.6 ± 9.1 cm; weight, 75.1 ± 16.6 kg; mean ± SD).

Overall Effect of Volume Status and Resistance Breathing

Absolute pulse pressure, SAP, and SV all decreased with LBNP ($P \leq 0.001$ for normovolemia vs. hypovolemia), and there was no ITD effect (all P-values ≥ 0.42) (Figure 11). MAP only decreased with LBNP without the ITD (P=0.006), while DAP did not change with LBNP (P=0.89) or resistance breathing (P=0.50). Finally, resting respiratory rates were approximately 12 breaths/min, and there was no effect on respiration rate with either LBNP (P=0.62) or ITD breathing (P=0.29).

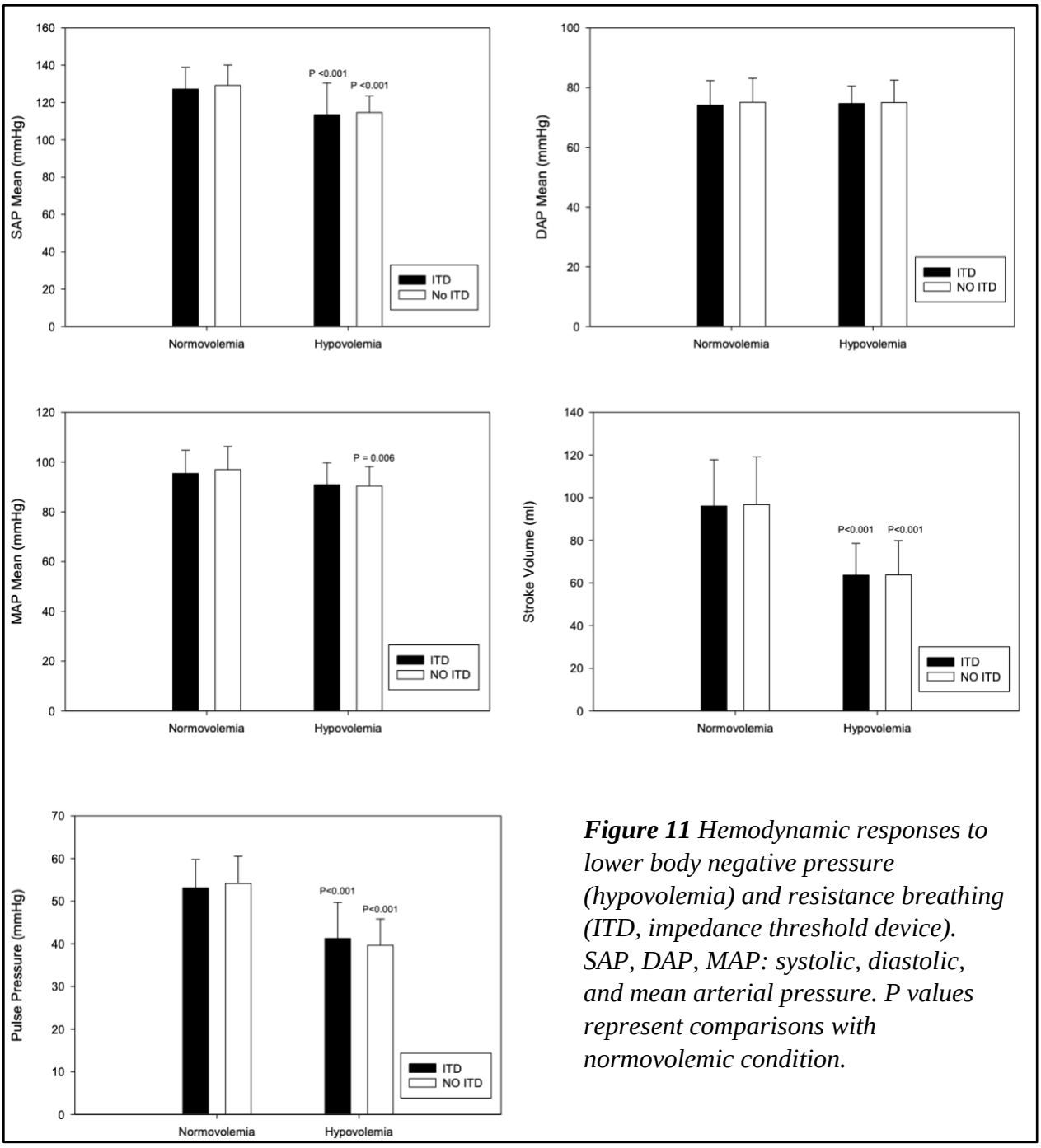

Figure 11 *Hemodynamic responses to lower body negative pressure (hypovolemia) and resistance breathing (ITD, impedance threshold device). SAP, DAP, MAP: systolic, diastolic, and mean arterial pressure. P values represent comparisons with normovolemic condition.*

Breath-by-Breath Responses to Resistance Breathing

