

AUTONOMIC FUNCTION RESPONSES COMPARING COLD PRESSOR AND TILT TABLE
TESTING IN A HEALTHY, NORMOTENSIVE POPULATION

A Thesis
by
JAMIE STARK INLOW

Submitted to the Graduate School
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

August 2015
Department of Health and Exercise Science

AUTONOMIC FUNCTION RESPONSES COMPARING COLD PRESSOR AND TILT
TABLE TESTING IN A HEALTHY, NORMOTENSIVE POPULATION

A Thesis
by
JAMIE STARK INLOW
August 2015

APPROVED BY:

Scott Collier, Ph.D.
Chairperson, Thesis Committee

Herman van Werkhoven, Ph.D.
Member, Thesis Committee

Alan Utter, Ph.D.
Member, Thesis Committee

N. Travis Triplett, Ph.D.
Chairperson, Department of Health & Exercise Science

Max C. Poole, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Copyright by Jamie Stark Inlow 2015
All Rights Reserved

Abstract

AUTONOMIC FUNCTION RESPONSES COMPARING COLD PRESSOR AND TILT TABLE TESTING IN A HEALTHY, NORMOTENSIVE POPULATION

Jamie Stark Inlow
B.S., James Madison University

Chairperson: Scott Collier, Ph.D.

The autonomic nervous system regulates unconscious body functions, such as blood pressure, heart rate, respiration, and thermoregulation. Testing for autonomic dysfunction can be dangerous or costly, though it is crucial for individual wellbeing and for certain diagnoses. Understanding how autonomic clinical testing works, and what correlations exist between different tests, may help make autonomic testing less invasive or dangerous. Therefore, the purpose of this study was to use head-up tilt testing, and cold pressor testing to evaluate autonomic function testing result differences in a young and middle-aged population. Ten total subjects, four males, and six females between the ages of 23 and 45 participated in a ten minute cold pressor test at $10\pm 1^{\circ}\text{C}$ tap water for ten minutes of submersion and a tilt table test with a five minute tilt at 80° . Vagal tone was measured and heart rate variability was interpreted using frequency data. While many results were insignificant, a more substantial drop was observed between the decrease in total power during the tilt table, than in the cold pressor test. These results indicate that the cold pressor test could serve as a safe pre-screening test before tilt table testing.

Table of Contents

Abstract	iv
Chapter 1: Introduction and Literature Review	1
Chapter 2: Methods	11
Chapter 3: Results.....	13
Chapter 4: Discussion.....	17
References	21
Vita	24

Chapter 1: Introduction and Literature Review

The autonomic nervous system regulates unconscious body functions, such as blood pressure, heart rate, respiration, and thermoregulation. Testing for autonomic dysfunction can be dangerous or costly, though it is crucial for individual wellbeing and for certain diagnoses. Understanding how autonomic clinical testing works, and what correlations exist between different tests, may help make autonomic testing less invasive or dangerous.

There are several methods for testing autonomic function, including the cold pressor test (CPT). In this test, subjects immerse their hand in water at a recommended temperature, usually of 10 degrees \pm 1°C (von Baeyer, Piira, Chambers, Trapanotto, & Zeltzer, 2005). Sympathetic response to this test, such as blood pressure or heart rate increase, has been said to serve as an indicator for cardiovascular risk later in life among healthy, normotensive individuals (Liu et al., 2009).

Tilt table testing is also done to measure autonomic nerve function. Head-up tilt (HUT) testing evaluates baroreflex function by providing an evaluation of heart rate and blood pressure in response to the tilt (Low, Tomalia, & Park, 2013). Patients with chronotropic incompetence may be at risk during HUT due to inability to increase heart rate to compensate for demand. Chronotropic incompetence exists in a wide range of 25-70% of patients with heart failure (Brubaker & Kitzman, 2011), thus making this population at risk during HUT. Possible risks of this test include syncope, changes in blood pressure, or palpitations as a result of changes in heart rate.

While all of the above tests are useful in measuring autonomic function, each test poses its own risks and benefits. Understanding how each test compares with one another, and if they each produce similar results, may help reduce potential dangers or discomfort when working with at-risk populations. Therefore, the purpose of this study was to use head-up tilt testing and cold pressor testing to evaluate autonomic function testing result differences in a young and middle-aged population. We hypothesize that CPT and HUT will elicit comparable results, thus making CPT a safer test for certain clinical populations, such as those with chronotropic incompetence. Analyzing the results of the tests will help to identify which test may be the safer option. This finding could allow CPT to be a safer, more cost efficient, and more comfortable test for patients undergoing autonomic testing.

