EFFECT OF CAFFEINE AND A PREWORKOUT SUPPLEMENT ON HEART RATE VARIABILITY BEFORE AND AFTER EXERCISE

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Cardiovascular health is negatively affected by overactivity of the sympathetic nervous system (SNS) during rest. Heart rate variability (HRV) has been used to predict SNS activity. The study investigated effects of a placebo, caffeine, and preworkout supplement (double blinded) on short term HRV before and after an acute bout of resistance exercise. Twelve subjects completed a trial with each supplement. Caffeine and exercise showed a significant decrease for Low Frequency (LF) Power normalized units (n.u.) ($p=0.005$) and a significant increase for High Frequency (HF) Power n.u. ($p=0.010$) immediately post exercise compared to exercise with placebo. Known effects that the combination of caffeine and exercise have on SNS activity do not agree with results found for HF Power n.u. and LF Power n.u using traditional interpretation of these indices for SNS activity. This suggests that the relationship between the cardiovascular autonomic control system and HRV is more complex than previously thought.

ACKNOWLEDGEMENTS

There are numerous people that have helped me complete this study and deserve recognition. I thank my advisor Dr. Dan Ewert for giving me support and inspiration, as well as instilling confidence in me to pursue this work. I also thank the rest of my supervisory committee members, Drs. Kyle Hackney and Jim Grier, for being so supportive and helpful when I needed it most. The many others I thank for helping me with this study include, but are not limited to Sherri Stastny, Michael Blake, Kara Stone, and Drew Taylor. I would not have been able to complete my work without all of you. I thank you all very much.

DEDICATION

I dedicate this work to my parents, Jerome and Colleen Gagnon. Of the many things I have learned from them, they have taught me to always believe in myself when taking on challenges in life.

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LIST OF ABBREVIATIONS

HRV .....Heart Rate Variability

HR .....Heart Rate

ECG.....Electrocardiogram

FFT.....Fast Fourier Transform

DFT .....Discrete Fourier Transform

AR .....Autoregressive

PSD .....Power Spectral Density

RMS .....Root Mean Squared

SSE.....Sum Squared Error

TP.....Total Power

VLF .....Very Low Frequency

LF .....Low Frequency

HF .....High Frequency

n.u.....Normalized Units

ANS.....Autonomic Nervous System

SNS .....Sympathetic Nervous System

PNS .....Parasympathetic Nervous System

SA .....Sinoatrial

AV.....Atrioventricular

RSA.....Respiratory Sinus Arrhythmia

CVD .....Cardiovascular Disease

GUI .....Graphical User Interface

IRB.....Institutional Review Board

FDA.....Food and Drug Administration

ANOVA .....Analysis of Variance

RM .....Rep Max

SD .....Standard Deviation

SE.....Standard Error.

MSE .....Mean Squared Error

LIST OF SYMBOLS

f_s ..... Sampling Frequency

a^k ..... Coefficients for Autoregressive (AR) Model of Order k

E_M ..... Prediction Error Variance for Autoregressive (AR) Model of Order M

R_j ..... Autocorrelation Value for Autoregressive (AR) Models With Lag j

λ_M ..... Reflection Coefficient for Autoregressive (AR) Models of Order M

A_M ..... Intermediate Matrix for Determining Autoregressive (AR) Model Coefficients for Model Order M

U_M ..... Intermediate Matrix for Determining Autoregressive (AR) Model Coefficients for Model Order M

V_M ..... Intermediate Matrix for Determining Autoregressive (AR) Model Coefficients for Model Order M

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1. INTRODUCTION

Heart rate variability (HRV), in general terms, is the amount of beat-to-beat variation in the heart rate. HRV has been shown to act as an indicator for cardiovascular health [1], [7], [9]. Some ECG machines include software to perform HRV analysis. However, there is no control over which HRV indices are measured, limiting the opportunity for thorough analysis and satisfactory comparison between most HRV studies.

HRV has recently been used as a non-invasive method for gaining insight into autonomic nervous system (ANS). While SNS activity is important for exercise because it increases heart rate, respiration rate, and blood flow to muscles, it is known that overactivity of the SNS during rest is often associated with multiple cardiovascular diseases (CVDs) such as hypertension and heart failure [6].

SNS activity can also be elevated through ingesting caffeine [10]. Many Preworkout supplements contain caffeine and advertise performance boosts through increasing SNS activity, however it is unknown what effects these supplements have on SNS activity either during rest or after an exercise bout. Apart from caffeine, preworkout supplements may contain other ingredients that have unknown effects on SNS activity. Furthermore, these supplements are unregulated by the Food and Drug Administration (FDA), making it difficult to determine the ingredients and the effects of these ingredients.

The purpose of this thesis was to determine the effect of a placebo, caffeine, and preworkout supplement on HRV, and, by inference, SNS activity. To accomplish this, an HRV software package was created to include the large number of standard HRV indices from [1]. Inclusion of the full set of standard indices increases the overlap of HRV indices among most other HRV studies for easier comparisons among studies.

2. BACKGROUND

Changes in HRV are widely believed to stem from the influence of both SNS and parasympathetic nervous system (PNS) activity on the sinoatrial (SA) node [8]. Although HRV actually measures variability from the atrioventricular (AV) node, it has been shown to very accurately reflect variability in the SA node [8]. A historical overview of the evolution of HRV research is summarized in [9].