With resistance breathing, there were no differences in the hemodynamic responses between volume conditions for the delta (Δ) or maximum values, except for SAP max and SV max, which were both higher with normovolemia vs. hypovolemia (table 2).

Table 2 *Arterial pressure and stroke volume responses to resistance breathing during normovolemia and hypovolemia*

	Normovolemia	**Hypovolemia**	**P-value**
ΔSAP (mmHg)	-2.0 ± 5.5	-1.2 ± 17.1	0.85
ΔDAP (mmHg)	-0.9 ± 3.4	-0.4 ± 8.3	0.78
ΔMAP (mmHg)	-1.6 ± 4.3	0.5 ± 9.8	0.37
ΔSV (ml)	-0.7 ± 4.2	-0.1 ± 9.7	0.78
Δ PP (mmHg)	-1.0 ± 3.0	1.6 ± 7.4	0.12
SAP Max (mmHg)	131.8 ± 12.1	124.0 ± 15.7	0.02
DAP Max (mmHg)	77.8 ± 8.7	80.4 ± 7.7	0.15
MAP Max (mmHg)	99.2 ± 10.0	98.2 ± 10.4	0.63
SV Max (ml)	105.0 ± 24.1	70.4 ± 16.2	<0.001

Paired t-test data presented as means ± standard deviation.

Breath-by-breath amplitude of SAP, DAP, and MAP were all higher with hypovolemia vs. normovolemia, while SV amplitude was lower with hypovolemia vs. normovolemia (figure 12).

