Assessing The Cardiovagal Baroreflex

Abrahm John Behnam

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Dr. Kevin Davy
Dr. David Samuels
Dr. Wally Grant
Dr. Demitri Telionis

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ABSTRACT

Abrupt decreases and increases in systolic arterial blood pressure produce baroreflex mediated shortening and lengthening, respectively, of the R-R interval. This phenomenon, otherwise known as the cardiovagal baroreflex, is best described by the sigmoid relationship between R-R interval length and systolic blood pressure. The linear portion of this relationship is used to derive the slope or gain of the cardiovagal baroreflex. Importantly, lower levels of cardiovagal baroreflex have been associated with poor orthostatic tolerance and an increased cardiovascular disease-related mortality. The most commonly used and accepted technique to assess cardiovagal baroreflex gain is the modified Oxford technique. Bolus injections of sodium nitroprusside followed by phenylephrine HCL are used to decrease and raise blood pressure ~15 mmHg, respectively. The baroreflex control of the cardiac vagal outflow can then be assessed by the relation of the R-R interval to systolic blood pressure. However, the modified Oxford technique does not always reveal the nonlinear nature of baroreflex relations. The reasons for this has been unclear. Thus, analysis of baroreflex gain when nonlinearities are not revealed is problematic. Five classifications of baroreflex trials have been identified: acceptable, threshold-heavy, saturation-heavy, linear-heavy, and random trials. A new method of gain estimation was developed that combines the strengths of the current methods of gain estimation with the knowledge of the classifications of baroreflex trials. Using this method, cardiovagal baroreflex gain assessment can be maximized if threshold-heavy, saturation-heavy, and random trials are filtered out of the analysis and the manual method is used to estimate gain on the remaining trials. In addition, a link seems to exist between the variability of δ and the variability in baroreflex gain between different subjects.
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PREFACE

This thesis is presented in the form of a journal paper because it is intended to be submitted for publication. The body of this paper consists of the journal paper with introduction, methods, results, discussion, and conclusion sections. Following the journal paper is the appendix composed of four sections. Appendix I contains an extended review of literature. Appendix II is the informed consent for participants. Appendix III gives instructions for carrying out the new method of gain estimation. Appendix IV details instructions for performing the Boltzmann fit in Origin 7. Finally, Appendix V contains the vita.
ASSESSING THE CARDIOVAGAL BAROREFLEX

INTRODUCTION

Sudden decreases and increases in systolic blood pressure results in baroreflex mediated shortening and lengthening of the R-R interval. This well-described phenomenon, known as the cardiovagal baroreflex, is best characterized by the sigmoidal relation between R-R interval length and systolic blood pressure. The linear portion of this relationship is used to derive the gain or slope of the cardiovagal baroreflex. Importantly, reduced cardiovagal baroreflex gain has been associated with poor orthostatic tolerance and an increased risk of dying from cardiovascular diseases.

The modified Oxford technique is a common approach for characterizing the nonlinear nature and estimating the gain of the cardiovagal baroreflex. Bolus injections of sodium nitroprusside followed by phenylephrine HCL are used to decrease and raise blood pressure ~15 mmHg, respectively. However, the modified Oxford technique does not always reveal the nonlinear nature of baroreflex relations. The reasons for this has been unclear. Thus, analysis of baroreflex gain when nonlinearities are not revealed is problematic. Accordingly, we sought to identify the different scenarios where a linear analysis of data from the modified Oxford technique would be incorrect. To overcome the restrictions of this technique, we further sought to develop an approach for assessing baroreflex gain that would take into account these limitations.

METHODS

Two hundred and fifty five baroreflex trials from 85 subjects were selected from our previous studies utilizing the modified Oxford technique. The subjects consisted of fifty three males and 32 females (age: 18-40 years) covering a wide range of aerobic fitness (V02max: 20.9 - 82.0 ml/kg/min) and total body adiposity (BMI: 17.8 - 35.4 kg/m²). All subjects were free of overt cardiovascular disease and were not taking cardioactive medications.

The nonlinear nature of each trial was characterized using the Boltzmann sigmoid (See Equation 1). This form of the function was chosen since it explicitly contains the parameters I_{RR,min} and I_{RR,max}, representing the minimum and maximum R-R interval. The other parameters BP_0 and δ represent the point of inflection and an inverse relation to curvature, respectively.
\[ I_{RR} = \frac{I_{RR,\min} - I_{RR,\max}}{1 + e^{(BP-BP_0)/\delta}} + I_{RR,\max} \quad (1) \]

Two common methods of gain estimation are peak gain and manual gain. Equation 2 uses the first derivative of the Boltzmann function taken at \( BP = BP_0 \) to obtain the peak gain.

\[ \frac{dI_{RR}}{dBP} = \frac{I_{RR,\max} - I_{RR,\min}}{4\delta} \quad (2) \]

Manual gain involves two steps: (1) incremental and symmetric removal of data points from both ends of the raw data, then (2) a linear regression is performed on the remaining data. This process is repeated until the correlation coefficient, \( r^2 \), is maximized and the resulting slope is the manual gain.

The parameter values and associated errors were measured using a least-square fit of Equation 1 in the scientific graphing and data analysis software, Origin 7. A plot of each trial along with the quantitative output of Origin 7 was recorded in Excel. Figure 1 illustrates the role of each parameter:

![Diagram](image)

Figure 1: An example of the least squares fit on an acceptable trial is shown along with the roles of each Boltzmann parameter.
Subjects were excluded if 1 or more of their 3 trials: (1) were unable to calculate the parameter errors for \( I_{RR,\text{min}} \), \( I_{RR,\text{max}} \), or \( \delta \) for the sigmoid fit in Origin 7.0, or (2) did not span the linear range defined by the peak and nadir of the second derivative of the Boltzmann function. The Boltzmann parameter values were compiled and their frequency distributions were observed for the 85 subjects with 3 acceptable trials.

A method of gain estimation was developed to minimize the number of subjects-trials unfit for further analysis. This method involved selective filtration of data within trials to isolate those suitable for manual gain estimation.

RESULTS

The nonlinear nature of the trials were well characterized by the Boltzman equation (\( r^2 = 0.966 \pm 0.030 \), \( P<0.05 \)). In general, the \( I_{RR,\text{min}} \), \( I_{RR,\text{max}} \), and \( BP_0 \) parameters were normally distributed. The \( \delta \) parameter, however, was positively skewed (Figure 2).

![Figure 2: Histogram of the \( \delta \) parameter showing an asymmetric distribution.](image)

The range of the \( \delta \) variability defined by its 95% confidence intervals (CI's) is illustrated in Figure 3. The three sigmoids all have \( I_{RR,\text{min}} \), \( I_{RR,\text{max}} \), and \( BP_0 \) set at their mean values and are
distinguished by δ plotted at its median, upper 95% confidence interval (CIU) and lower 95% confidence interval (CIL).

Figure 3: Plot of Boltzmann sigmoid range for filtered trials with $I_{RR,\text{min}}$, $I_{RR,\text{max}}$, and δ fixed at their mean values and the median of δ variable about its upper 95% confidence interval (CIU) and lower 95% confidence interval (CIL).

NEW METHOD OF GAIN ESTIMATION

In the process of fitting the Boltzmann sigmoid, five classifications of baroreflex trials were observed: acceptable, threshold-heavy, saturation-heavy, linear-heavy, and random trials. Cut off values were set for the $I_{RR,\text{min}}$, $I_{RR,\text{max}}$, and δ parameters to quantitatively define these classifications. These values were selected because they excluded the majority of the tail of the skewed distributions and more liberal cut-offs did not drastically change the number of subjects included for analysis. Acceptable trials were defined as trials with all parameter errors falling within the limits of the cut off values. Threshold-heavy, linear-heavy, and saturation-heavy trials are defined as trials whose data is primarily concentrated in either the threshold, linear, or
saturation regions, respectively. When the data is limited to one region of the sigmoid, the parameter errors associated with that region will be within the cut off limits and the parameter errors associated with the other two regions will be outside of the cut off limits. Random trials are distinguished from acceptable trials by parameter errors for the linear region which fall outside of the acceptable range. The limits of each classification are listed in Table 1.

<table>
<thead>
<tr>
<th>Classifications of Baroreflex Trials</th>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>I_{RR,min} % error</td>
</tr>
<tr>
<td>Acceptable</td>
</tr>
<tr>
<td>Threshold-heavy</td>
</tr>
<tr>
<td>Saturation-heavy</td>
</tr>
<tr>
<td>Linear-heavy</td>
</tr>
<tr>
<td>Random</td>
</tr>
</tbody>
</table>

The total sorted trials consisted of 5 threshold-heavy, 23 saturation-heavy, 20 linear-heavy, 24 random trials, and 183 acceptable trials. Threshold-heavy, saturation-heavy, and linear-heavy trials are examples of when the modified Oxford technique covers only part of the full range of the nonlinear function. Examples of these classifications are shown in Figure 4.
Figure 4: Examples of four classifications of data using the Boltzmann fit in Origin 7.0 that are may be problematic in estimating gain: (A) threshold-heavy trial, (B) saturation-heavy trial, (C) linear-heavy trial, and (D) random trial.

The new method of gain estimation involves selective filtration of data trials to exclude subjects with threshold-heavy, saturation-heavy, and random trials. Therefore, subjects with only linear-heavy trials remain in the pool of acceptable trials for analysis. The manual gain estimation is then used on the selectively filtered data. The new method was applied to the original unfiltered pool of subjects. 46 subjects fell into the acceptable category, 9 of which were purely linear-heavy. The new gain estimation process is detailed in Figure 8:
DISCUSSION

The modified Oxford technique was developed to overcome limitations of previous approaches including the Valsalva’s maneuver and bolus injections of various agents to raise acute arterial blood pressure, i.e., the so called Oxford technique. A major strength of the modified Oxford technique is that it provides the opportunity to characterize the entire sigmoid relation between systolic blood pressure and R-R interval. In so doing, the most linear region of the baroreflex relation can be objectively defined by the second derivative of the sigmoid function. The latter was accomplished in 72% of the trials in the present study. Consistent with the observation of others, a sizeable fraction (~28%) of the trials could not be characterized by a sigmoid relation with identifiable threshold, linear, and saturation regions in the present study.