Typical HRV analysis consists of many numerical indices and is not characterized by a single index [1]. HRV analysis consists, most commonly, of two types of analysis: time and frequency domain analysis [1]. The purpose of these types of analysis is to describe beat-to-beat variability in numerous ways with the goal of gaining insight to the SNS and PNS activity [9].

It is known that increased SNS activity increases heart rate while increased PNS activity decreases heart rate [9]. It has also been shown that heart rate changes occur synchronously with the respiration rate, otherwise known as respiratory sinus arrhythmia (RSA), providing information about SNS and PNS activity. Time domain analysis attempts to measure the variability of RSA as a measure of the relationship between SNS and PNS activity.

It has also been shown that beat-to-beat variability does not occur as one single frequency, such as by the respiration rate, but has been shown to occur at numerous frequencies. This is the basis for the use of frequency domain analysis. Although very controversial, changes in heart rate that occur at frequencies greater than 0.04 Hz but less than 0.15 Hz have been shown reflect the combination of both SNS and PNS activity while changes in heart rate that occur at frequencies greater than 0.15 Hz but less than 0.40 Hz reflect PNS activity. Frequency domain analysis attempts to measure the contributions of various frequencies to overall beat-to-beat variability.

To perform these analyses, the timing of a series of heart beats must be measured. This is typically achieved by measuring the electrical activity of the heart via an ECG waveform. There are two main lengths of time commonly used for HRV analysis: short term HRV analysis (5 minutes) and long term HRV analysis (24 hours).

Once a recording is completed, the R wave of each QRS complex is detected. Once each normal R wave is detected, the RR intervals (time between each RR interval in milliseconds) are determined. After visual confirmation that there are no abnormalities in the RR interval calculations, and the abnormal (or ectopic) R waves and subsequent RR intervals are rejected as part of analysis, the RR intervals are termed "NN" intervals (normal RR intervals),.

The plot of the NN intervals vs time is commonly referred to as the "tachogram," and will be referred to as the tachogram in this thesis. The time and frequency domain analysis methods are determined from the NN data in the tachogram.

One idea brought forth by [3] noted the NN interval's mathematical dependence on the heart rate (HR). They suggested a need for a correction procedure, which they developed [3]. The correction involves dividing each NN interval by the average NN interval. The end result is a unitless tachogram that no longer depends on its average value.

2.1. Time Domain HRV Analysis

Time domain analysis consists of determining statistical characteristics directly from the data on the tachogram. Some typical time domain indices that represent the time domain methods are shown in Table 1. All of those time indices are suggested for use by [1].

Table 1. Summary of Standard Time Domain Indices Used for HRV Analysis.

Index	Units	Description	Definition
Mean NN	ms	The average of all NN intervals	$\frac{1}{n}\sum_{i=1}^{n} NN_i$
Max NN	ms	The maximum of all NN intervals	-
Min NN	ms	The minimum of all NN intervals	-
SDNN	ms	The sample standard deviation of all NN intervals	$\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(NN_i-\mu)^2}$
rMSSD	ms	The root mean square of successive differences	$\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(NN_{i+1}-NN_i)^2}$
Ln(rMSSD)	-	The natural logarithm of the rMSSD	$\ln(rMSSD)$
pNNxx	ms	The percentage of ΔNN intervals that differ by more than xx ms	–
Triangular Index	ms	The total number of NN intervals divided by the max of the density distribution of NN intervals	The total number of NN intervals divided by the max of the density distribution of NN intervals with a bin size of $\frac{1}{128}\ s = 7.8125\ ms$
Mean ΔNN	ms	The average of all ΔNN intervals	$\frac{1}{n}\sum_{i=1}^{n}\Delta NN_i$
SDSD	ms	The sample standard deviation of successive differences (standard deviation of all ΔNN intervals)	$\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(\Delta NN_i-\mu)^2}$

2.2. Frequency Domain HRV Analysis

Frequency domain analysis consists primarily of determining the signal power of the tachogram over various frequency ranges [1]. Determining the signal power of a frequency range of the tachogram is achieved by integrating the power spectral density (PSD) of the tachogram over a desired frequency range. Commonly, the PSD is determined by two different methods:

non-parametric and parametric methods. Non-parametric methods consist of determining the PSD using a fast Fourier Transfer (FFT), and parametric methods consist of determining the PSD using an autoregressive (AR) model. Some typical frequency domain indices are shown in Table 2.

Table 2. Summary of Standard Frequency Domain Indices Used for HRV Analysis.

Index	Units	Description	Frequency Range (Hz)
Total Power (TP)	ms^2	Variance of all NN intervals	Approximately equal to $f \leq \sim 0.4$, but includes all frequencies.
Very Low Frequency (VLF) Power	ms^2	Power in the VLF range	$0 \leq f \leq 0.04$
Low Frequency (LF) Power	ms^2	Power in the LF range	$0.04 \leq f \leq 0.15$
High Frequency (HF) Power	ms^2	Power in the HF range	$0.15 \leq f \leq 0.40$
LF:HF Ratio	ms^2	Ratio between LF and HF Power	
Low Frequency (LF) Power	n.u.	Normalized Power in the LF range. Equal to LF divided by the TP minus the VLF.	
High Frequency (HF) Power	n.u.	Normalized Power in the HF range. Equal to HF divided by the TP minus the VLF.	