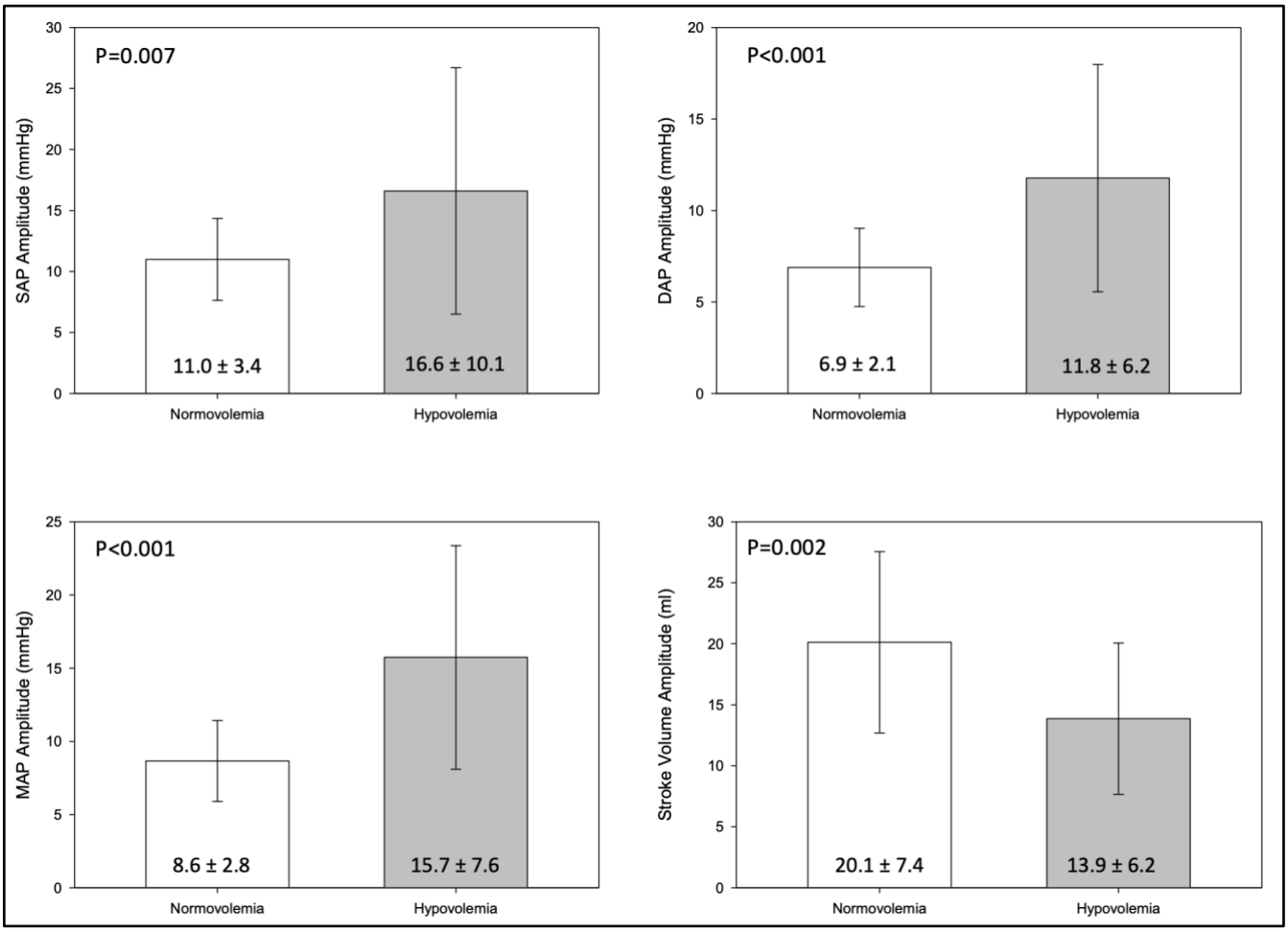

Figure 12 *Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP) and stroke volume amplitude responses to resistance breathing under normovolemic and hypovolemic conditions.*

Receiver Operating Characteristic (ROC) Curve Analysis

Sensitivity and specificity of traditional vital sign methods for detecting hypovolemia from normovolemia were evaluated by calculating the ROC AUC comparing the normovolemia condition without the ITD to the hypovolemic condition without the ITD (Figure 13). The ROC AUC for heart rate was 0.95, 0.85 for SAP, and 0.46 for respiratory rate.