Review of Literature

Water Immersion Skin Wrinkling

The WISW phenomenon occurs through the process of vasoconstriction, though there are several theories of the exact mechanism. The most recent theory consists of a five stage process theorized by Wilder-Smith (2015). Sweat pores on the palms and fingertips allow water to reach the sympathetic nerve fibers that innervate the digit vasculature via glomus bodies (Wilder-Smith, 2015). The introduction of water through the sweat pores increases sympathetic nerve firing, thus shrinking the glomus bodies and causing vasoconstriction of the digital pulp. Negative pressure from loss of volume causes the skin to be pulled inward and skin wrinkling occurs (Wilder-Smith, 2015).

This physiological response is also dependent on the characteristics of the water being used. Temperature, tonicity, pH, and immersion time are key factors in this response. The sympathetic nervous system is responsible for hand and foot temperature regulation (Wilder-Smith, 2015), meaning it is vital to keep these variables constant during a WISW test. Lower water temperatures have been found to cause a decrease in skin wrinkling, likely due to a decrease in water diffusion (Wilder-Smith, 2015). New research on this matter is based on the findings of older research that 40°C is the optimal water temperature for skin wrinkling and skin wrinkling is expected to occur in as little as 3.5 minutes (Cales & Weber, 1997).

Water tonicity has also been shown to play a role in WISW. Tsai and Kirkham (2005) tested wrinkling on 14 healthy volunteers between the ages of 26 and 31 using four different solutions at $30 \pm 1^\circ\text{C}$: Distilled water, 0.9% saline, 10% saline, and 20% saline. Time to wrinkling for distilled water and 0.9% saline showed no significant difference at 11.5 minutes. In the more hypertonic solutions significant results were seen. The average time to wrinkling with the 10% solution was between 29.9 and 31 minutes and over 60 minutes for the 20% saline solution. The increased length of time for wrinkling to occur in the water and 0.9% saline solutions was likely due to the decrease in solution temperature.

Skin wrinkling is also affected by pH and time. Water pH can be increased by adding more basic materials, such as soap, which increases water-binding capacity, causing an increase in time to wrinkling (Tsai & Kirkham, 2005). In healthy individuals, time to wrinkling is dependent on the above-mentioned variables: Temperature, tonicity, and pH.

Many factors are involved to stimulate skin wrinkling, though it is agreed that undamaged sympathetic nerve fibers are needed to cause water immersion skin wrinkling (Tsai & Kirkham, 2005). Skin on denervated digits does not show skin wrinkling. Older

studies have found that patients with nerve damage from motor accidents, or deep lacerations contributing to nerve damage, showed no skin wrinkling on digits with loss of feeling (O'Riain, 1973). Decreased skin wrinkling was also observed in diabetic patients, but no correlation was found with cardiovascular tests of autonomic function (Clark, Pentland, Ewing, & Clarke, 1984).

The involvement of water immersion skin wrinkling and the sympathetic nervous system raises questions about its correlation with other measures of autonomic testing. The sympathetic nervous system plays a large role in the control and integration of the cardiovascular system (Wallin & Charkoudian, 2007). Sympathetic neural innervation of the heart and peripheral circulation originates from the intermediolateral cell column of the spinal cord. Postsynaptic neurons have long axons that extend to organs such as the heart, blood vessels, and sweat glands, and cause vasoconstriction. (Wallin & Charkoudian, 2007). As mentioned earlier, it has been hypothesized that skin wrinkling is caused by vasoconstriction (Wilder-Smith, 2004). Wilder-Smith (2004) proposed that water diffusion through sweat glands may trigger sympathetic skin nerve fibers to vasoconstrict, resulting in negative digit pulp pressure, thus causing wrinkling to occur from skin being drawn down unevenly from varying tautness. If vasoconstriction is the cause of skin wrinkling, this raises the question of cardiovascular sympathetic nervous system correlation with water immersion skin wrinkling.

Tilt Table Testing

Head-up tilt table testing evaluates subject heart rate and blood pressure response when tilted head up. Blood pressure is monitored beat-to-beat throughout the entire test, or

manually every minute. While protocol changes depending on the test administrator, the subject is monitored supine for a period of time, followed by a quick head up tilt (HUT) at 70-90°. The subjects are then lowered back to the supine position until they return to baseline measurements. The normal response to this test should result in a heart rate increase of 10-30 beats per minute and a drop in systolic blood pressure of less than 30 mm Hg (Novak, 2011).