2.2.1. Non-Parametric Method: Fast Fourier Transform (FFT)

The FFT method calculates the discrete Fourier transform (DFT) of a discrete signal sampled at equal intervals using an FFT algorithm. The PSD, as determined by the FFT method, displays magnitudes at frequencies over the frequency range of zero Hz to one-half of the sampling frequency (Nyquist's theorem) with a frequency spacing of $\Delta f = \frac{1}{T} = \frac{1}{N/f_s} = \frac{f_s}{N}$ where T is the length of the signal in seconds, N is the number of data points, and f_s is the sampling frequency. Sometimes, the PSD may have a small frequency resolution (large Δf) which makes

it difficult to see the contribution of various frequencies to the PSD. It is possible to increase the frequency resolution (decrease Δf), if desired, by performing "zero padding." Zero padding consists of adding zeros to the end of the signal. Zero padding increases N, therefore increases frequency resolution (decreases Δf). Once the PSD has been determined, the PSD is integrated, such as by the trapezoidal method (the integration method is arbitrary), over a desired frequency range to determine the signal power over a chosen frequency range. Zero padding can also be used to create a Δf that makes integration more convenient, such as making sure that the Δf allows for there to be data points at specific frequencies of concern (the end points for the frequency ranges for HRV analysis).

2.2.2. Parametric Method: Autoregressive (AR) Modeling

Determining the PSD by AR modeling consists of creating an AR model which is used as a prediction model to model a signal based on a linear combination of previous data points, each with a specific weight. The chosen number of previous data points used in the model is the AR model order. The PSD, as determined by the AR model, displays the magnitudes at frequencies over the frequency range of zero Hz to one-half of the re-sampling frequency (Nyquist's theorem). Unlike the PSD by FFT, the PSD by the AR method is a continuous function and can be plotted with any frequency spacing Δf with no modification to the data of the interpolated tachogram such as zero padding. This allows for easier integration, such as by the trapezoidal method, of the waveform as well to determine the signal power over a chosen frequency range, since integrating the continuous PSD may be very difficult.

2.2.3. Interpolated Tachogram (Cubic Spline)

An issue with determining the PSD by either FFT or AR modeling is that both methods require that the set of data points be spaced at equal intervals, which is not the case of the

tachogram. To represent the tachogram with data points at equal intervals, some method of interpolation must be performed. A common method of interpolation is to create a cubic spline and re-sample the cubic spline at some chosen re-sampling frequency. Note that the sampling frequency of the ECG is typically not equal to the re-sampling frequency of the cubic spline.

2.2.3.1. Cubic Spline Re-Sampling Frequency

It is important to choose an appropriate re-sampling frequency to satisfy the needs of the frequency domain analysis. One constraint in the choosing of the re-sampling frequency is determined by applying Nyquist's theorem. According the Nyquist's theorem, the re-sampling frequency must be more than twice as large as the highest frequency of concern. The highest frequency of concern is 0.4 Hz (the upper bound on the high frequency range for HRV analysis). Therefore the first constraint is that the re-sampling frequency $f_s > 2 * (0.4\ Hz) = 0.8\ Hz$.

The second constraint comes from properties of the PSD by both the FFT and AR model methods. For the FFT method, the frequency spacing is directly proportional to the re-sampling frequency, therefore the smaller the re-sampling frequency the smaller the frequency spacing. A small frequency spacing is always preferred over a large frequency spacing because it gives a more accurate representation of the PSD, and it also allows for more accurate integration when estimating the power of a chosen frequency range.

For the AR method, if the re-sampling frequency is large, the model order of the AR model must also be large to examine frequency ranges of concern for HRV, resulting in a more complex and undesirable model. The shape of the PSD, as far as the number of peaks is the same no matter the re-sampling frequency. But what does change is the actual frequencies at which these peaks occur. With a low re-sampling frequency, the first peak on the PSD may occur at 0.1 Hz and the last peak may occur at 0.3 Hz (both within ranges of concern for HRV), but with a

high re-sampling frequency, the first peak on the PSD may occur at 10 Hz and the last peak may occur at 30 Hz (not within ranges of concern for HRV). The conclusion is that, to avoid having a large AR model order, it is desirable to have a small re-sampling frequency. Some common re-sampling frequencies are 2-5 Hz [8]. All of those re-frequencies satisfy the constraints discussed above.

2.2.3.2. Interpolated Tachogram with Zero Mean

Before the PSD of the interpolated tachogram is determined by the FFT or AR modeling method, the mean of the interpolated tachogram is typically subtracted from each data point on the interpolated tachogram, creating an interpolated tachogram with zero mean. If the mean from the interpolated tachogram is not removed, there will be a very large signal power at 0 Hz. If the total power of the interpolated tachogram were to be determined (by integrating the entire frequency range from 0 Hz to the one-half of the re-sampling frequency), the integration would involve the very large value at 0 Hz. There is no interest in determining the signal power contribution at 0 Hz, just the signal power associated with nonzero frequencies, hence the term heart rate variability.