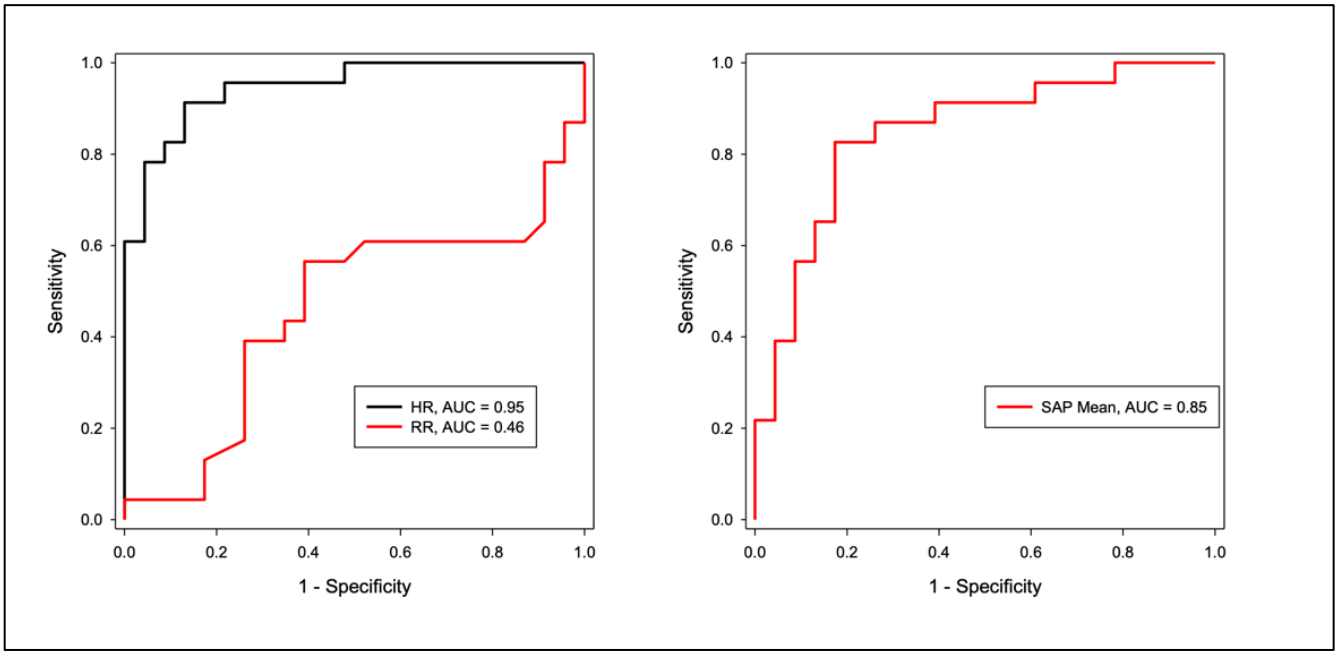

Figure 13 *Receiver Operating Characteristic (ROC) curves for the traditional vital signs of heart rate, respiratory rate (RR) and systolic arterial pressure (SAP). The normovolemia/no ITD condition and the hypovolemia/no ITD condition were compared.*

The novel hypovolemia detection methods were also evaluated via ROC curve analysis comparing the normovolemic condition with the ITD with the hypovolemic condition with the ITD (Figures 14 and 15). The mean, max, min, and amplitude of each measurement was assessed. The HR response had the highest ROC AUC of 0.91, and the ROC AUC was >0.85 for stroke volume mean, max, and min, pulse pressure mean, and MAP amplitude.