We identified several scenarios where linear gain estimation may be misleading. Two percent of the total trials were classified as “threshold-heavy” and 9% of the total trials were classified as “saturation-heavy”. The use of a linear regression to characterize these trials would produce gain estimates which would likely underestimate baroreflex gain. Gain estimation reaches a peak value at BP<sub>0</sub>, the point of inflection located at the center of the linear region. Ideally, gain estimations using linear regression should be made from trials covering most, if not
all, of the linear region. These trials have a resting BP (midpoint) which lies close to BP₀ and covers a section of the sigmoid arc with the majority of its range overlapping the linear region. Many trials cover a slightly different section of the sigmoid arc, with their resting BP at a value different than BP₀; as resting BP strays from BP₀ in either direction, the magnitude of gain estimation decreases in concurrence with less overlap of the linear region. The data ranges of both threshold-heavy and saturation-heavy classifications span a section of the sigmoid with resting BP values far from BP₀ and minor overlapping of the linear region. Therefore, threshold-heavy and saturation-heavy trials are likely to underestimate gain.

Nine percent of the total trials were characterized as “random” based on the low % errors for I_{RR,min} and I_{RR,max} (threshold and saturation regions) and large δ % error. The appearance of random trials consisted of prominent threshold and saturation regions with little or no transition region between the two extremes. In these cases, the linear region comprised of at most 2-3 data points which fell within a narrow systolic BP range of less than 5 mmHg. The use of a linear regression would thus overestimate gain beyond physiological limits.

The final scenario was defined as “linear-heavy” and comprised 8% of the total trials. Linear-heavy trials were characterized by having large % errors for I_{RR,min} and I_{RR,max}, and low δ % error. The appearance of linear-heavy trials consisted of a prominent linear region with no clear indication of threshold and saturation regions. This makes a linear regression without the need of truncation possible. Linear-heavy trials exclusively overlap the linear region; however, the extent of this overlap is unclear because the entire range of the sigmoid is not defined. The distinguishing feature of linear-heavy trials from the other of the scenarios is linear estimation of gain is feasible.

With knowledge of the above scenarios, a method of gain estimation was developed in order to minimize subject-trials excluded by previous methods. The first step was to develop a means of filtering out the threshold-heavy, saturation-heavy, and random trial scenarios unfit for linear gain estimation. The parameter errors which defined each classification were used in defining the limits of the filter. The second step was to apply a gain estimation method using a linear regression with variable truncation on the remaining subject-trials. The manual gain method was chosen over other existing methods because it is not affected by parameter errors in the nonlinear regions and flexibly truncates the data to isolate the linear region. This flexibility allows for linear-heavy trials to be included in the data analysis and therefore maximizes the
number of subject-trials included for analysis. The details of this gain estimation method are illustrated in Figure 8.

In the process of developing an alternative approach to gain assessment, the frequency distributions were analyzed for each sigmoid parameter. To do this objectively, the statistical analysis was limited to trials whose BP range spanned the “most linear” region of the SBR—RR relation, as defined above. The skewed frequency distribution of the $\delta$ parameter stood out from the other normally distributed parameters. This evidence links the variability in the $\delta$ parameter to the variability in gain between subjects.

A major use of the $\delta$ parameter is in the calculation of the peak gain from the Boltzmann sigmoid. In equation 2, peak gain is calculated from the slope of the Boltzmann sigmoid at $\delta$; the R-R interval range is divided by 4 times the $\delta$ parameter. Therefore, one possible recommendation for calculations of peak gain when using a nonlinear analysis is for the BP range revealed by the modified Oxford technique to be greater than 4 times the value of $\delta$. In many cases, a BP range of $4\delta$ may not be covered by the modified Oxford technique (i.e. linear-heavy trials). Therefore, the entire sigmoid range may not be fully expressed in all trials, and these should be left out of the analysis for a more accurate estimation of peak gain.

CONCLUSION

Five classifications of baroreflex trials have been identified: acceptable, threshold-heavy, saturation-heavy, linear-heavy, and random trials. A new method of gain estimation was developed that combines the strengths of the current methods of gain estimation with the knowledge of the classifications of baroreflex trials. Using this method, cardiovagal baroreflex gain assessment can be maximized if threshold-heavy, saturation-heavy, and random trials are filtered out of the analysis and the manual method is used to estimate gain on the remaining trials. In addition, a link seems to exist between the variability of $\delta$ and the variability in baroreflex gain between different subjects.
REFERENCES


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APPENDIX I - Literature Review

I.1 - BACKGROUND

Autonomic Innervation of the Heart

The heart is innervated by both sympathetic and parasympathetic vagal fibers. Sympathetic efferent nerves exist throughout the atria and ventricles including the conduction system of the heart. Vagal nerves are located throughout the atrial myocardium and sparsely in the ventricular muscle. Despite overlapping which occurs between the nerve fibers, the sinoatrial (SA) and atrioventricular (AV) nodes are primarily innervated by the right and left vagus nerves, respectively. Sympathetic stimulation of the heart leads to increased heart rate, increased force of contraction, and increased conduction velocity. Parasympathetic stimulation of the heart (vagal tone) has opposite effects, resulting decreased heart rate, decreased force of contraction, and decreased conduction velocity. Heart rate is also governed by the activity of the SA node. The automatic nature of heart rate is in response to the conductance changes in Ca$^{++}$, Na$^+$, and K$^+$ of the SA nodal cells. Unlike nerve cells and muscle cells, SA nodal cells have no true resting potential. Instead, they continuously generate regular, spontaneous action potentials. If the heart were left untouched by neurohumoral influences, the spontaneous firing rate of the SA node would lead to an intrinsic heart rate of 100-115 beats/min.

When the heart is at rest, efferent vagal influences dominate over sympathetic influences of the heart. Vagal tone continuously works to depress the SA node firing rate by increasing K$^+$ and decreasing Ca$^{++}$ and Na$^+$ conductance, lowering the resting heart rate to the range of 60-80 beats/min. During this process, vagal tone also increases the time for the SA nodal cells to reach threshold which hyperpolarizes SA nodal cells and promotes electronic stability of the heart. Vagal tone also plays an important role in restoring resting heart rate post exercise, and abnormalities in heart rate recovery is a potential indicator of cardiovascular mortality.
Arterial Baroreceptor Physiology

The arterial baroreceptors are stretch responsive receptors located in the walls of the aortic arch and at the bifurcation of the carotid arteries. Their activity alters vagal tone and regulates the autonomic innervation of the heart. Activation of the baroreceptors occurs when the vessel walls stretch in response to an increase or decrease in arterial blood pressure. Once activated, the baroreceptor's signal is sent to the medulla in the brainstem from both of its locations. The baroreceptors in the aortic arch are innervated by the aortic nerve, that later combines with the vagus nerve (X cranial nerve) traveling to the brainstem. The baroreceptors in the bifurcation of the carotid sinus are innervated by the sinus nerve that later synapses with the brainstem. The response of the cardiac center in the medulla leads to a change in autonomic outflow that is received by the heart to modulate its activity.\(^8\)

Figure 6: Arterial baroreceptor and autonomic innervation of the heart.
Cardiovagal Baroreceptor Regulation of Arterial Blood Pressure

The stretch responses of cardiovagal baroreceptors play a direct role in the short term regulation of arterial blood pressure. This process involves a negative feedback mechanism. The following flowchart illustrates an example how this system responds to an increase in arterial blood pressure under normal conditions:

![Flowchart showing arterial baroreceptor short-term regulation of blood pressure](image)

Figure 7: Arterial Baroreceptor short-term regulation of blood pressure.

An increase in arterial blood pressure causes the walls of the aortic arch and carotid sinus to stretch until the pressure exceeds some threshold level where baroreceptor firing begins. Once this initial threshold is breached, firing begins at some value substantially above zero. As pressure increases, afferent nerve traffic increases as more nerves are recruited to accompany the firing rates of the individual fibers with higher pressure thresholds. These signals travel up the sinus and vagal nerves and are received by the brainstem. The cardiac center of the medulla causes the autonomic nervous system to decrease sympathetic outflow with a concurrent increase in parasympathetic outflow (vagal tone). The heart and blood vessels respond by negative inotropy, bradycardia, and vasodilatation. These responses work together to decrease cardiac output and systemic vascular resistance to eventually decrease arterial blood pressure to normal levels.
When arterial pressure decreases, the opposite effects occur to compensate for the blood pressure change. The vessel walls of the aortic arch and carotid sinus recoil, leading to a decrease in baroreceptor firing with a concurrent decrease in afferent nerve traffic. These signals travel up the sinus and vagal nerves and are received by the brainstem. The cardiac center of the medulla causes the autonomic nervous system to increase sympathetic outflow with a concurrent decrease in vagal tone. The heart and blood vessels respond by positive inotropy, tachycardia, and vasoconstriction. These responses work together to increase cardiac output and systemic vascular resistance to eventually increase arterial blood pressure back to normal levels.