2.3. Correction for Mean Heart Rate (HR)

The non-linear relationship between NN intervals and heart rate (HR) was pointed out by [3], but ignored by many if not most HRV researchers. Equation 1 describes the relationship between an NN interval and heart rate. This relationship is also shown in Figure 1.

$$NN\ Inteval\ (ms) = \frac{60\ bpm}{HR\ (bpm)} * 1000\ ms. \quad (1)$$

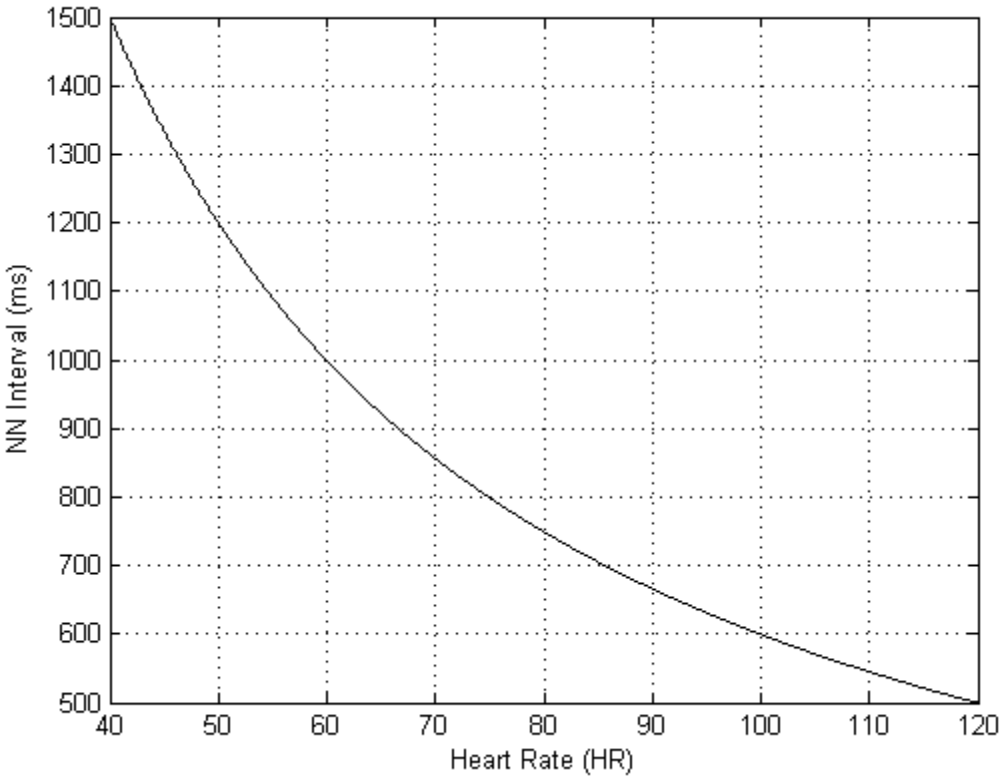

Figure 1. Relationship Between the NN Interval and Heart Rate. The figure shows how an NN interval is inversely proportional to the HR. At low HRs, the NN intervals are long; at high HRs, the NN intervals are short. The figure is a re-creation of a figure found in [3].

At low HRs, a small fluctuation in HR creates a large change in NN interval. At high HRs, the same small absolute fluctuation in HR creates a much smaller absolute change in NN interval. This suggests that the variability in NN intervals is dependent on the HR [3]. Figure 2 shows how the variability in HR creates a different variability in NN intervals, dependent on the HR.