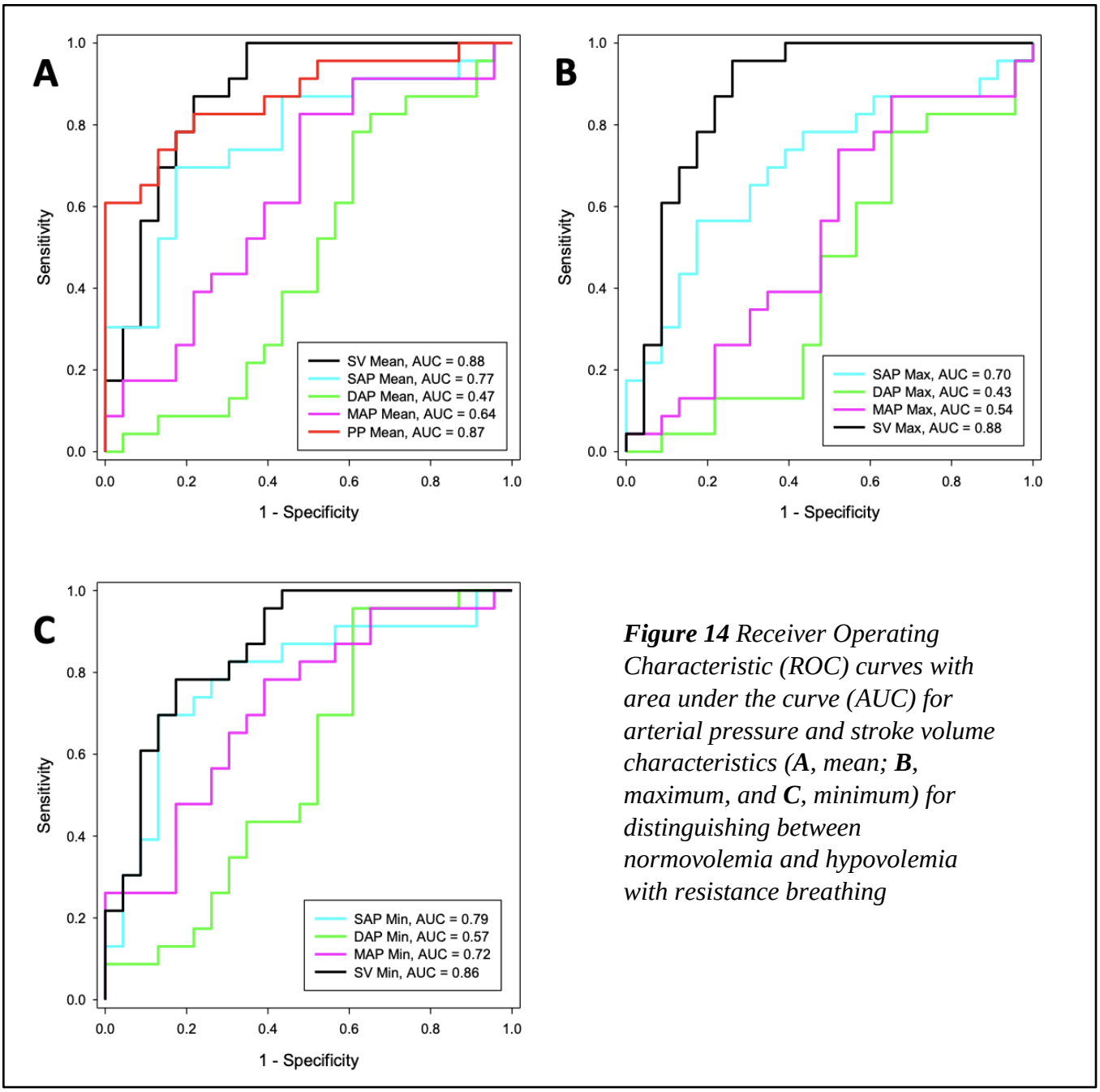

Figure 14 *Receiver Operating Characteristic (ROC) curves with area under the curve (AUC) for arterial pressure and stroke volume characteristics (**A**, mean; **B**, maximum, and **C**, minimum) for distinguishing between normovolemia and hypovolemia with resistance breathing*