Complexities of the Cardiovagal Baroreflex

The process nerve recruitment as baroceptors are activated plays an important role in the complexity and nonlinearity of the baroreflex relationship. Each receptor has its own stretch or pressure threshold for activation. When pressure exceeds this threshold, receptor firing begins abruptly and some fixed initial frequency. The number of firing impulses begins to increase linearly over a mean arterial pressure range between ~60-120 mmHg. As the pressure increases, more nerves are recruited to accompany the firing rates of the individual fibers with higher pressure thresholds. Also, baroreceptor discharge rates increase as pressure is raised above threshold levels. Firing frequency increases more steeply with earlier than with later increases of pressure, and then reaches some maximum saturation frequency. Once the saturation frequency is exceeded, a variety of responses occur in the individual fibers. Some individual fibers tend to continue to increase their firing rates, while others remain constant, decline, or begin firing intermittently. Thus, the process nerve recruitment adds great variability to the baroreflex relation.\(^8\)
Several other factors contribute to the non-linearity and complexity of the baroreflex:

**Adaptation** - involves the influence of the viscoelastic factors of the coupled elastin, collagen, smooth muscle, and amorphous ground material on baroreflex function. Adaptation occurs instantly, and continues as long as the new level of stretch is maintained. Adaptation is present after sudden reductions of pressure ('creep'), but may have a slower time course than that which occurs during sudden elevations of pressure.  

**Hysteresis** - refers to the rate sensitivity that is evident in the midst of baroreceptor firing at the onset of systole, when the rate of pressure change and arterial expansion is the greatest. Hysteresis is suggested by the observation that for identical pressures, firing rates are greater when pressure is rising than when it is falling. An example is how R-R intervals and sympathetic firing respond differently to rising and falling pressures.

**Off response** - occurs when baroreceptors fire in systole and are silent in late diastole during usual resting levels of arterial pressure. The duration of the neural silence is a direct function of the extent of abrupt pressure reductions, and that firing does not resume at all if the pressure falls below the fiber's threshold level. Firing stops altogether, and then resumes at a low frequency which gradually increases to the expected level.

**Pulsatile pressure** - pressure changes in actual blood flow are pulsatile, whereas the preceding factors deal with step increases or decreases in pressure. Arterial pressure is lower when pulsatile pressure is substituted for steady pressure. As pulsatile pressure increases, systolic rates of baroreceptor firing increase with steadily diminishing increments. As pulse pressure increases, diastolic firing rates fall to zero, and the duration of diastolic silence increases.

**Rate of Pressure Change** - baroreceptor sensitivity to the rate of pressure change is a potential, yet unproven assumption.
Resetting - results from viscoelastic relaxation occurring within the walls of arteries, which leads to progressive reductions of strain on the baroreceptors during pressure elevation. Resetting occurs when the pressure changes are maintained for longer periods of time. The precise time when adaptation becomes resetting has not been defined.\textsuperscript{8}

Vasoconstrictors - (i.e. adrenaline or nor adrenaline) have potential influence over baroreceptor function as well, but it is not clearly defined.\textsuperscript{8}

The process of baroreceptor and nerve fiber recruitment, changes in baroreceptor artery dimensions, pressure, and tension can all lead to changes in afferent nerve activity. The human cardiovagal baroreflex response to changes in arterial pressure is a sigmoid relationship with threshold, linear, and saturation regions. The complexity of this relation is attributed by the aforementioned factors. Adaptation and resetting play a role due to the viscoelasticity of blood vessels. Pulsatile blood flow and the accompanying rates of pressure change further complicate responses. There are differences in the firing rates of rising and falling pressures showing evidence of histeresis. An off-response exists that reveals the baroreceptor firing during systole and a neural silence during diastole. Finally, the complexity is strengthened by the potential influence of vasoconstrictors and the rate of pressure change over baroreceptor activity.\textsuperscript{8}

**Cardiovagal Baroreflex Relation (Systolic BP – RR interval relation)**

The nonlinearity of the Systolic BP – R-R interval relation has been investigated using asymmetric and symmetric logistic functions. Asymmetric logistic equations have been shown to better characterize the baroreceptor reflexes in animal studies\textsuperscript{9,16}, but symmetric logistic functions are more appropriate in describing the human cardiovagal baroreflex.\textsuperscript{14} The symmetric baroreflex sigmoid describes how the R-R interval (heart period) varies with changes in systolic blood pressure (SBP) and this curve consists of threshold, linear, and saturation regions. Because the inverse of the R-R interval is heart rate, variations in the heart period are directly related to variations in heart rate. Cardiac neural activity regulating blood pressure is characterized using only SBP because the off-response of baroreceptor firing causes a neural silence during diastole and limits major neural activity to occur during systole.\textsuperscript{8} Therefore, the baroreflex curve
essentially reveals the relationship between changes in blood pressure and the compensations made by heart rate. The graph below illustrates the baroreflex curve and its physiological parameters under ideal conditions.

Figure 8: SBP—R-R Interval Relation

The threshold and saturation parameters set the physiological limitations of the response and are critical in defining the nonlinearity of the relationship. The Operating Point (OP) marks the point of inflection—the point where the sigmoid changes concavity. The parameter which is of most interest is the gain (sensitivity). This parameter occurs near the normal mean arterial pressure and is maximized at the OP. One method of calculating gain is shown on the figure as Maximum gain. More on this method and other common gain estimation methods will be discussed later.
Methods of Assessing the Cardiovagal Baroreflex:

**Computer Assessment**

**Time Domain Approach**

There are two methods of computer analysis to assess the baroreflex relation. The first is the Time Domain approach which involves calculation of regressions between spontaneously occurring ascending or descending ramps of arterial pressure and subsequent R-R intervals. Continuous recordings of arterial pressure, electrocardiograms, and muscle sympathetic nerve activity are examined visually to find ramps of three or more increasing arterial pulses that are preceded and caused by bursts of sympathetic activity. A linear regression is then performed on the sequences of systolic blood pressure and RR interval changes to derive the gain.  

**Frequency Domain Approach**

The second approach is the frequency domain approach, which is based upon the observation that high-frequency R-R interval changes are associated with arterial pressure changes occurring also at high frequencies. Thorough processing of data is used to quantify the relation between high-frequency changes in R-R interval and arterial pressure recordings. Discrete measurements of sequential R-R intervals and arterial pressures are initially subjected to a fast Fourier transformation to derive power spectra. Cross-spectral analysis is later used to determine the frequency range over which high correlations exist between R-R intervals and arterial pressure. The data is finally filtered to exclude changes outside the frequency range and the slope is calculated from the resulting regression.

**Advantages**

The main advantage of both of these techniques is that they do not involve interventions. The benefit of this is the independence from foreign or naturally occurring drugs or
neurotransmitters, or unnatural neck pressure changes. With out the use of interventions, they do not perturb the reflex that they measure.⁸

Disadvantages

The downfall of this method is that it can be used only to study baroreflex modulation of sinus node function and not for baroreflex control of arterial pressure or sympathetic activity. They both require relatively long (1 to 5 min) periods of recording. Their temporal resolution does not compete with that of other approaches which can measure responses occurring over only seconds. Such computer measurements require a stationary time series during data acquisition and therefore may not be applicable to more thorough physiological studies. Without intervention, a major drawback of these methods is that they delineate only a portion of the sigmoid baroreceptor-cardiac reflex relation and are not sufficient to express the full non-linear nature of the baroreflex.⁸

Valsalva's Maneuver

The Valsalva maneuver triggers hemodynamic responses by voluntary straining which causes elevations of intra-thoracic and intra-abdominal pressure. The test is heterogeneous in that the methods used and results obtained are widely variable.⁸ Despite differences between methods, there are four main phases of Valsalva maneuver which are universally accepted:¹³

1. Brief rise of arterial pressure and reduction of heart rate immediately after the onset of straining
2. Fall, and later partial or complete recovery, of arterial pressure to baseline levels, and a cardioacceleration during the period of straining
3. Sudden, very brief further reduction of arterial pressure and elevation of heart rate immediately following the release of straining
4. Terminal, sustained elevation of arterial pressure above control levels and concomitant slowing of heart rate
Advantages

The Valsalva maneuver is attractive because it is safe\textsuperscript{27, 19}, can be performed without sophisticated equipment, and yields reproducible, quantitative results.\textsuperscript{19, 2} The quantitative information acquired can give insight to both sympathetic and vagal baroreflex systems. Histeresis is not an issue during and after straining because in both cases pressure changes are from low to high.\textsuperscript{13} The range of pressures that can be used is vastly greater than other techniques. Quantitative results have closely correlated to those derived after bolus phenylephrine injections (Oxford Technique).\textsuperscript{8}

Disadvantages

A major disadvantage of the Valsalva maneuver is its difficulty to quantify the intensity of stimulation in the multiple receptor areas involved; the intensity of stimulation of the baroreceptors in the aortic arch may not be the same as the intensity felt by the baroreceptors at the carotid bodies. The procedure is subject to reflex adjustments which can minimize measured pressure changes. There is no agreement on a standard Valsalva maneuver, therefore the magnitude of the responses depends on the particulars of the test used and is thus inappropriate to make quantitative comparisons between different studies using different methods. For example, straining can occur at different stages of respiration for a variety of durations with hard to quantify intensities. Intensities and durations of strain are determined by the subjects themselves, which makes interpretation of the results subjective. The intensities of stimulation of receptor areas in normal versus patients with cardiovascular diseases may not match. As straining is sustained, the level of exertion along with fatigue increase and breathing patterns are altered adding more complexity.\textsuperscript{8}

Neck Suction/Pressure

The Neck Suction technique is based on the principle that afferent baroreceptor traffic can be increased or decreased by positive or negative pressure outside of a baroreceptor artery, respectively.\textsuperscript{1} These pressure changes are applied to a chamber fixed over or around the neck and
are transmitted to the carotid arteries. Nearly linear increases or decreases of carotid artery diameter result.\textsuperscript{17} The responses are assessed with electrocardiographic R-R or P-P intervals, heart rate changes, muscle sympathetic nerve activity, ventricular stroke volumes, and plasma neurotransmitter concentrations.\textsuperscript{8}