Change in posture requires the delivery of blood to major organs. Monitoring the body's ability to adapt to an upright postural change can tell a lot about the state of the sympathetic nervous system. Pressure changes help regulate this system, particularly through baroreflex physiology. The baroreflex is made up of arterial high pressure receptors, located in the carotid sinus and aortic arch, and cardiopulmonary low pressure receptors, located in the atria and ventricles (Lambert & Lambert, 2014). The initial response to upright postural change is a decrease in venous return, resulting in the demand for an increase in heart rate to compensate for decreasing pressures and to ensure pressure does not drop too low. This baroreflex helps maintain the normal tilt response by increasing peripheral vasoconstriction via sympathetic nerve activity. Decreasing venous return during upright postural changes also leads to a decrease in cardiac output, due to a decrease in preload, or cardiac stretch of the heart before contraction. An increase in preload will result in an increase in cardiac output, and vice versa.

Baroreflex sensitivity and cardio-vagal activity act together to produce an autonomic response during HUT. The vagus nerve is responsible for carrying a variety of signals to and from the brain that regulate many of the body's unconscious demands, such as heart rate regulation. Cardio vagal baroreflex sensitivity can be determined by the change in arterial

pressure, by measuring R – R intervals (Ramirez-Marrero, Charkoudian, Zhong, Hesse, & Eisenach, 2007). Ramirez-Marrero et al. (2007) studied baroreflex sensitivity and sympathetic activity via norepinephrine (NE) plasma responses in a group of 51 normotensive, healthy, young adults between the ages of 19 and 39 years old. Results showed an inverse relationship between baroreflex sensitivity and NE plasma increases during HUT, which indicates that more responsive vagal mechanisms, or baroreceptor sensitivity, may require less of a sympathetic response during HUT (Ramirez-Marrero et al., 2007). Other research regarding the relationship between baroreflex sensitivity and sympathetic response has found similar responses. Cardiac output was measured in 18 healthy men between the ages of 26 and 29 and findings showed that cardiac output and stroke volume were shown to have an inverse relationship with baroreflex control of muscle sympathetic nerve activity (Charkoudian et al., 2005). High resting cardiac output in healthy subjects may be a limitation to baroreflex sensitivity, though sympathetic response is highly variable between different individuals.

Failure to maintain vitals during postural changes may be the result of orthostatic intolerance. Syncope and postural orthostatic tachycardia syndrome (POTS) are often diagnosed during a tilt table test. POTS is characterized by an increase in heart rate of 30 beats per minute within 10 minutes of standing in the absence of orthostatic hypotension (Lambert & Lambert, 2014) and is more common among women and younger populations (Ives & Kimpinski, 2014).

Large increases in heart rate as a response to HUT may not always indicate orthostatic intolerance. Ives and Kimpinski (2013) study evaluated 120 healthy subjects between the ages of 14 and 76 with no history of orthostatic intolerance or autonomic

dysfunction. The median change in heart rate increment between the ages of 14 and 25 was 33.6 beats per minute, 27.4 beats per minute between the ages of 26 and 40, and 11.7 beats per minute between the ages of 41 and 76 years old. Younger individuals were found to have higher heart rate increments during HUT, but those with greater postural heart rate increments did not demonstrate a greater degree of orthostatic intolerance (Ives & Kimpinski, 2013). Further research indicates that higher postural heart rate increments during HUT does not indicate reduced autonomic function, or the likelihood to develop POTS later in life (Ives & Kimpinski, 2014).

Cold Pressor Test

The cold pressor test (CPT) is another non-invasive measurement that tests autonomic function by stimulating the sympathetic nervous system. During this test, one of the hands is submerged in cold water (0-10°C) for a specified amount of time (1-10 minutes, depending on water temperature). Blood pressure response is monitored during the whole test to assess cardiovascular health by measuring the ability of the sympathetic nervous system to vasoconstrict at the affected site. Healthy, normotensive individuals should respond to this test with a sustained increase in blood pressure as a result of increased peripheral resistance as the sympathetic nervous system becomes excited, though heart rate response may be less defined (Mourot, Bouhaddi, & Regnard, 2009). Older research that evaluated differences in cold pressor responses between 26 – 67 year old normotensive and hypertensive patients concluded that young, normotensive individuals often have heightened systolic and diastolic blood pressures during this test (Benetos & Safar, 1991). With an increase in age, the sympathetic activity decreases, hence the decrease in normotensive response among the older

population in Benetos' and Safar's (1991) findings. Age is a determining factor and these findings suggest that this test should not be used as a predictor for hypertension.