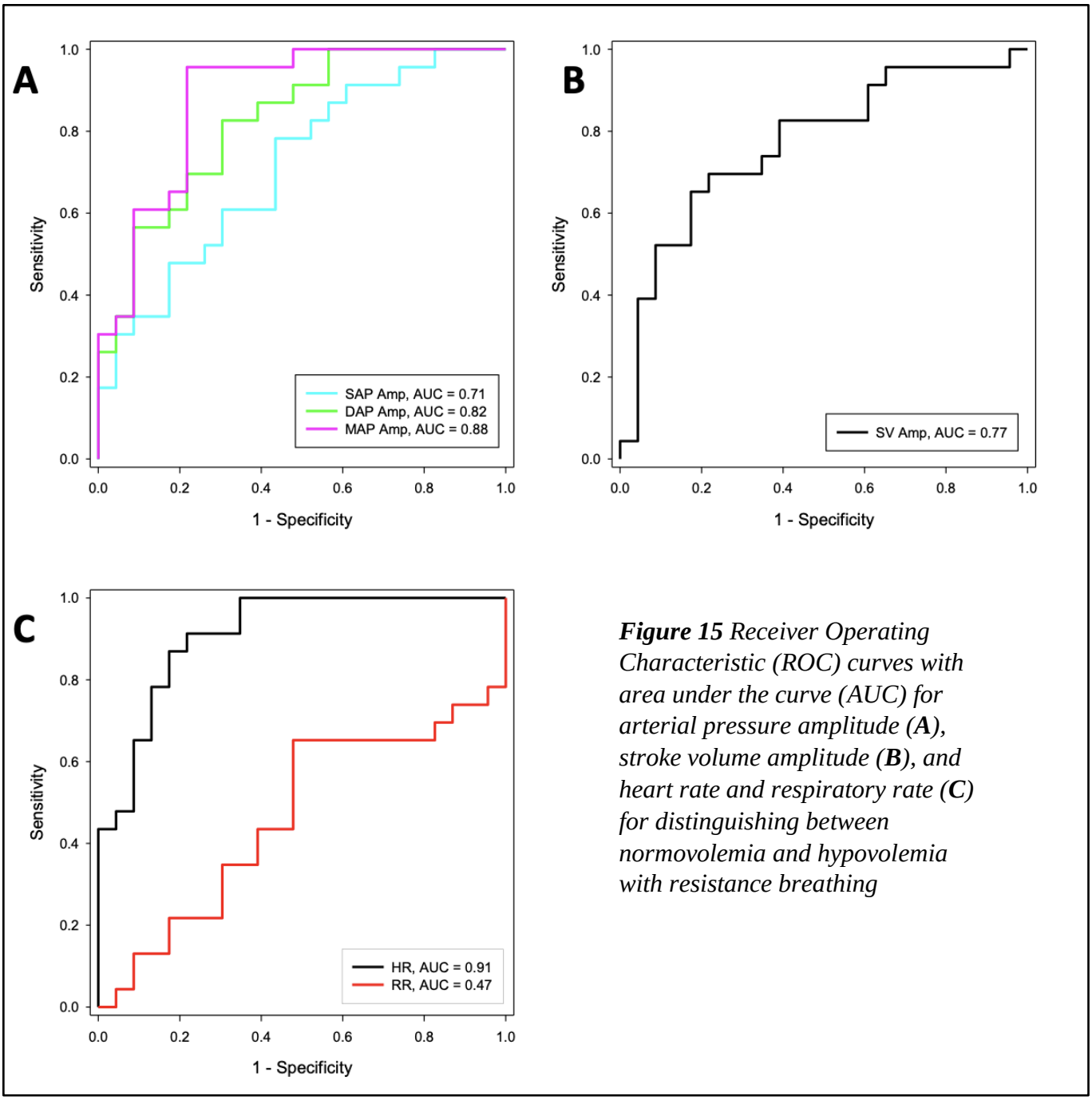

Figure 15 *Receiver Operating Characteristic (ROC) curves with area under the curve (AUC) for arterial pressure amplitude (**A**), stroke volume amplitude (**B**), and heart rate and respiratory rate (**C**) for distinguishing between normovolemia and hypovolemia with resistance breathing*

Discussion

The objective of this study was to determine if inspiratory resistance breathing could be used for the detection hypovolemia in healthy human subjects. The key findings of this study were: 1) decreases in absolute arterial pressures and stroke volume with LBNP were not affected by resistance breathing; 2) there was no effect of resistance breathing on the absolute change in arterial pressures or stroke volume between normovolemia and hypovolemia; 3) breath-by-breath arterial pressure amplitudes were higher with resistance breathing during hypovolemia vs. normovolemia, while stroke volume amplitude was lower under the same conditions; 4) ROC curve analysis revealed high sensitivity and specificity for some of the novel metrics with resistance breathing, suggesting diagnostic potential for these measurements for detection of volume status.

An interesting finding was the decrease in stroke volume amplitude while the amplitude of arterial pressure (systolic, diastolic, and mean) increased. A possible explanation for the reduced stroke volume amplitude could be the reduction in venous return caused by the LBNP stimulus. The reduced venous return means less blood is returning to the heart during each breath. Therefore, across each breathing cycle there will be less cardiac preload and subsequently a reduced stroke volume. While stroke volume amplitude was lower with ITD breathing during hypovolemia, arterial pressure amplitude was higher. A potential explanation for these differential responses may be related to the relationship between arterial pressure, flow, and systemic vascular resistance (i.e., Ohm's law). Cardiac output is the product of stroke volume and heart rate, and cardiac output can be multiplied by systemic vascular resistance to calculate MAP (assuming negligible effects of right atrial pressure). A prior study demonstrated an increase in systemic muscle sympathetic nerve

activity (MSNA) on a breath-to-breath basis, measured via peroneal nerve microneurography, in response to ITD breathing as shown in figure 16 (18). The subsequent cyclic vasoconstriction from increased MSNA with each breath on the ITD may be the cause of the increased arterial pressure amplitudes by cyclically increasing and decreasing systemic vascular resistance. These concepts may also explain how MAP (without the ITD) decreased without an accompanying decrease in DAP. Increases in SVR due to resistance breathing may have protected DAP from change while the decreases in SV produced decreases in SAP and ultimately MAP.