Advantages

The greatest advantage of the neck suction technique is the finer control of pressure changes which occur over a wider range or pressures than the pharmacological approach. This makes it more likely to express the entire sigmoid baroreceptor-stimulus response relation. In addition, this technique is noninvasive and does not include drug interventions. This technique also holds the ability to track changes in both arterial pressure as well as heart rate, unlike with drug interventions.\textsuperscript{8}

Disadvantages

A drawback to the Neck Suction technique includes the lack of a true physiological response that includes the contribution of chemoreceptor activity to sinus node responses.\textsuperscript{7,21} More evidence supporting the lack of a physiological response involves sinus node and muscle sympathetic nerve responses to neck suction that are directly opposite (vagal-cardiac traffic is increased and muscle sympathetic nerve traffic is decreased).\textsuperscript{32} These responses to neck suction do not appear to be governed by cerebral blood flow; they appear to be purely mechanical.\textsuperscript{21} The sudden shifts in pressure change themselves are not physiological. The neck chambers are uncomfortable to wear and alter respiration, both aspects which can alter baroreflex response. Subjects are aware of pressure changes that exceed about 5 mmHg.\textsuperscript{8} There is a possibility that the pressure transmitted to the carotid sheath may not be the same as those registered in the neck chamber. Issues exist with the catheter coating and placement.\textsuperscript{21} The rate of neck chamber pressure change varies from study to study and the duration of the baroreflex stimuli applied by different groups is highly variable.\textsuperscript{31} Adaptation and resetting of the baroreflex may apply more to suction than to pressure. These and other drawbacks involve variability between the methods, intensity details, physiological reliability of the Neck Suction technique.\textsuperscript{8}
Pharmacological Approach

Oxford Technique

The Oxford technique is a traditional pharmacological approach that involves a bolus injection of phenylephrine HCL to raise blood pressure ~15 mmHg. Vasoactive drugs are injected in bolus to avoid baroreflex resetting during infusions that may reduce vagal responses. The slope of the linear regression of R-R interval on systolic blood pressure (gain) is considered to provide an index of arterial baroreflex responsiveness. The Oxford technique treats the baroreceptor stimulus-sinus node response relation as a linear function; unlike the non-linear sigmoid which provides a more accurate description of the baroreflex curve. Another drawback of Oxford technique is its limited pressure changes which do not span the entire sigmoid reflex arc. Other potential problems involve subjects with resting heart rates that lie towards the upper part of the linear region; thus, a bolus injection of phenylephrine HCL would raise blood pressure into the saturation region and yield a much lower gain estimation.

Modified Oxford Technique

The Modified Oxford technique was created to address limited pressure changes of the Oxford technique which is also a major drawback of the Time domain, Frequency domain, and Valsalva approaches. This technique involves bolus injections of sodium nitroprusside and phenylephrine HCL to lower and raise blood pressure ~15 mmHg. The initial injection of sodium nitroprusside lowers pressure below the threshold range, and the subsequent bolus injection of phenylephrine increases pressure throughout the threshold, linear, and possibly saturation ranges.

Advantages

Other advantages that the Modified Oxford has over the previously mentioned techniques include employing the ultimate physiological baroreceptor stimulus by using drugs. The baroreceptors are stimulated with naturally occurring arterial pulses and rates of pressure change
over a natural range of pressures. This method involves no voluntary physical exertion or patient co-operation and is unobtrusive. The respiration during pressure change can also be accounted for. Baroreceptor historessis influences are avoided because R-R intervals are measured only when pressure is rising. 

Disadvantages

A major disadvantage with any pharmacological approach is the use also is the drug strength. Though the use of drugs can deliver a more natural response under physiological conditions, drugs have the potential to perturb the reflex that it is measuring and alter respiratory drive. In addition, baroreflex control of arterial pressure can not be assessed because it is the variable being perturbed by the bolus injection of drugs. If repeated injections of vasoactive drugs exceed the subject's metabolic and excretory capacities, the agents will accumulate, and alter baseline autonomic activity.

I.2 - CURRENT METHODS OF GAIN ESTIMATION

The methods of gain estimation all deal with preliminary data processing which involves binning the data. The data is averaged over 3 mmHg increments to reduce variability due to respiration. During inspiration, sympathetic activity and R-R intervals decline in parallel to arterial pressure; during expiration, sympathetic activity and R-R intervals increase in parallel, as arterial pressure falls. The baroreflex mechanism would dictate that vagal activity changes in parallel with arterial pressure. Such variability is minimized by binning the data and does not affect the overall non-linearity of the relationship.

Manual ("Traditional") Gain

The Manual ("traditional") method of gain assessment involves removing the data points from both ends to remove threshold and saturation regions and isolate the linear region. Data points could be removed by setting arbitrary values for truncation or by visual inspection of the linear region. For arbitrary truncation of data points, the peak and
nadir of the second derivative of the logistic function is commonly used to define the linear range. Once the data is cropped, a linear regression is performed on the data to obtain the gain. For removal of data points by visual inspection, a linear regression is fit to the data and the $r^2$ correlation coefficient is observed. This process is continued until the threshold and saturation regions are excluded and $r^2$ fit is maximized. An example of this process is illustrated below:

![Figure 9: Example of the Manual Gain Estimation approach with arbitrary truncation of the threshold and saturation regions.](image)

Some positive attributes of this method involve the ability to obtain gain calculations from data trials when computer-based methods are unable to process the data. This may be due to large errors in data sets where sigmoid fits are made to data sets that only span the linear range of the sigmoid. A related advantage of the manual gain estimation method is that more subject trials are utilized and therefore data trials can be used to calculate gain. A few issues with this method involve performing a linear fit on a very complex non-linear function and the idea that data is excluded in the process. Also, calculation takes a long time since each individual data set has to be manually fit and $r^2$ maximized.

A few significant assumptions have to be made to avoid these issues. One is that the data obtained covers a symmetric range of the baroreflex curve. Because data is removed from both
ends symmetrically, if the available data range spans mostly the threshold or saturation section of the sigmoid, the gain estimations will be fairly low. Another assumption is that the data points removed belong to the threshold and saturation regions and not the "linear region" of the sigmoid. Even after the data is binned, variability still exists by respiration and this may be deceiving when viewing a data set. If the subject has a very stubborn and robust baroreflex that does not respond with flexibility to the drugs, the entire data set could already exist only on the linear portion. Therefore, manually excluding removing data points of such an individual and trying to maximize the $r^2$ fit to data could be misleading as good data points might be thrown away. The last assumption involves maximizing the $r^2$ value as indication of when to stop removing data points. Because of the variability involved with the individual data points and the ambiguity of which region(s) the data range covers, maximizing the $r^2$ value may not be the most reliable method of isolating the linear region where gain exists. Determination of where this region exists is sometimes done by hand or visual inspection and therefore the same trial could be processed by two different people and yield slightly different results.

Maximum Gain

Maximum gain is obtained by first fitting a sigmoid function to the binned data using Origin 7. The first derivative of the resulting Boltzmann sigmoid is taken at the OP to find the maximum gain of the baroreflex relationship. An example of the sigmoid fit is illustrated below:
The Maximum gain estimation method is attractive because the logistic sigmoid function is fit to the entire data set. The influence of error variances of the individual data points are minimized because none of the data points are excluded. Other methods (manual and 2nd derivative gain) exclude data points and the variability of the remaining data points holds more influence on the regression that is fit to the data.

The Maximum gain is a reliable technique because not only the \( r^2 \) fit, but also the errors of each of the Boltzmann sigmoid parameters are known. Only relying on the \( r^2 \) value to determine the fit can be misleading. Consider two scenarios:

1. Data that spans only the linear region with a very good \( r^2 \) sigmoid fit
2. Data that spans all three (threshold, linear, and saturation) regions and has a good \( r^2 \) sigmoid fit

Gain is calculated using both the threshold and saturation regions in addition to other Boltzmann parameters. Scenario (1) would have very large errors associated with the threshold and saturation parameters. Therefore, the gain calculations would carry the threshold and saturation errors and result in gain estimations with large error. The better scenario is (2). Since the data spans the threshold and saturation regions, smaller errors will be associated with the saturation and threshold parameters. Therefore the gain calculation from (2) will have a much smaller error and will be more representative baroreflex relation. Linear regression fits to baroreflex data such as with the manual method do not account for this aspect of expressing the non-linearity of the sigmoid and could potentially have large errors associated with their gain estimations.

A drawback of the Maximum gain estimation is that it calculates the maximum possible gain value which occurs at the OP; it is likely that a patient's resting point falls above or below this point, which translates to a lower gain value.

2\textsuperscript{nd} Derivative ("Remodeled") Gain

2\textsuperscript{nd} Derivative ("Remodeled") gain takes ideas from the two previously mentioned gain estimation methods. A linear fit is performed to the data points like the Manual gain method, but
first, the data points are truncated to only include values which occur in a mathematically defined linear region. To define the linear region, a sigmoid fit is performed to the binned data, similar to the Maximum gain method. Then, the second derivative is taken of the sigmoid function and the SBP points where the peak and nadir occur are recorded. The SBP range between where the peak and nadir of the second derivative occurs is defined as the linear region. Once the data is truncated, a linear regression is fit to the remaining data to define the 2\textsuperscript{nd} Derivative gain. An example of this process is illustrated below:
The 2nd Derivative gain estimation compensates for the many assumptions that need to be made for the Manual gain method. It also yields a gain value lower than the Maximum gain, which may better explain the true gain of an individual.

The drawback involves working with a truncated data set. As mentioned earlier, the variability of the individual data points have greater influence on the regression fit when less data is used.