While this test may not be useful for predicting hypertension, recent evidence shows that it may identify subjects that are prone to weight gain and are at risk for other cardiovascular issues (Grewal, Sekhon, Walia, & Gambhir, 2015). Obese individuals with a body mass index (BMI) of greater than 30 are shown to have a decreased sympathetic response to CPT due to decreased baroreflex function and individuals with low resting muscle sympathetic activity may be at risk for weight gain due to lower metabolic rate (Grewal et al., 2015). While we did not analyze BMI for this study, this information poses some questions for future autonomic function research.

Autonomic Function Test Comparisons

While all of the previously mentioned tests are used to assess autonomic functioning, interpretation of results may not always be standard for each person, and how all of these tests compare with one another is largely unknown. Interpreting skin sympathetic nerve activity can be very difficult, as indicated by current and lack of research. Skin sympathetic nerve activity responses to baroreflex stimuli have shown varying results. Studies that have measured skin sympathetic nerve activity in the leg have shown no change with baroreflex stimuli, though a study in which it was measured in the arm during body heating, combined with lower body negative pressure, showed a decrease in skin sympathetic nerve activity (Dodt, Gunnarsson, Elam, Karlsson, & Wallin, 1995). Use of autonomic function tests could help draw comparisons between skin sympathetic nerve activity and baroreflex stimuli response. Very little research has been done to explore this relationship and better

understand the causes behind skin wrinkling, or how skin sympathetic nerve activity may relate to this phenomenon.

To our knowledge, this has only been evaluated once in a previous pilot study (van Barneveld, van der Palen, & van Putten, 2010). Twenty healthy subjects with a mean age of 26 years old were used, as well as 15 patients with an average age of 58 years old. The patients used were referred for syncope, dizziness, diabetes mellitus, and Parkinson's disease. All subjects underwent HUT testing and WISW. Only 4 subjects showed abnormal results, though there was a significant correlation between the results of HUT testing and WISW. With that being said, only one subject showed an abnormal WISW test, though 3 patients already had wrinkled hands at baseline and also showed abnormal HUT results. This pilot test concluded that WISW could be used as a screening test before HUT testing.

The limited age population used in the study done by van Barneveld et al. (2010), as well as limited results, shows the need for further research in this area. As stated earlier, this pilot study is the only current study that has investigated the correlation between HUT testing and WISW testing. In addition, there is a need to research the similarities and differences of results between CPT, WSW, and HUT testing. Understanding the use of each test, how each test may differ from one another, and what each test is specifically useful for, will help eliminate risk and discomfort for some populations in future settings.

Heart Rate Variability

Heart rate variability (HRV) can serve as a marker for vagal tone, or sympathetic control. HRV is the beat-to-beat changes in heart rate and it can be measured by examining the variation in R-R intervals on an ECG. As vagal tone decreases, HRV decreases, which

happens as a result of aging, though on the contrary, with regular physical activity HRV increases due to an increase in vagal tone (Kawachi, 1997).

Frequency can help provide information about HRV through the measurement of power. Total power (TP) is the total measurement of frequencies between 0 and .4 Hz, which reflects overall autonomic activity. Low frequency (LF) ranges between 0.04 and 0.15 Hz and generally reflects sympathetic activity, while high frequency (HF) ranges between 0.15 and 0.4 Hz and measures vagal activity, or parasympathetic control (Medicore, 1996). The LF/HF ratio represents the balance between sympathetic and parasympathetic control, where higher values indicate sympathetic domination and lower values indicate parasympathetic domination (Medicore, 1996).

Chapter 2: Methods

Subjects

Ten total subjects, four males, and six females between the ages of 23 and 45 voluntarily participated in this study. The subjects were normotensive and none were taking medications at the time of testing. The subjects were informed of the study details and all potential risks before signing an informed consent. Appalachian State University's Institutional Review Board (IRB) approved this study.

Testing Protocol

All subjects reported to the Charleston Forge Vascular Biology and Autonomic Studies Laboratory for a one-time visit. Subjects were required to abstain from fluids that contain caffeine for 12 hours before testing. Upon arrival, blood pressure, height, and weight were measured and recorded.

During HUT testing, subjects were observed in the supine position in a dimly lit room until baseline hemodynamic measurements were acquired. After baseline, subjects were observed for ten minutes in the supine position, five minutes at an 80° tilt, and ten minutes back in the supine position. Subjects were to be lowered back to the supine position if systolic pressures dropped more than 20 mmHg, diastolic dropped more than 10 mmHg, or if heart rate did not compensate for the postural change. Returning the subject back to supine position due to these conditions was recorded as an abnormal result.