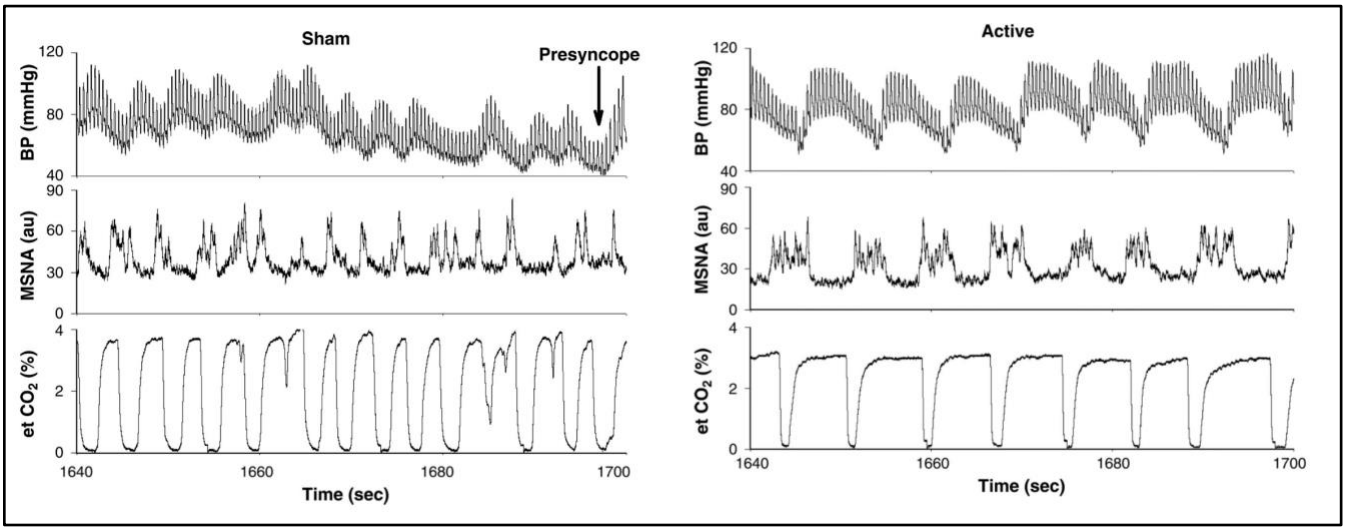

Figure 16 *Effect of breathing without ("Sham") and with ("Active") inspiratory resistance on muscle sympathetic activity (MSNA) during central hypovolemia* (18).

To further examine the sympathetic responses to resistance breathing (with and without hypovolemia), future studies could be conducted with additional measures such as MSNA or circulating catecholamines from blood samples.

The high ROC AUC for the heart rate and SAP responses were not surprising considering the methodological approach we used to explore this question (which was not designed to examine this specific hypothesis *a priori*). Increased heart rate is a very sensitive reflex response to reductions in central blood volume, which consistently decreases with application of LBNP (see figure 13). Accordingly, in our controlled experimental setting, there are few alternative explanations for an increase in heart rate compared with a physician in the clinical setting trying to decide if their patient is hypovolemic, such as the psychological stress or pain experienced during a traumatic injury, or the effects of unknown drugs and toxins in the patient's circulation. The high ROC AUC for systolic arterial pressure may be explained by the comparison of only two extremes in volume status in this study (normovolemia vs. hypovolemia), and baroreflex compensation was already compromised with the magnitude of hypovolemia utilized (see figure 11). To better evaluate the sensitivity of systolic arterial pressure, future investigations could utilize earlier stages of LBNP when systolic arterial pressure is relatively stable due to effective reflex compensation. Indeed, this experimental design would also be appropriate for assessment of all of the novel metrics used in the present investigation to determine their sensitivity and specificity for detection of mild central hypovolemia.