SIGMOID FUNCTION

The 4-parameter Boltzmann sigmoid function was chosen to use for the Maximum gain and 2nd Derivative gain. Symmetric sigmoid functions, such as the Boltzmann, have shown to better represent the human cardiovagal baroreflex.14 The illustration below shows the logistic function and the parameters associated with it:
The parameters $A_1$ and $A_2$ correspond to the threshold and saturation values, respectively. The other parameters $x_0$ refers to the operating point, and $dx$ is related to the width of the function. The Boltzmann was also chosen because of its simplicity and the direct correspondence between the function parameters and the physiological parameters.
REFERENCES


DATE: September 18, 2006

MEMORANDUM

TO: Kevin P. Davy
Brenda M. Davy
Madlyn Frisard

FROM: David M. Moore

SUBJECT: IRB Full Review Continuation 3: “Abdominal Fat and Autonomic-Circulatory Control in Humans[Part I: Abdominal Fat and Autonomic-Circulatory Control in Humans, Part II: Weight Gain and Autonomic-Circulatory Control in Humans]”, OSP #04019808, IRB # 05-457

This memo is regarding the above referenced protocol which was previously granted approval by the IRB. The proposed research, having been previously approved at a convened IRB meeting, required full IRB review prior to granting an extension of approval, according to the specifications authorized by 45 CFR 46.110 and 21 CFR 56.110. The above referenced protocol was submitted for full review continuation and approval by the IRB at its most recent meeting. Pursuant to your request, I, as Chair of the Virginia Tech Institutional Review Board, have, at the direction of the IRB, granted approval for this study for a period of 12 months, effective September 13, 2006.

Approval of your research by the IRB provides the appropriate review as required by federal and state laws regarding human subject research. As an investigator of human subjects, your responsibilities include the following:

1. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

2. If re-approval is not obtained (unless the study has been reported to the IRB as closed) prior to the expiration date, all activities involving human subjects and data analysis must cease immediately, except where necessary to eliminate apparent immediate hazards to the subjects.

cc: File
OSP
II.2 - Informed Consent for Participants

Department of Human Nutrition, Foods and Exercise, Virginia Tech

TITLE: Abdominal Fat and Autonomic-Circulatory Control in Humans

INVESTIGATORS: Kevin P. Davy, Ph.D. and Brenda M. Davy, Ph.D.

MEDICAL DIRECTORS: Jose Rivero, M.D.
                   Donald Zedalis, M.D.

FUNDING SOURCE: American Heart Association

PURPOSE:
The amount and location of body fat can influence cardiovascular health and function. The build up of fat in the abdominal region is associated with elevated risk of getting high blood pressure. The reason(s) for this elevated risk is/are unclear. However, altered autonomic-cardiovascular function (how the nervous system influences how the heart and blood vessels work) is one possible reason. The general purpose of the present study is to try and learn how the amount and location of body fat influences autonomic-cardiovascular function.

METHODS:
You are being asked to participate in all of the sessions of the study described below. If you agree to participate in this study you will first be required to complete a personal health history questionnaire. The results of your medical history and study tests may be discussed with the study medical director to determine your eligibility. Based on our evaluation of the questionnaire you may then be eligible to become a study subject. Eligible candidates will be non-smoking males or females between the ages of 18 and 80 years who do not have diabetes as assessed by a medical history or the diabetes test. Your blood pressure also must be less than 159/99 mmHg. You will not be eligible to participate in the study if you use any medication that might influence your heart, lungs, blood vessels or kidneys. Three hundred people will be included in this study.
There are two parts to the study. The first is a comparison of subjects with higher versus lower levels of total body fat and abdominal fat. You will be divided into one of two groups according to your body mass index. The body mass index is calculated from your height and weight. You will either be placed in a group whose body mass index is less than or equal to 25 or a group whose body mass index is greater than or equal to 30 but less than or equal to 40.

The second part of the study will involve weight loss and weight maintenance. If your body mass index is greater than or equal to 25 but less than or equal to 40 then you may be asked to be involved in a weight loss group or a control group. We will also use the results of your body fat measurement to determine your eligibility. Your participation in one of these groups will be determined by randomization, a procedure similar to flipping a coin. If you are placed in the weight loss group, you will receive instruction on how to change your existing diet to reduce the amount of fat and calories so that you lose 10% of your initial body weight over a 12-16 week period. The individuals in the control group will be asked not to change any of their dietary or physical activity habits. You will have the additional option to receive a supply of weight loss shakes and meal bars at no cost (SLIMFAST FOODS CO) to assist with your weight loss efforts for the duration of the study. You will be asked to come to the War Memorial Hall every 1-3 days to be weighed and to discuss with a dietitian any problems you may be experiencing with your weight loss program. You may also be provided with weight loss shakes or bars or other foods to assist with your weight maintenance efforts. For a one-month period before and after the weight loss or control group, you will be asked to eat your normal diet so that your sodium intake and body weight remain stable.

There will be approximately 30 visits if you participate in the weight loss but not weight maintenance component of the study. The actual number and order of visits may depend on your schedule and the availability of the study staff. The order may differ from the order of appearance in this document.

After weight loss you will be randomized to one of two weight maintenance groups. The first group (Group 1) will receive instructions on how to maintain weight loss for up to a one year period and return every three months to meet with a member of the research staff to be weighed. During this time you will be able to discuss any problems you may be experiencing in maintaining weight loss. The second group (Group 2) will also receive instructions on how to maintain weight loss for up to one year. However, if you are randomized to this group you will
be asked to return at least once every two weeks to be weighed and discuss any problems with weight maintenance. You will be asked not to change your physical activity habits regardless of which you group you are in. All of the sessions described below will be repeated again following the weight maintenance period.

The total time commitment will be approximately 18 months if you participate in both the weight loss and weight maintenance aspects of the study. If you are assigned to Group 1, there will be a total of approximately 50 visits. If you are assigned to Group 2, there will be a total of approximately 70 visits. The actual number and order of visits may depend on your schedule and the availability of the study staff. The order may differ from the order of appearance in this document.

Session 1

- Medical History – You will be asked to complete a medical history questionnaire. This procedure is used to screen for pre-existing disease or other reasons you should not participate in this study. Your height and weight will also be measured at this time. Your body weight will be measured on a standard balance scale and will include the weight of light indoor clothing or hospital gown without your shoes. Your waist, hip, and neck circumference will be measured using a measuring tape.
- Overnight Fast - You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- Catheter and Blood Draw – A small plastic tube will be inserted into an arm vein to draw blood (approximately 2 tablespoons) to measure the levels of cholesterol and glucose. An additional 3 teaspoons will be frozen for other blood tests which may include levels of blood hormones which influence your cardiovascular system. The tests will be restricted to those relevant to the research project described. Any blood samples remaining after 10 years will be destroyed.
- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.
- Oral Glucose Tolerance Test for Diabetes – For this procedure you will be asked to drink a very sweet sugar solution (75 grams of glucose). Blood samples will be taken both before
and four times (total=5 blood samples) after you drink this solution. The purpose of this procedure is to determine whether you have normal glucose control after drinking this sweet tasting drink (oral glucose tolerance). This procedure will take approximately 2 hours of your time. If your glucose response indicates you may have diabetes you will not be able to continue participation in this study and you will be referred to your personal physician.

- Sodium Excretion – You will collect all of your urine for a 24-hour period. We will give a container to bring with you for this purpose. We will measure the amount of salt in your urine. This collection will take approximately 10 minutes of your time over the course of a day. You will be asked to return the container provided to you on the following day.

Session 2

- Sleep Test – you may be asked to have a sleep evaluation to determine whether you have abnormal breathing while you sleep. If you have this evaluation done, you will be asked to either come to the sleep laboratory at Sleep Disorders Network Allergy and Asthma Associates of Southwest Virginia located in Christiansburg on an evening close to the time you usually go to sleep. If the evaluation is performed at the sleep laboratory in Christiansburg, you will be required to sleep there overnight. In either case:
  - You will have the electrical activity in your brain and the electrical activity of the muscles in your face, and eye movement monitored with small pads with wire that will be attached to your scalp and skin.
  - A sensor attached to your ear or finger will monitor the oxygen level in your blood.
  - A sensor placed just under your nose and mouth will monitor the airflow.
  - Your breathing will be monitored with a plastic belt placed around your chest and abdomen.
  - Your heart rate will be measured from electrodes placed on your chest.

A physician will determine if the results of your sleep study indicate you have sleep apnea. If you do have sleep apnea, you will be referred to your personal physician or a doctor will be recommended to you. Your physician will want to determine whether further tests are needed and if you need medical treatment. If your sleep study is abnormal, you may still be able to participate in other parts of this study. However, we will need written approval from your
personal doctor to continue in the study. The approximate time required is one night or 7–10 hours.

This session will not be required for individuals with a body mass index less than 25 kg/m$^2$.

Session 3

- **Pregnancy Test** - If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.

- **Body Composition** – This test is to measure your body fat. You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 45 minutes and there is no pain associated with the procedure. This procedure will be performed once at the beginning of the study and a second time at the end of your weight loss program.

- **Physical Examination** – If you are a male over 45 years of age or female over 55 years of age, you will be required to have a physical examination by a medical doctor. The doctor will ask you questions about your health history and will listen to your heart and lungs with a stethoscope. The doctor may also palpate your pulse in your neck or wrist or feel for swelling in your ankles.