CPT was done on the left hand in $10\pm 1^{\circ}\text{C}$ tap water for ten minutes of submersion. Subjects immersed their hand up to their wrist in a pitcher of water while lying on the tilt table in a supine position. Temperature was monitored throughout the entire test using a glass mercury thermometer, and the water was stirred every two minutes to prevent warmer temperatures around the hand. Hemodynamics were monitored during the entire HUT and CPT. These two tests were done in a random order.

Hemodynamic Measurements

Beat-to-beat blood pressure was monitored continuously during the HUT and CPT using the Finometer®. Arterial pressure and heart rate were monitored through a finger cuff placed on the middle finger of the subject. Heart rhythms were monitored continuously using a 3-lead electrocardiogram. Electrodes were placed on the right and left clavicle and on the fifth intercostal space below the left pectoral muscle at the anterior axillary line.

Data Analysis

All data was reduced and analyzed using WinCPRS by Absolute Aliens Oy.

Chapter 3: Results

Cold pressor testing and tilt table testing were successfully performed on all subjects, except for one. Beat-by-beat blood pressure was unable to be measured during HUT, so subject data were omitted. Subject data were age 28 ± 8 years, height 173.22 ± 12.78 centimeters, and weight 76.66 ± 24.74 kilograms. Baseline data and hemodynamic data during testing for HUT and CPT is shown in tables 1 and 2.

For both CPT and HUT, increases were seen in both systolic and diastolic blood pressure. From baseline measurement, HUT systolic blood pressure increased from 118.2 ± 6.7 mmHg to 128.55 ± 8.3 mmHg and diastolic pressure increased from 66.88 ± 4.9 mmHg to 81.44 ± 9.7 mmHg. Similarly, CPT showed similar blood pressure increases from baseline with an increase in systolic pressure from 122.1 ± 11.9 mmHg to 132.3 ± 10.6 mmHg and increase in diastolic pressure from 68.7 ± 8.9 mmHg to 76 ± 5.7 mmHg. These increases lead to increases in mean arterial pressure (MAP) in both tests, which is shown below.

Cardiac output (CO) showed an opposite relationship between the two tests, due to the nature of the tests. HUT yielded a slight overall decrease in CO, while CPT showed a slight increase. Stroke volume (SV) showed the same results, with a decrease in HUT and an increase in CPT. In both tests, low frequency (LF) power decreased and high frequency (HF) power decreased. In addition, the LF/HF ratio and total peripheral resistance (TPR) showed an increase in both tests. Total Power (TP) decreased from 9579.22 ± 9141.99 ms² to

1653.77±1446.30 ms² during HUT. During CPT, TP decreased from 2942.63±2650.75 ms² to 1597.56±1171.37 ms².

Table 1: Tilt Table Hemodynamic Responses

	HUT 1	HUT 2
Systolic BP(mmHg)	118.2±6.7	128.55±8.3
Diastolic BP(mmHg)	66.88±4.9	81.44±9.7
HR (bpm)	58.88±10.1	76±11.8
CO (mL/min)	5.81±1.2	5.56±1.8
SV (mL)	101.88±29.7	74.66±23.4
MAP (mmHg)	86.77±5.1	100.33±9.9
TPR (mmHg·min·mL⁻¹)	0.941±0.2	1.202±0.4
LF (ms^s)	0.602±0.18	0.696±0.18
HF (ms²)	0.379±0.16	0.291±0.17
LFHF%	203.47±123.8	362.21±275.3
TP (ms²)	9579.22±9141.99	1653.77±1446.30

HUT1 indicates baseline measurements, while HUT2 indicates peak measurements during tilt

Table 2: Cold Pressor Hemodynamic Responses

	CP 1	CP 2
Systolic BP(mmHg)	122.1±11.9	132.3±10.6
Diastolic BP(mmHg)	68.7±8.9	76±5.7
HR (bpm)	59.5±11	59.3±10.4
CO (mL/min)	5.62±1.04	5.805±1.3
SV (mL)	98±28.01	101±28.9
MAP (mmHg)	90.33±11.5	98.67±5.9
TPR (mmHg·min·mL⁻¹)	0.954±0.4	1.075±0.3
LF (ms²)	0.569±0.13	0.649±0.13
HF (ms²)	0.413±0.13	0.328±0.20
LFHF%	166.94±116.1	347.39±360.5
TP (ms²)	2942.63±2650.75	1597.56±1171.37