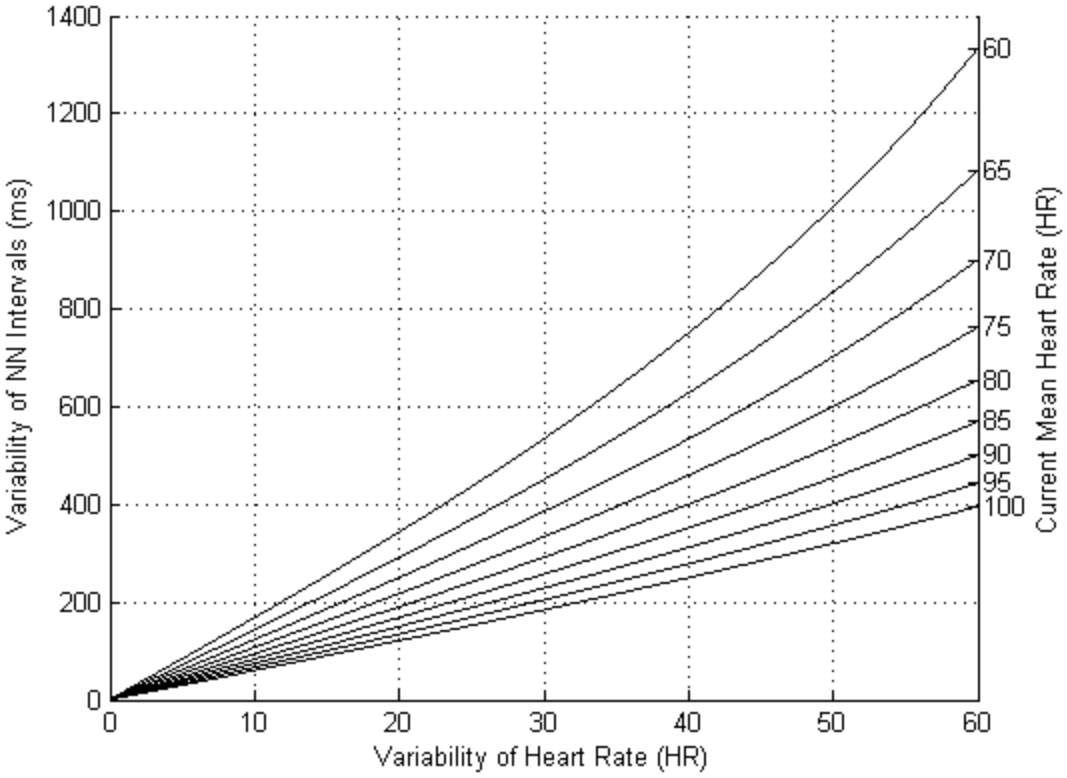

Figure 2. Relationship Between Variability in NN Intervals and Variability in Heart Rate at Specific HRs. The top line represents a HR of 60 bpm and each line below it represents a 5 bpm increase in HR up to 100 bpm. This demonstrates that the variability in HR maps to a variability in NN intervals, but the mapping depends on the current HR (i.e. for a current HR of 60 bpm, a variability in HR of 30 bpm maps to a variability in NN intervals of ~520 ms, but for a current HR of 100 bpm, a variability in HR of 30 bpm maps to a variability in NN intervals of ~190 ms). The figure is a re-creation of a figure found in [3].

Dividing all NN intervals by the average NN and the corresponding HRs by the average HR, creates a linear relationship between the variability in HR and the variability in NN [3]. Because of the division, the remaining values are unitless. The relationship is shown in Figure 3.

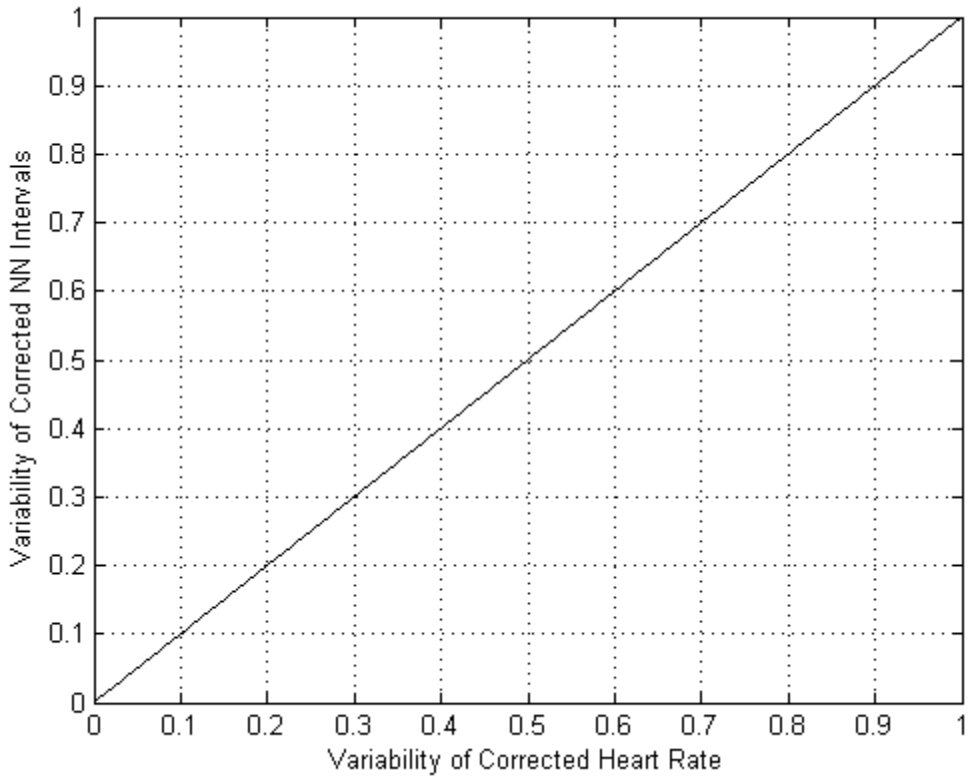

Figure 3. Relationship Between Variability in NN Intervals and Variability in HR after Correction for Mean HR. This demonstrates that after a correction for mean HR, a variability in HR maps to variability in NN intervals, but the variability in NN intervals is no longer dependent on the current HR. The figure is a re-creation of a figure found in [3].

Dividing the tachogram by the average NN interval creates a linear relationship between the variability in HR and the variability in NN interval for all HRs [3]. Because of the mathematical properties of the correction, the result of this correction amplifies HRV for tachograms with an average NN interval less than 1000 ms (HR of 60 bpm), and suppresses HRV for tachograms with an average NN interval greater than 1000 ms.

3. METHODS

The goal of this thesis was to determine the how a placebo, caffeine, and preworkout supplement affected SNS activity as determined by HRV analysis. The effects were tested during rest prior exercise and after the conclusion of an exercise bout. The study was completed with IRB approval from NDSU (Protocol #HE15244).

The group of subjects used in this study all fit a specific criteria as discussed below. Each subject came in 4 separate sessions (a familiarity session and 3 trial sessions), each separated by a minimum of 48 hours.

The familiarity session consisted of each subject completing a questionnaire to confirm that they qualify for the study, filling out an informed consent form, and performing an isometric muscle contraction for both elbow flexion and extension on a Biodex to determine the peak maximal force isometric muscle force for both elbow flexion and extension. This peak maximal force was then used to determine the load for the exercise protocol described below.

Each trial session involved the consumption of either a placebo, caffeine, or preworkout supplement followed by an exercise protocol. Each subject completed a trial with each of the three supplements. The study was double blinded (neither the researcher nor the subject knew the content of the supplement consumed, which was randomly coded for later decoding and data interpretation) so that there was no psychological influence on the subject during each trial. It was during the trial sessions that ECG recording were completed for HRV analysis at the specific times discussed below. Each trial started at a scheduled time between 6:00 and 9:30 AM and the subjects were told to not eat the morning prior to each trial.