- **Graded Exercise Test** – This procedure involves walking or running on a motorized treadmill while the electrical activity of your heart (electrocardiogram or ECG – a tracing of your heartbeats) and blood pressure is being monitored. The angle of the treadmill will increase every 2 minutes and the test will end when you are either too exhausted to continue or at approximately 8 – 12 minutes. The test is used to determine your fitness level and as a screening test for heart disease. This test will also be a practice session. You will be asked to return to repeat this test. On the second visit you will also be asked to breathe through a mouthpiece and wear a nose clip so that we can measure how much oxygen you take up during exercise. The physical examination and graded exercise test may take place in either War Memorial Hall at Virginia Tech or at the office of the Heart Specialists of Southwest Virginia (directions will be provided).
- Diet and Physical Activity Questionnaires – You will be asked to complete three questionnaires. The first two are food intake questionnaires, which will be used to determine your average intake of calories, fat, protein, carbohydrate, fiber, fruits and vegetables you eat during the month. You will also be asked to remember everything you ate on the previous day and write it down. This should take approximately 15-20 minute. For the second food questionnaire, you will be asked to write down everything you eat for a 4-day period. This should require approximately 10-20 minutes of your time each day. The third questionnaire is to estimate your usual physical activity level, which will require about 15 minutes to complete.

- Physical Activity Monitor – you will be asked to wear a small monitor to measure your physical activity performed during a 24 hr period. The monitor is slightly larger than a watch and will clip to your belt or waistband and will not interfere with your normal daily activity.

Approximate time required is 2 hours.

Session 4

- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.

- Overnight Fast: You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.

- Arterial Blood Pressure, Heart Rate, and Breathing – A continuous recording of the blood pressure in your arteries will be made by placing a small cuff around your middle finger while your hand is maintained at heart level. Heart rate will be measured by placing three electrodes on your chest and reading the signal of electrical activity from your heart. You will be asked to pace your breathing to a clock at a rate of 15 breaths per minute. A loose fitting plastic belt will be placed around your waist to measure your breathing movements.

- Sympathetic Nervous System Activity – This test involves measuring the activity of one of your nerves on the side of the knee or arm. Two small needles will be placed through your skin on the side of your knee or arm. The position of one of the needles will be moved back
and forth through your skin while a very small electrical impulse (1-2 volts) is passed through the needle. The needles may be inserted up to an inch below the skin of your leg or arm. This search procedure will continue until the electrode being moved causes your foot or hand to twitch. This procedure will take between 5-60 minutes. When a foot or hand twitch is observed, measurements will begin and continue during the procedure described below.

- **Catheter and Blood draw** – A small plastic tube will be inserted in your arm to draw blood (approximately 3 tablespoons). We will measure norepinephrine, angiotension II, angiotensinogen, aldosterone, all hormones that influence your cardiovascular system. We will also measure growth hormone and cortisol. These are hormones that influence your metabolism (how your body burns calories and produces body heat) and cardiovascular (the heart, blood vessel and lungs) system. The plastic tube (catheter) will be left in your arm for the study described below.

- **Arterial Baroreflex Study** – This measures the relationship between changes in blood pressure and the change in heart rate and sympathetic nervous activity. You will be given an injection of a drug - 100 to 150 micrograms sodium nitroprusside - that causes your blood vessels to dilate (get larger), into a large forearm vein that will lower your blood pressure. Sixty seconds later, you will be given an injection of a second drug – 100 to 150 micrograms of phenylephrine HCL. This drug causes your blood vessels to get smaller and will raise your blood pressure. The amount of each drug to be injected will begin with a small amount, and may be increased if your blood pressure does not change at least 15 points. These two drugs will be injected a total of three times. A time period of at least 20 minutes will separate each series of injections. In addition, a small pencil shaped blood pressure measuring device will be pressed gently against your neck for a short time.

**Blood Flow in Heart and Arteries** – the blood flow and diameter in the arteries in your neck, arm and leg will be measured with an ultrasound machine. An ultrasonic machine is sort-of like radar – a low frequency radio wave that bounces off the tissues and sends a picture back to a “TV-like” screen. A mobile hand unit used will be pressed gently against an artery in your neck, arm and leg. The amount of blood that your heart pumps in one beat and in one minute will be measured with another ultrasound probe. For these measurements, the probe will be pressed gently against two different places on your chest.
- Cold Pressor Test – You will be asked to cover your hand up to wrist level in a bucket of iced cold water for 2 minutes. This will provide information about your sympathetic nervous system and circulation not provided by the arterial baroreflex study. Increased activity of the sympathetic nervous system is the normal body response to fear, stress and surprise. The heart rate goes up, the lungs expand for more air intake and you get an energy rush. You may withdraw your hand from the water at any time if the cold water becomes too painful or uncomfortable.

- Handgrip – You will be asked to perform three brief (about 5 seconds) maximal squeezes on a handgrip device and then an isometric handgrip at less than half your maximal grip strength for approximately 2-3 minutes. This will not be exhausting and will provide additional information about your sympathetic nervous system and circulation responses to a different form of stress.

The approximate time required for these tests will be approximately 3 hours.

Session 5

- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.

- Overnight Fast - You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.

- Computed Tomography Scan – The amount of total fat, fat around your internal organs, and the fat under the skin in the abdominal area will be measured by computed tomography (CT scan). The CT scan imaging will be performed at Montgomery Regional Hospital. For this procedure, you will be asked to lie still on a table. An x-ray machine (the CT scanner) will rotate around you and the table will move back and forth slightly making it possible to take X-rays from several angles. The actual x-ray time is approximately 2 minutes or less. You will be lying on the table for approximately 15 to 30 minutes. The approximate time required for the entire procedure is one hour. A longer period of time may be required due to heavy scheduling and/or emergency need of the CT scan at the Montgomery Regional Hospital.
Session 6

- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.
- Overnight Fast - You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- Resting Energy Expenditure – After fasting for 12 hours you will come to the clinic between 7:00 and 8:00 a.m. You will lie quietly on a hospital-type bed and after a 15-minute period of quiet rest a clear bubble-type hood will be placed over your head in order to collect all of the air that you breathe out. This hood will not disturb your natural breathing pattern. This test will tell us how many calories you burn at rest.

Session 7

- Pregnancy Test - If you are female you will be required to have a pregnancy test. This will require approximately 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.
- Overnight Fast - You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- Arterial Blood Pressure, Heart Rate, and Breathing – A continuous recording of the blood pressure in your arteries will be made by placing a small cuff around your middle finger or wrist while your hand is maintained at heart level. Heart rate will be measured by placing electrode pads on your chest and reading the electrocardiograph (ECG – a tracing of your heartbeats) signal. You will be asked to pace your breathing to a clock at a rate of 15 breaths per minute. A loose fitting plastic belt will be placed around the upper abdomen to measure your breathing movements.
- Sympathetic Nervous System Activity – The measurements of sympathetic nervous system activity involves measuring the activity of one of your nerves on the side of your knee or arm. Two small microelectrodes (small needles) will be placed through your skin. The position of one of the electrodes will be move back and forth through your skin while a very small electrical impulse (1-2 volts) is passed through the electrode. The needles may be
inserted up to an inch below the skin of your leg or arm. This search procedure will continue until the electrode being moved causes your foot or hand to twitch. This procedure will take between 5 – 60 minutes. When a foot or hand twitch is observed, measurement of the activity of the sympathetic nervous system will begin and continue during the procedure described below.

- **Forearm or Leg Blood Flow** – The amount of blood flow to your arm or leg will be measured in two ways. First, we will place a small flexible piece of plastic around your arm or leg and blood pressure cuffs around your upper and lower arm. The cuffs will be inflated and deflated periodically. Second, we will use an ultrasound machine, which produces sound waves to measure your arm or leg blood flow. The two techniques will be used together to get the most accurate measurement.

- **Lower Body Negative Pressure** – Your lower body (up to your waist) will be sealed in an airtight box which is attached to a vacuum cleaner. When the vacuum cleaner is turned on a negative pressure is created inside the box and this causes some of the blood in your body to move into your legs. This causes your sympathetic activity to increase. This procedure will be performed at 3 to 4 different levels of negative pressure to increase your sympathetic activity. We will then measure your sympathetic activity and how the blood flow in your forearm and/or leg changes.

- **Catheter and Blood Draw** – A small plastic tube (catheter) will be inserted into an arm vein to draw blood (approximately 2 tablespoons) to measure the levels of norepinephrine in your vessels. Norepinephrine is a substance secreted by your sympathetic nerves and causes your blood vessels to get smaller.

Session 8

- **Ambulatory Blood Pressure**: Your blood pressure will be measured over the course of an entire day (daytime and nighttime) using a cuff placed around your arm and a small computer type device placed around your waist. Your blood pressure will be measured at 20 minute intervals during the daytime and 30-minute intervals at night. You will be asked to stop and remain still while the cuff inflates around your arm. You will wear this cuff and computer type device for an entire day and then return it to War Memorial Hall at the end of that period.
The approximate time for the test is 24 hours.

SUMMARY OF SUBJECT RESPONSIBILITIES

- Provide an accurate history of any health problems or medications you use before the study begins.
- Inform the experimenters of any discomfort or unusual feelings before, during or after any of the study sessions.
- Be on time and attend all of the scheduled experiment sessions.
- Follow all participant instructions for each session.
- Record the food you eat and your physical activity as instructed by the study investigators.
- Carefully follow the diet provided by the research dietitian and refrain from starting an exercise program during the study.
- Follow instructions on consuming any food provided to you.
- Inform the study investigators if you are pregnant or become pregnant during the study.

RISKS OF PARTICIPATION

- Catheter and Blood Draw: Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this persists for only a short time. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruises. The risk of a blood clot forming in the vein is about 1 in 200 (0.005%), while the risk of infection or significant blood loss is 1 in 1000 (0.001%). There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the catheter is removed. If you feel faint during or after a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff who are experienced in catheter placement and blood draws will minimize these risks.

- HIV/AIDS: Your blood will be tested for the presence of HIV if one of the study investigators is exposed to your blood. There will not be any cost to you for this test. The results will be sent to your primary care physician or the study medical director, Dr.
Jose Rivero, if you do not have a primary care physician. He/she will discuss them with you and provide you with the necessary referral for further evaluation and/or counseling if your results are positive. The results of your test will remain confidential.