CP1 indicates baseline measurements, while CP2 indicates peak measurements during submersion

Table 3: Tilt Table and Cold Pressor Hemodynamic Response Differences

	HUT	CP
Systolic BP (mmHg)	+10.35	+10.22
Diastolic BP (mmHg)	+14.56	+7.23
HR (bpm)	+17.12	-0.23
CO (mL/min)	-0.25	+0.19
SV (mL)	-27.22	+3.00
MAP (mmHg)	+13.56	+8.34
TPR (mmHg·min·mL⁻¹)	+0.255	+0.121
LF (ms²)	+0.094	+0.080
HF (ms²)	-0.088	-0.085
LF:HF (%)	+158.74	+180.45
TP (ms²)	-7925.45	-1345.07

Table 4: Resting Cold Pressor and Tilt Table vs. Peak Response Comparison

	CP SYS	CP DIA	CP MAP	CP HR	CP SV	CP CO	CP TPR	CP LF	CP HF	CP LF:HF	CP TP
P value	0.0867	0.0822	0.0791	0.8243	0.4893	0.3499	0.1974	0.3656	0.0620	0.1683	0.2326
	HUT SYS	HUT DIA	HUT MAP	HUT HR	HUT SV	HUT CO	HUT TPR	HUT LF	HUT HF	HUT LF:HF	HUT TP
P value	0.0369	0.0009	0.0021	0.0003	0.0004	0.5122	0.0348	0.0216	0.0360	0.1527	0.0366

P-values (two-tailed) measured between HUT baseline and peak values and CPT baseline and peak values

Table 5: Cold Pressor and Tilt Table Peak Response Comparison

	Sig. (2-tailed)
CPSYS-TTSYS	0.379
CPDIA-TTDIA	0.543
CPMAP-TTMAP	0.372
CPHR-TTHR	0.692
CPSV-TTSV	0.611

Chapter 4: Discussion

In healthy subjects, similar findings were discovered between both HUT and CPT. Blood pressure increases during CPT occur from a sympathetic response to the cardiovascular system as a response to the sudden cold stress. Vasoconstriction of the affected site can cause a sudden increase in heart rate, thus increasing stroke volume.

Unlike the CPT response, the increase in blood pressure during HUT is a response to postural change. The initial response to the upright postural change is for blood pressure to drop while heart rate increases to compensate for the change and to ensure that pressures do not become too low. Heart rate increases from 10-30 bpm are considered normal (Novak, 2011), which was seen in this study. As adaption occurs, pressures rise to account for the change. Decreasing venous return during upright postural changes also leads to a decrease in cardiac output, due to a decrease in preload, or cardiac stretch of the heart before contraction. This could explain why cardiac output slightly decreased during HUT, though the change was not significant because baseline measurements were compared to hemodynamic measurements taken five minutes into the upright tilt. The decrease in cardiac output may also explain the decrease in stroke volume.

Total peripheral resistance (TPR) is the resistance to blood flow in the vascular system. A greater increase in TPR in HUT than in CPT was likely due to the postural change in HUT. Many factors affect TPR, such as blood viscosity or vessel diameter, though gravitational influences may be the cause for the increase in TPR during HUT. While the

heart is doing more work to adjust for falling pressures, as pressures begin to adjust and increase, TPR increases, as well.

Low frequency and high frequency data indicate the amount of heart rate variability (HRV) within the time period of data capture. HRV is beat-to-beat alterations in heart rate, which is said to decrease during acute stress (Kawachi, 1997). Aging also lowers HRV with time, though physical activity increases HRV (Kawachi, 1997). Based on this information, the subjects in this study should have fairly high HRV, which indicates vagal activity. Total power (TP) indicates overall autonomic function, while low and high frequency data individually show sympathetic and parasympathetic control. The low frequency and high frequency components of the data make it easier to interpret HRV in the subjects of this study. The high frequency portion is directly related to respiration rate, while the low frequency portion is mediated by sympathetic intervention of the vagus and cardiac nerves (Kawachi, 1997), though there is still mystery behind the exact mechanisms of these two frequencies and its application to clinical research.