3.1. Preworkout Procedure

One of the important aspects of the experiment was the selection criteria for the subjects. Also, it was necessary to determine what would be used as well as the doses for the placebo, caffeine, and preworkout supplements. Once the experiment was completed, which also included numerous ECG recordings, methods of HRV analysis had to be determined.

3.1.1. Subject Selection

The 12 subjects selected for this study were all males aged 18 to 35 years. Each subject also must have reported that they participated in upper and lower body resistance exercise 2-3 days per week for the past 6 months. It was also required that each subject reported that they were a habitual caffeine user, which was defined as consuming 100-500 mg of caffeine daily. A subject was excluded if they:

- Were a current smoker.
- Were currently taking any prescription medications that interact with caffeine.
- Were currently taking anabolic steroids.
- Have had any current or previous cardiovascular, musculoskeletal, or neurological medical problems.
- Were known to have had allergic reactions to drugs, chemicals, or food ingredients including milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, and soybeans.
- Had consumed other dietary supplements (other than vitamins) within the past 30 days.

3.1.2. Supplement

The study consisted of three supplements: a placebo, caffeine, and preworkout. The placebo supplement consisted of a corn starch mix with a fruit punch flavored, 5 calorie sweetened drink (Crystal Light, Northfield, IL). The caffeine supplement consisted of a commercially available caffeine pill, ground, using a mortar and pestle in 350 mg doses. It was then mixed with the same fruit punch flavored drink as the fruit punch flavored drink as was the placebo supplement. The preworkout supplement used in this study was the Arnold "pre-workout" powder (MusclePharm). A 1 scoop (6 gram) dose of the supplement was used for the preworkout supplement in this study. The label on the back of this particular preworkout supplement is shown in Figure 4.

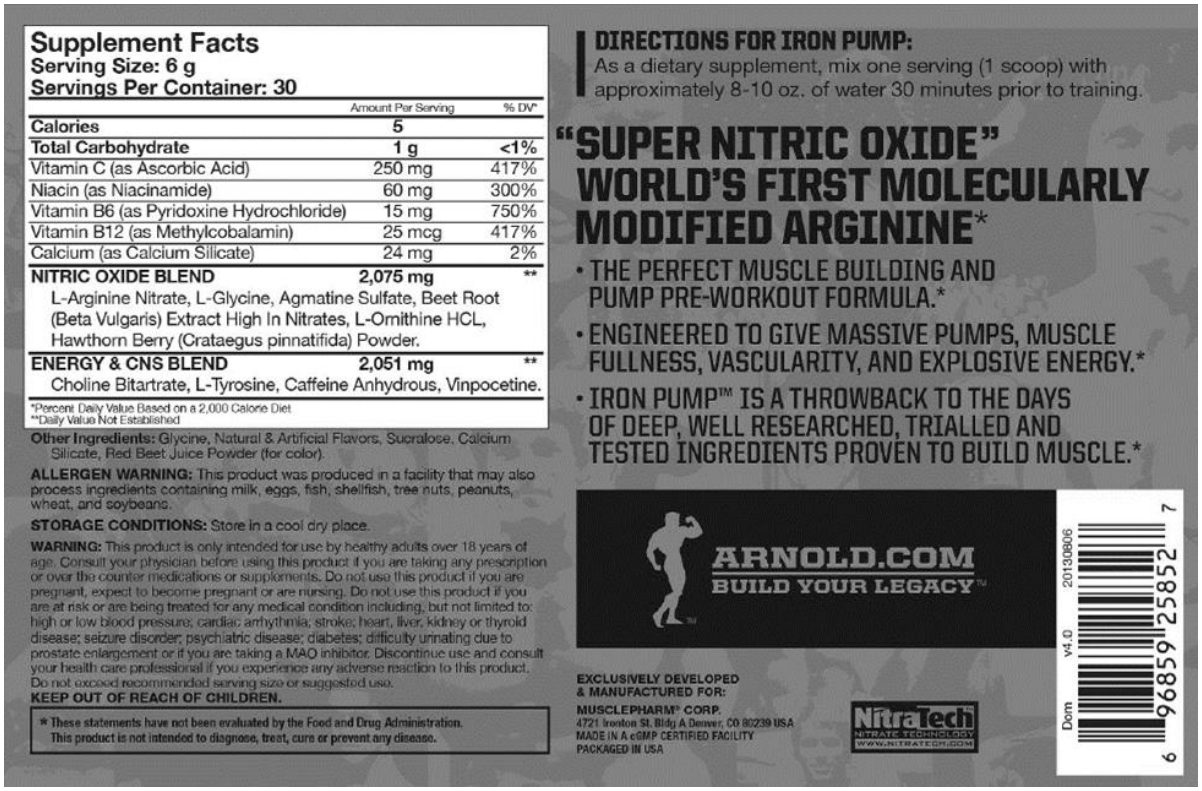

Figure 4. Label for Arnold "Pre-Workout" Supplement (MusclePharm), Which was Used as the Preworkout Supplement for this Study. The label includes details about the ingredients that are claimed to be in the supplement.