- Oral Glucose Tolerance: Because this procedure requires the placement of the catheter in a vein in each arm, the risks here are identical to those stated above. In addition, there is a small risk of low blood sugar occurring during or after the test. If this happens, orange juice (with table sugar) or some other sugar containing food will be given to you.

- Sleep Study: There are no known risks associated with sleep studies. However, you may not sleep very well the night of the study since it is an environment different from your home.

- Graded Exercise Test: Maximal exercise testing may cause fatigue, muscle strains, an irregular heart beat (dysrhythmias) and a change in blood pressure. There is a 0.01% chance of death and a 0.04% risk of heart attack requiring hospitalization. The mouthpiece and noseclip may be uncomfortable to wear.

- DEXA Scan: The amount of radiation that you will receive in the DEXA exam (combined with the CT scan) is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, however the exact increase in such risk is not known.

- CT scan: The amount of radiation that you will receive in the CT scan (combined with the DEXA exam) is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is less than that received from a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The total amount of x-ray exposure from both the DEXA and CT scan is less than a chest x-ray. The radiation in this study is not expected to greatly increase these risks, however the exact increase in such risk is not known. You should know that the CT scan for this study is for research purposes and not for diagnosis. The CT scan will be not be reviewed or saved for future purposes by Montgomery Regional Hospital.
Sympathetic Nervous System Activity: Some subjects experience a temporary (seconds) pain and discomfort while the microelectrodes are being inserted into the skin. After the procedure, there is a small risk of numbness, “pins and needles”-type of sensation, or pain that may last 1-3 days. In very rare cases, numbness, pins and needles type sensations, or pain in the leg or arm has lasted several weeks or months (1 to 3 in 1000 or 0.001 to 0.003%). It is also possible that permanent nerve damage could occur. The principal investigator of this project has performed this procedure over 300 times and only one individual (0.003%) has experienced pins and needles sensations for 7 to 10 days. All of these problems can be minimized by only having experienced individuals perform this technique. In addition, by minimizing the time to find the nerve to less than 60 minutes, the risk of unpleasant after-effects is reduced even more.

Drug Infusions: Because this procedure requires the insertion of a catheter, the risks here are identical to those described above under catheters. In addition, the infusion of nitroprusside could cause low blood pressure and nausea, sweating or a sudden elevation in heart rate. These feelings should pass within 1-2 minutes. There is an extremely low risk that your blood pressure would drop so low after the nitroprusside injection that you faint. You should know that a physician will not be on site if this were to occur. Our emergency plan would involve raising your legs to help blood flow return to your head, calling 911 to activate an emergency response from the Virginia Tech Rescue Squad, and continued monitoring of your blood pressure. The Virginia Tech Rescue Squad would provide rapid response to an emergency call at War Memorial Hall. They would initiate emergency treatment which might include intravenous fluids and additional medical treatment to maintain your blood pressure. You would then be driven by ambulance to the emergency room at the hospital. However, the amount of nitroprusside used in the present study was selected to lower blood pressure by approximately 15 points or mmHg. This is similar to how much your blood pressure falls when an individual rises from a lying down to a standing position. The infusion of phenylephrine may result in a headache, restlessness, a sudden decrease in heart rate, and/or rarely an irregular heart beat. These feeling or symptoms, if they occur, usually pass within a few minutes. There is also a small risk that some
phenylephrine will leak out from the catheter site causing severe constriction of the surrounding small blood vessels. This may result in an inadequate blood supply to the surrounding tissues and eventual death of that tissue if untreated. Using a large vein in your arm minimizes this risk. However, if this problem occurs, you will be referred to a physician for immediate treatment. In the event of an emergency, the Virginia Tech Rescue Squad will be contacted.

There is a remote possibility that you may have an allergic reaction to the medications or its vehicle. If this happens during the study, we will call 911 to initiate an emergency response by the Virginia Tech Rescue Squad. They may give you another medication to treat this allergic response and then transport you to the nearest hospital emergency room. It is important to inform us if you have any known medication allergies before you participate. If you have a history of allergies to phenylephrine or nitroprusside you will be no allowed to participate in this aspect of the study. If you have an allergic reaction to the medications during the study, you will not be allowed to participate further in this aspect of the study.

These risks are slightly increased because we will repeat both drug infusions two times. It should be emphasized that the amount of these drugs you will be receiving is very small and rapidly broken down and eliminated by your body. This lowers the risk of any adverse reactions to the drugs. The principal investigator has performed over 150 of these tests with no adverse events.

- **Lower Body Negative Pressure:** There is a very small risk of feeling nausea or fainting. The study will be stopped if you begin to feel an upset stomach, like you might faint, if your heart rate or blood pressure suddenly drops. You will be monitored continuously by the study staff to avoid any of these situations.

- **Cold Pressor Test:** This procedure may be painful and will cause your heart rate and blood pressure to increase.

- **Handgrip:** This may cause some discomfort and fatigue in your hand (similar to holding a heavy suitcase for several minutes) or forearm and will cause your heart rate and blood pressure to increase. There is a low risk of some temporary soreness in the day or two after the procedure. This test is not meant to continue until exhaustion.
- Pregnancy: You should not become pregnant during this study because of the exposure to x-rays and study drugs. If you are capable of having a child you must have a negative pregnancy test before each session that may pose a risk to an embryo or fetus (x-ray exposure or medication injection). You must agree to use an effective method of birth control, such as abstinence, condom use, oral contraceptives or use of an IUD to ensure that you will not get pregnant. Otherwise, you must be surgically sterile. If you become pregnant during this study, you must notify your study investigator immediately. There may be unforeseen risks to the embryo or fetus in the event that you become pregnant.

- Ambulatory Blood Pressure: The cuff inflation may cause some discomfort and mild bruising in your arm. The cuff inflation may cause you to have difficulty sleeping.

- Weight Gain: Weight gain is common following weight loss programs. It is possible that you will gain some or all of the weight you lost during the study. We can make no promises or commitments on the long term success of maintaining your weight loss. This is a possibility that you should consider before you agree to participate.

- It is not possible to identify all potential risks in an experiential study, however the study doctors and study staff will take all possible safeguards to minimize any known and potential risks to your well-being. We believe the overall risks of participation are minimal. All of the procedures are well established and used routinely in the study investigators laboratory.

- Side effects are possible in any research study despite high standards of care, and could occur through no fault of your own or the study doctors or the study staff.

**BENEFITS OF PARTICIPATION**
Your participation will provide you with:

- Information on your body composition and aerobic fitness.
- Information on your blood pressure, cholesterol and glucose tolerance

**COMPENSATION**
We will pay you $100 each time you complete all the sessions 2-8 described above. If you dropout of the study or are unable to complete the study you will be paid $15 per session for
those individual sessions you complete. If you complete each of these sessions three times you will receive a total of $300.

CONFIDENTIALITY
The data from this study will be kept strictly confidential. No data will be released to anyone but those working on the project without your written permission. Data will be identified by subject numbers, without anything to identify you by name. In the event that your exercise test indicates you may have a heart problem or if your sleep test indicates that you stop breathing during your sleep, Dr. Rivero or Dr. Zedalis may want to share this information with your doctor but will request your written approval first.

FREEDOM TO WITHDRAW
You are free to withdraw from the study at any time for any reason. Simply inform the experimenters of your intention to cease participation. Circumstances may come up that the researcher will determine that you should not participate or continue as a subject in the study. For example, inability to perform certain key measurements because of your body weight or size, lack of compliance to instructions, failure to attend testing sessions, or illness could be reasons for the researchers to stop your participation in the study. You may be able to participate in the entire study even if you choose not to participate in certain sessions/tests. However, if you choose not to participate in Session 1 and/or the graded exercise tests then you will not be able to participate in the entire study. These sessions/tests are included to determine if it is safe for you to participate in the study.

INJURY DURING PARTICIPATION IN THIS STUDY
Neither the researchers, the University nor Montgomery Regional Hospital have money set aside to pay for medical treatment that would be necessary if injured as a result of your participation in this study. Any expenses that you incur including emergencies and long term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

APPROVAL OF RESEARCH
This research has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech, and by the Department of Human Nutrition, Foods, and Exercise. Montgomery Regional Hospital Institutional Review Board has also approved this research. You will receive a copy of this form to take with you.

SUBJECT PERMISSION
I have read the informed consent and fully understand the procedures and conditions of the project. I have had all my questions answered, and I hereby give my voluntary consent to be a participant in this research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time.

If you have questions, you may contact:
- Principal Investigator: Kevin Davy, Associate Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-3487; After hours: 540-230-0486
- Chairman, Institutional Review Board for Research Involving Human Subjects:
  David Moore, (540) 231-4991
- Department of Human Nutrition, Foods and Exercise reviewer:
  Robert Grange, (540) 231-2725
- Chairman, Institution Review Board, Montgomery Regional Hospital:
  Chris Riegert: (540) 951-1111

Name of Subject (please print)________________________________

Signature of Subject________________________________________  Date_________
An optional part of this study involves the collection of an additional blood sample to conduct DNA testing. DNA is the principal chemical component of our genes. Our genes contribute to characteristics such as hair color, height, and blood type. Differences between individuals arise from small variations in the DNA of one or more genes. This testing will try to determine if any variations are present in genes that may play a role in how the nervous system influences heart and blood vessel function. For example, a small variation in the gene responsible for making angiotensin II, an important cardiovascular hormone, may be related to differences in nervous system activity between individuals participating in this study.