The results of this study showed an increase in LF and a decrease in HF, which indicates a healthy vagal response among the subjects of this study. Previous studies have shown similar results among healthy (and unhealthy) subjects (Mehlsen, Kaijer, & Mehlsen, 2008; Montano et al., 1994), though numbers are variable. The decrease in the HF component occurs due to the sympathetic excitation that happens upon immediate tilt, thus causing an increase in the LF components as vagus and cardiac nerves become stimulated through this intervention. Large decreases in TP were seen in both tests, though HUT yielded a decrease almost six times that of CPT. The extreme change in vagal tone in HUT is responsible for this immense change.

In addition to the LF and HF components, the LF:HF ratio can provide information on sympathovagal balance, where increases in this ratio have been said to represent a shift to sympathetic dominance, and decreases represent a shift to parasympathetic dominance (Billman, 2013). Both CPT and HUT yielded increases in the LF:HF ratio, thus accurately representing this theory.

In comparison to HUT results, CPT showed similar findings. In a normal response to CPT, individuals should show a sustained increase in BP along with an increase in peripheral resistance (Mourot et al., 2009). This was the case among the subjects of this study, though HR remained generally constant, which may explain why CO remained constant during the test, as well. LF, HF and LF:HF findings were also reflective of HUT subject data.

In conclusion, it is evident that both HUT and CPT are sufficient measurements for testing autonomic function, particularly sympathetic influence when a stressor is introduced. Using healthy, young subjects helped to determine similarities between the two tests and helped to lower any underlying risks. Findings suggest that CPT can serve as a safer, lower risk test than HUT, thus making CPT a safe potential screening test before conducting HUT. HUT successfully gauges baroreflex health, thus making it the gold standard when identifying those with chronotropic incompetence, though screening individuals with CPT before beginning HUT could decrease risk factors before beginning the test. Those with drastic drops in TP during a CPT will likely respond very poorly to HUT, thus eliminating this risk with a pre-screening. Though much of the results were insignificant, it was clear that the two tests both accomplished the same task and CPT may be a safe pre-screening test for HUT.

In the future, using colder water and decreasing the time of the CPT may produce different results. The water used in this study was slightly warmer than a true CPT, which could have played a role in affecting sympathetic influence, though results show a true CPT response. Researching other tests, such as water immersion skin wrinkling, would also be useful in comparison to HUT and CPT. Though there is risk to HUT, it is useful in diagnosing syncope-related illnesses, but reducing risk and finding other alternate, useful tests is ideal for future research.

References

- Benetos, A., & Safar, M. E. (1991). Response to the cold pressor test in normotensive and hypertensive patients. *Am J Hypertens*, 4(7 Pt 1), 627-629.
- Billman, G. E. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol*, 4, 26. doi: 10.3389/fphys.2013.00026
- Brubaker, P. H., & Kitzman, D. W. (2011). Chronotropic incompetence: causes, consequences, and management. *Circulation*, 123(9), 1010-1020. doi: 10.1161/circulationaha.110.940577
- Cales, L., & Weber, R. A. (1997). Effect of water temperature on skin wrinkling. *J Hand Surg Am*, 22(4), 747-749.
- Charkoudian, N., Joyner, M. J., Johnson, C. P., Eisenach, J. H., Dietz, N. M., & Wallin, B. G. (2005). Balance between cardiac output and sympathetic nerve activity in resting humans: role in arterial pressure regulation. *J Physiol*, 568(Pt 1), 315-321. doi: 10.1113/jphysiol.2005.090076
- Clark, C. V., Pentland, B., Ewing, D. J., & Clarke, B. F. (1984). Decreased skin wrinkling in diabetes mellitus. *Diabetes Care*, 7(3), 224-227.
- Dotz, C., Gunnarsson, T., Elam, M., Karlsson, T., & Wallin, B. G. (1995). Central blood volume influences sympathetic sudomotor nerve traffic in warm humans. *Acta Physiol Scand*, 155(1), 41-51. doi: 10.1111/j.1748-1716.1995.tb09946.x
- Grewal, S., Sekhon, Ts, Walia, L., & Gambhir, R. (2015). Cardiovascular response to acute cold stress in non-obese and obese healthy adults. *Ethiop J Health Sci*, 25(1), 47-52.
- Ives, C. T., & Kimpinski, K. (2013). Higher postural heart rate increments on head-up tilt correlate with younger age but not orthostatic symptoms. *J Appl Physiol (1985)*, 115(4), 525-528. doi: 10.1152/jappphysiol.00292.2013
- Ives, C. T., & Kimpinski, K. (2014). Higher heart rate increments on head-up tilt in control subjects are not associated with autonomic dysfunction. *Clin Neurophysiol*, 125(10), 2109-2114. doi: 10.1016/j.clinph.2014.02.014
- Kawachi, I. (1997). Heart Rate Variability. Retrieved June 11, 2015, from <http://www.macses.ucsf.edu/research/allostatic/hearttrate.php>