In regards to the novel metrics we explored in this study, the highest ROC AUCs were for stroke volume mean, max, and min, pulse pressure mean, and MAP amplitude (all >0.85), which can be considered as relatively high for the detection of hypovolemia. The ROC AUCs for the other novel metrics were much lower, with some only slightly more predictive than the flip of a coin (i.e., 0.50). While the AUC for heart rate with resistance breathing was highly sensitive and specific (ROC AUC of 0.91) if offered no advantage for detection of volume status over the heart rate

response without resistance breathing (ROC AUC of 0.95). The ROC AUC for respiratory rate while using the ITD did not discriminate between volume status (AUC ROC of 0.47), which suggests that increases in respiration rate due to hypovolemia were not affected by resistance breathing.

Methodological Considerations

LBNP is a validated experimental model of hemorrhage in regards to eliciting central hypovolemia (10), but it does not incorporate all aspects of this complex physiological condition. Actual hemorrhage is often accompanied by additional stress stimuli (such as pain, anxiety, tissue damage) which can lead to an augmented autonomic response beyond the response elicited by the hypovolemic stress alone. While isolating the effects of central hypovolemia is an advantage of the LBNP method, it does not account for the impact that these additional changes in sympathetic nervous activity may have on vascular tone. Future studies could modify the magnitude of sympathetic nerve activity (e.g., through the use of techniques such as hand grip exercise) to more closely mimic these effects, followed by subsequent assessment of resistance breathing for volume status detection.

Another key difference between the LBNP model of central hypovolemia and actual hemorrhage is that blood volume is lost to the external environment with actual hemorrhage, but during LBNP there is a translocation of blood volume to the lower body, which delays the transit of blood back to the heart. This translocation of blood volume into the lower limbs during LBNP, rather than removal of blood from the circulation, results in increased hematocrit (9) as fluid moves out of the

circulation and into the interstitial spaces; this is in contrast to a decrease in hematocrit with actual hemorrhage (10). Hemoconcentration resulting from increased hematocrit may result in increased blood viscosity (η), and an attenuated decrease in blood pressure, according to Poiseuille's Law:

$$\Delta P = \frac{8\eta \ell Q}{\pi r^4}$$

On the other hand, however, decreases in flow (Q) from the LBNP stimulus would result in a decrease in ΔP. As such, it is difficult to measure the magnitude of these opposing effects on arterial pressure and stroke volume with the LBNP stimulus, but it is important to acknowledge this potential limitation of LBNP as an experimental model of hemorrhage, which may affect the translation of our findings to the actual hemorrhage condition.

A clinical limitation of using resistance breathing, such as the ITD, is that hemostasis must be achieved prior to its application so as to not exacerbate the hemorrhagic injury. In this study, the progressive LBNP protocol continued even after the ITD was applied, which differs from the clinical scenario where bleeding would be stabilized first. These limitations must be considered as further studies are designed to assess the effectiveness of resistance breathing as a diagnostic tool under actual hemorrhage conditions. For example, further investigation into the breath-by-breath hemodynamic responses used in this study could be explored in animal models of actual hemorrhage. Furthermore, future studies could be designed to simulate a condition of hemostasis with LBNP by reducing the chamber pressure until a target reduction in stroke volume is achieved (absolute or relative to individual baseline), then keeping that pressure constant throughout the experiment.

As this investigation was a retrospective analysis of previously collected data, we were unable to adapt our approach to account for some of these methodological considerations. However, further examination of these variations in experimental design does pose an exciting line of investigation in this field.

Conclusion

In this proof of principle investigation, we assessed whether resistance breathing could be used as a novel diagnostic tool for clinicians in the determination of volume status following a hemorrhagic injury. We observed increases in the amplitude of arterial pressure and decreases in stroke volume amplitude in response to resistance breathing during central hypovolemia. We also observed high sensitivity and specificity for a select number of hemodynamic measures in detection of volume status with resistance breathing. Future studies should be conducted to address the limitations of this retrospective analysis, including exploration of this approach in animal models of actual hemorrhage to continue the pursuit of improved methods for detection of volume status.

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