METHODS:
To obtain this blood sample a small plastic tube will be inserted into one of your arm veins to draw blood. You will be asked to avoid eating for 12 hours prior to this visit so that the other test results being conducted (for example, cholesterol and glucose levels) will not be influenced by the food you eat or by the normal digestion process. Three teaspoons of blood will be collected and the white blood cells in that sample will be frozen for DNA testing at a later time. Tests performed on these samples will be restricted to relevant genetic tests or procedures available during a 10 year storage period. The reason to retain these samples is that new genetic
tests may be identified that may provide helpful information about differences in autonomic-cardiovascular function. The samples will be destroyed after this time. The genetic testing may take place at a commercial laboratory or academic institution other than Virginia Tech. The results of the genetics testing performed as a result of your participation in this study become the sole property of Virginia Tech and you give up your right to any diagnostic tests that may be developed directly or indirectly as a result of this study.

SUMMARY OF SUBJECT RESPONSIBILITIES

- Provide an accurate history of any health problems or medications you use before the study begins.
- Inform the experimenters of any discomfort or unusual feelings before, during or after any of the study sessions.
- Be on time and attend all of the scheduled experiment sessions.
- Follow all participant instructions for each session.
- Inform the study investigators if you are pregnant or become pregnant during the study.

RISK OF PARTICIPATION

- Genetic Testing: There is an extremely small risk that the results of your genetic testing could impact the employability of you or family members, the insurability of you or your family members by private insurance companies. However, we will take several steps to minimize these risks. The results of your genetic testing information will be coded and held confidential. The results of your genetic tests will not be shared with you, your family members or your personal physician nor will we make samples, tested or not, available to family members for their own use in genetic testing. The results of these tests will be kept in a locked cabinet in the investigators office.

- Catheter and Blood Draw: Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this persists for only a short time. You may have some pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 cases, a small amount of bleeding under the skin will cause a bruise. The risk of a blood clot forming in the vein is about 1 in 200, while the risk of infection or significant blood loss is 1 in 1000. There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the catheter is removed. If you feel faint during or after
a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff who are experienced in catheter placement and blood draws will minimize these risks.

- HIV/AIDS: Your blood will be tested for the presence of HIV if one of the study investigators is exposed to your blood. There will not be any cost to you for this test. The results will be sent to your primary care physician or the study medical director, Dr. Jose Rivero, if you do not have a primary care physician. He/she will discuss them with you and provide you with the necessary referral for further evaluation and/or counseling if your results are positive. The results of your test will remain confidential.

- It is not possible to identify all potential risks in an experiential study, however the study investigators and study staff will take all possible safeguards to minimize any known and potential risks to your well-being. We believe the overall risks of participation are minimal. All of the procedures are well established and used routinely in the study investigators laboratory.

- Side effects are possible in any research study despite high standards of care, and could occur through no fault of your own or the study investigators or the study staff.

BENEFITS OF PARTICIPATION
There is no direct benefit to you in having these genetic tests performed. None of the genetic test results will be provided to you or to any other physician who is treating you or may treat you in the future. The study investigator will not notify you, your physician, or any third party of any genetic test results, even if this could benefit you now or in the future.

COMPENSATION
You will not be paid for your involvement in this genetic testing study.

CONFIDENTIALITY
This blood sample collected from you will be handled in a coded (your name will not be associated with the sample), confidential way and used to make a bank of your DNA. The principal investigator will keep the key linking the coded sample container to your name in his
office in a locked cabinet. Only the principal investigator will have access to that key to maintain your confidentiality.

FREEDOM TO WITHDRAW
Participation in this part of the study is optional and entirely up to you. The samples will be stored in a freezer in War Memorial Hall, Department of Human Nutrition, Foods, and Exercise at Virginia Tech. If you agree to participate in this part of the study, but change your mind at any time in the future, please contact the study investigator and he will locate the remaining genetic blood sample(s) and destroy them.

INJURY DURING PARTICIPATION IN THIS STUDY
Neither the researchers, the university nor Montgomery Regional Hospital have money set aside to pay for medical treatment that would be necessary if injured as a result of your participation in this study. Any expenses that you incur including emergencies and long term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

APPROVAL OF RESEARCH
This research has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech, and by the Department of Human Nutrition, Foods, and Exercise. Montgomery Regional Hospital Institutional Review Board has also approved this research. You will receive a copy of this form to take with you.

SUBJECT PERMISSION
I have read the informed consent and fully understand the procedures and conditions of the genetic testing. I have had all my questions answered, and I hereby give my voluntary consent to be a participant in this aspect of the research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time or have the samples collected destroyed at my request.

If you have questions, you may contact:
- Principal Investigator: Kevin Davy, Associate Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-3487; After hours: 540-230-0486
Chairman, Institutional Review Board for Research Involving Human Subjects:
David Moore, (540) 231-4991

Department of Human Nutrition, Foods and Exercise reviewer:
Robert Grange, (540) 231-2725

Chairman, Institution Review Board, Montgomery Regional Hospital:
Chris Riegert: (540) 951-1111

Name of Subject (please print)______________________________________

Signature of Subject_____________________________________________  Date________
APPENDIX III - Instructions for the New Method of Gain Estimation

The following instructions will guide you through the preferred method of sorting trials.

Step 1: Run the Boltzmann fit for each individual trial using Origin 7.

(for detailed information on how to use Origin 7, refer to Appendix B below)

Step 2: Copy the plot and parameter output to a new excel file and save.

Step 3: Repeat Steps 1 and 2 for all trials.

Step 4: Compile the parameters of each trial in a new excel file in the following format:

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<th>E</th>
<th>F</th>
<th>C</th>
<th>H</th>
<th>A1 value</th>
<th>A1 error</th>
<th>A2 value</th>
<th>A2 error</th>
<th>x0 value</th>
<th>x0 error</th>
<th>dx value</th>
<th>dx error</th>
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<td>R²</td>
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<td></td>
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</table>

Step 5: Eliminate any trials where not all the subject parameters and associated parameter errors are known.

Step 6: Sort trials in excel using the following criteria:

- Random trials = if absolute value (dx % error) > 100
- Threshold-heavy trials = if absolute value (A2 error / A2 value)*100 > 50
  and if absolute value (A1 error / A1 value)*100 < 50
- Saturation-heavy trials = if absolute value (A1 error / A1 value)*100 > 50
  and if absolute value (A2 error / A2 value)*100 < 50
- Linear-heavy trials = if absolute value (A1 error / A1 value)*100 > 50
  and if absolute value (A2 error / A2 value)*100 > 50
*In excel, label a column for each of these criteria that will evaluate each of the trials by outputting “1” if it is true and nothing if it is false.

The modified excel sheet will look like the following:

![Excel Sheet](image)

Step 7: Keep any trials marked as Linear-heavy (even if it is also marked as Random) and delete any trials marked as Saturation-heavy, Threshold-heavy, and Random.

Step 8: Perform a linear regression to estimate gain on all the trials using the manual method of truncation.
APPENDIX IV - Instructions for the Boltzmann fit

The following instructions will guide you through the Boltzmann fit using Origin 7.

Step 1: Open Origin 7 program.
Step 2: Choose “Open Excel…” from the File drop down menu.
Step 3: Select the baroreflex excel file and open as an “Origin Worksheet”.

![Origin Worksheet with Excel file]
Step 4: Make sure that all the columns are free of any labels and only contain the numerical data.

- The first column should contain the SBP data, A(X)
- The second column should contain the R-R interval data, B(Y)

Highlight the B(Y) column.
Step 5: Click on the “Analysis” tab on the top and select “Fitting Wizard…” after highlighting the “Non-linear Curve Fit” option.

Step 6: Choose the default settings for the “Select Data” window by clicking “Next”
Step 7: For the “Select Function” window, set the Category to “Growth/Sigmoidal” and the Function to “Boltzmann”, then click “Next”.

Step 8: Select the default settings for the “Weighting” window by selecting “Next”

Step 9: Select the default settings for the “Fitting Control” window by selecting “Next”
Step 10: Click on “Finish” for the Results window and the plot will appear in a new window.
Step 11: Select the “View” tab on the top and choose to view the “Results Log”. 
Step 12: The Results Log will appear in the lower right of the window. The parameter and error values can be easily cut and pasted from here to excel to record the data.

Step 14: Cut and paste the plot and parameter output into a new excel sheet and save.
APPENDIX V - Vita

Abrahm John Behnam

EDUCATION

M.S. School of Biomedical Engineering Sciences (SBES), May 2007
Virginia Polytechnic Institute and State University and
Wake Forest University

B.S. *Magna Cum Laude* Engineering Science and Mechanics (ESM), December 2004
Virginia Polytechnic Institute and State University

HONORS

Magna Cum Laude in ESM
Virginia Tech Dean’s List
Pratt Scholarship

WORK EXPERIENCES

Open House Director, ESM Recruiting Committee (Fall 2003, 2004)
Physics Instructor, Kaplan Test Prep and Admissions (Fall 2004 - Spring 2006)
Graduate Teacher Assistant, Virginia Polytechnic Institute (Fall 2004 - Spring 2006)
Violin Teacher, Performing Arts Institute of Virginia (February 2005 - September 2006)

PROJECTS

Developing a Refined Approach of Cardiovagal Baroreflex Gain Assessment
(Spring 2005 - Spring 2007)
Design and Construction of a Third Generation Mock Cardiovascular Circulatory Device
(Spring 2002 – Fall 2004)

ACTIVITIES

Graphic Designer, (2000 - present)
Volunteer, Good Samaritan Hospice, Roanoke (2002 - 2005)
Counselor, Camp Rainbow, Roanoke (Summer 2003 & 2004)
Shadowed Dr. Nelson, Cardiac Surgeon, Western Maryland Health System (Summer 2002)
Shadowed Ashley Ross, M.D./PhD. Student, Johns Hopkins University (Winter 2001)
Volunteer at Western Maryland Health System (Summer 2001)
Officer, Virginia Tech Table Tennis Club Team (Fall 2004 - present)