3.1.3. Procedure

Before the experiment started, each subject was prepped with the placement of 10 ECG electrodes to allow for a 12 lead ECG recording. Once prepped, each subject was told to sit upright in a chair and relax. ECG "monitoring" was started, and the experiment was postponed until the subject's heart rate seemed to reach a baseline. Once this occurred, the procedure shown in Figure 5 was followed. Each ECG recording measurement was 5 minutes long, which is the typical length of time for a short-term HRV analysis [1].

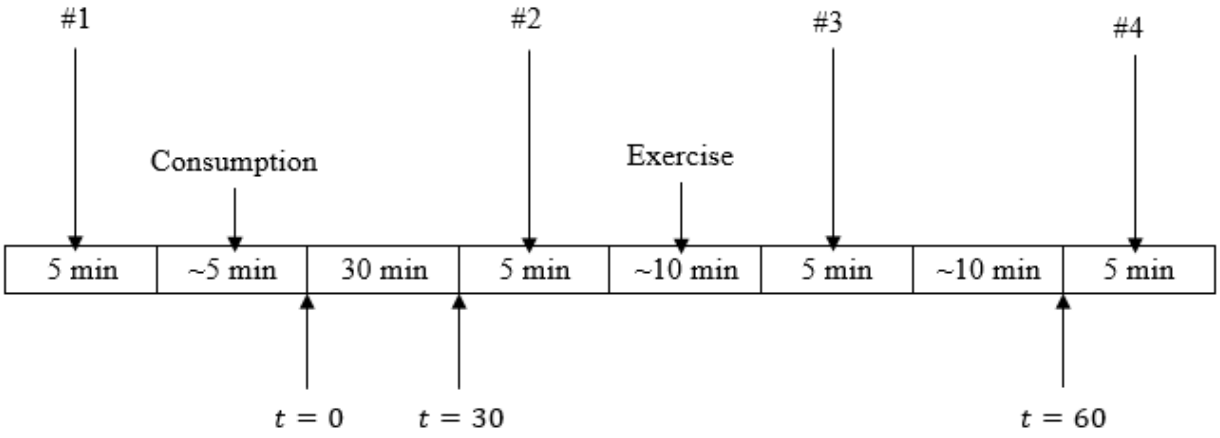

Figure 5. Experimental Procedure. The numbers at the top of the figure represent the 5 minute ECG measurements that were taken. Measurement #1 is taken prior to supplement consumption. Measurement #2 is taken 30 minutes post supplement consumption. Measurement #3 is taken immediately post exercise. Measurement # 4 is taken 60 minutes post supplement consumption. Each block on the timeline refers to the length of time that passed during each portion of the procedure.

3.1.3.1. Exercise Protocol

The exercise protocol consisted of 5 sets of 10 reps of elbow flexion and extension on the Biodex. There was a one minute recovery period between each of the 5 sets. The force load was set at 50% of the peak maximal isometric force for both elbow flexion and extension that was determined during the familiarity session.

3.2. Heart Rate Variability (HRV) Measures

The ECG recordings were performed using an PC-ECG 1200S (Norav) with a sampling frequency of 500 Hz. There were three built-in filters that were used during the recording (low pass filter for high frequency noise, 60 Hz notch filter, and a filter for ridding the signal of noise from muscle contractions). The data files were extracted from the machine for HRV analysis. HRV analysis consisted of creating a tachogram and performing time and frequency domain analysis on the tachogram. Because performing these tasks consists of many complex components, an interactive software package was created in MATLAB to calculate all measures easily and efficiently. Because the software package uses many built-in MATLAB functions, it was necessary to validate the performance of the built-in MATLAB functions. Validation was completed by simulating a tachogram and determining HRV from the tachogram using the MATLAB functions and comparing the results to HRV as determined by hand calculations. The validation is shown in Appendix D.

3.2.1. Tachogram (NN Intervals vs Time)

To perform time and frequency domain analysis on an ECG recording, the tachogram must be determined. There are a few steps that must be performed to determine the tachogram including R wave peak and RR interval detection as well as ectopic beat and RR interval rejection, and plotting of the normal RR intervals (NN intervals) vs time. If the MATLAB software package either did not detect an R wave or RR interval or detected an R wave or RR interval that was not in fact an R wave or RR interval, the software package offered a graphical user interface (GUI) feature so that these things could be interactively modified. A screenshot of the GUI is shown in Figure 6.