- Lambert, E., & Lambert, G. W. (2014). Sympathetic dysfunction in vasovagal syncope and the postural orthostatic tachycardia syndrome. *Front Physiol*, *5*, 280. doi: 10.3389/fphys.2014.00280
- Liu, Z., Hesse, C., Curry, T. B., Pike, T. L., Issa, A., Bernal, M., . . . Eisenach, J. H. (2009). Ambulatory arterial stiffness index is not correlated with the pressor response to laboratory stressors in normotensive humans. *J Hypertens*, *27*(4), 763-768. doi: 10.1097/HJH.0b013e328324eb27
- Low, P. A., Tomalia, V. A., & Park, K. J. (2013). Autonomic function tests: some clinical applications. *J Clin Neurol*, *9*(1), 1-8. doi: 10.3988/jcn.2013.9.1.1
- Medicore. (1996). Heart Rate Variability Analysis System. (3.0), 1-41.
- Mehlsen, J., Kaijser, M. N., & Mehlsen, A. B. (2008). Autonomic and electrocardiographic changes in cardioinhibitory syncope. *Europace*, *10*(1), 91-95. doi: 10.1093/europace/eum237
- Montano, N., Ruscone, T. G., Porta, A., Lombardi, F., Pagani, M., & Malliani, A. (1994). Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation*, *90*(4), 1826-1831.
- Mourot, L., Bouhaddi, M., & Regnard, J. (2009). Effects of the cold pressor test on cardiac autonomic control in normal subjects. *Physiol Res*, *58*(1), 83-91.
- Novak, P. (2011). Quantitative autonomic testing. *J Vis Exp*(53). doi: 10.3791/2502
- O'Riain, S. (1973). New and simple test of nerve function in hand. *Br Med J*, *3*(5881), 615-616.
- Ramirez-Marrero, F. A., Charkoudian, N., Zhong, L., Hesse, C., & Eisenach, J. H. (2007). Balance between sympathetic response to head-up tilt and cardiac vagal factors in healthy humans. *Clin Auton Res*, *17*(4), 227-230. doi: 10.1007/s10286-007-0427-y
- Tsai, N., & Kirkham, S. (2005). Fingertip skin wrinkling – the effect of varying tonicity. *The Journal of Hand Surgery: British & European Volume*, *30*(3), 273-275. doi: http://dx.doi.org/10.1016/j.jhsb.2004.12.010
- van Barneveld, S., van der Palen, J., & van Putten, M. J. (2010). Evaluation of the finger wrinkling test: a pilot study. *Clin Auton Res*, *20*(4), 249-253. doi: 10.1007/s10286-010-0071-9
- von Baeyer, C. L., Piira, T., Chambers, C. T., Trapanotto, M., & Zeltzer, L. K. (2005). Guidelines for the cold pressor task as an experimental pain stimulus for use with children. *J Pain*, *6*(4), 218-227. doi: 10.1016/j.jpain.2005.01.349

Wallin, B. G., & Charkoudian, N. (2007). Sympathetic neural control of integrated cardiovascular function: insights from measurement of human sympathetic nerve activity. *Muscle Nerve*, *36*(5), 595-614. doi: 10.1002/mus.20831

Wilder-Smith, E. P. (2004). Water immersion wrinkling--physiology and use as an indicator of sympathetic function. *Clin Auton Res*, *14*(2), 125-131. doi: 10.1007/s10286-004-0172-4

Wilder-Smith, E. P. (2015). Stimulated skin wrinkling as an indicator of limb sympathetic function. *Clin Neurophysiol*, *126*(1), 10-16. doi: 10.1016/j.clinph.2014.08.007

Vita

Jamie Stark Inlow, formerly known as Jamie Elizabeth Stark before marriage, was born in Dayton Ohio to Eric and Cindy Stark. After many moves, Jamie graduated from Wilson Memorial High School in Fishersville, Virginia in 2009 and immediately went on to pursue her Bachelor of Science at James Madison University, where her father is a professor. Jamie completed her degree in May of 2013 and went on to pursue her Master's in Clinical Exercise Science at Appalachian State University. She received a graduate assistantship working in the Learning Assistance Program working with "at risk" student athletes.

Jamie has now received a position as Component A Director for the College STAR program at Appalachian State University. Jamie and her husband live in Boone, North Carolina with their cat, Arya.