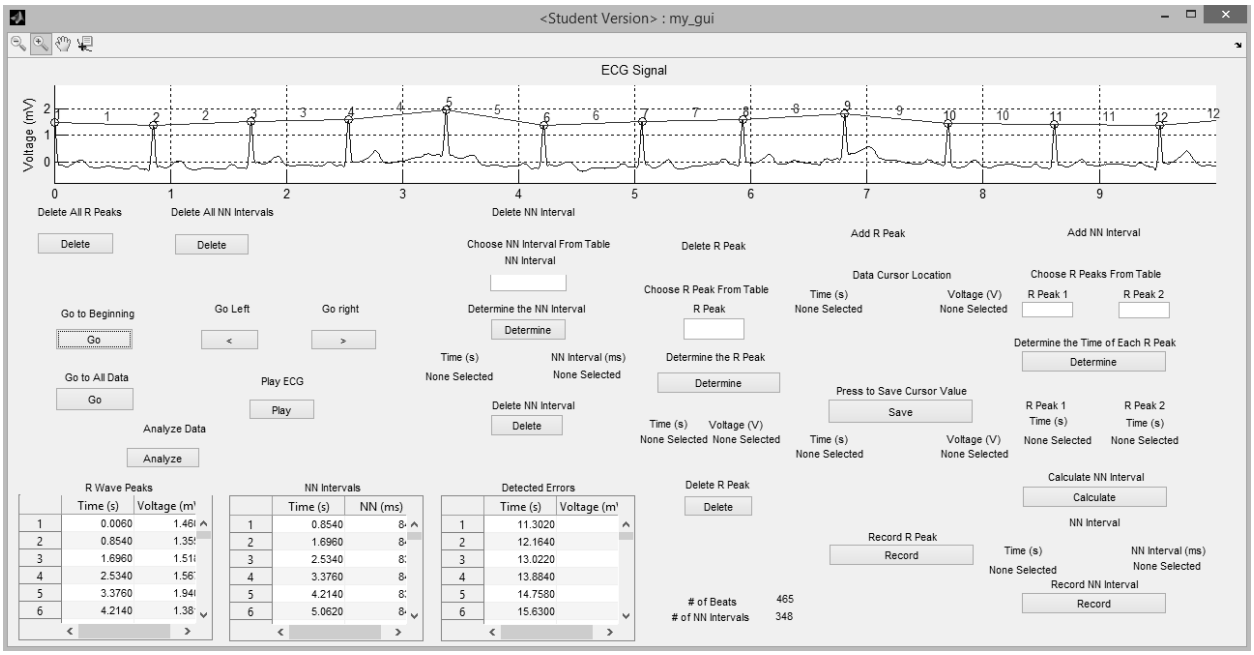

Figure 6. Graphical User Interface (GUI) Within MATLAB-Created HRV Software Package. The GUI consists of a plot showing the ECG, detected R wave peaks with numbers, detected NN intervals with numbers, and controls for interactively editing the data. The GUI offers controls for adding R wave peaks and NN intervals as well as offers controls for deleting previously detected R wave peaks and NN intervals.

3.2.1.1. R Wave Peak Detection and Rejection of Ectopic/Missing Heart Beats

The RR intervals were determined by calculating the amount of time between R wave peaks. After visual inspection and editing of the R wave peaks and RR intervals, the remaining normal RR intervals (NN intervals) were kept. The ECG, detected R wave peaks, and NN intervals were plotted. If any errors were detected, they were interactively changed. Once everything seemed correct, the tachogram could be used for HRV analysis.

An example of a tachogram from a 5 minute ECG recording taken during this study is shown in Figure 7.

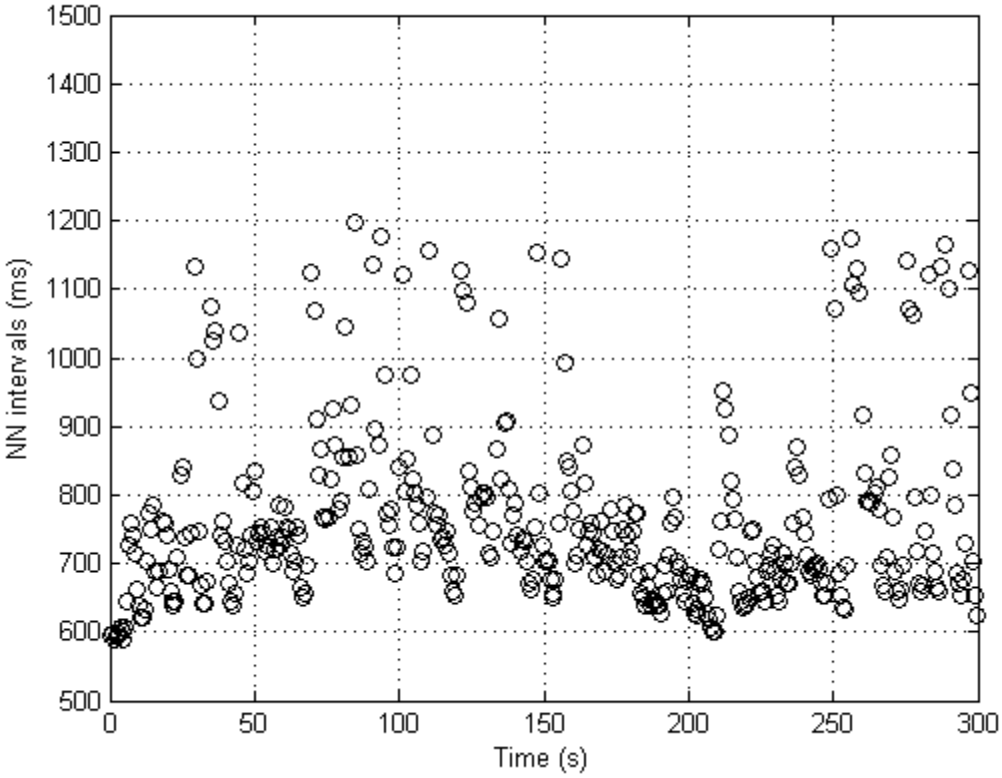

Figure 7. Sample Tachogram (NN intervals vs Time). The sample tachogram was from a 5 minute ECG recording completed during the study.

3.2.2. Time Domain Analysis

The time domain indices in Table 1 were determined directly or indirectly from the tachogram. The time domain indices determined indirectly from the tachogram include those that use information about the ΔNN intervals. Therefore, the ΔNN intervals of the tachogram were plotted vs time. An example of the ΔNN intervals vs time from a 5 minute ECG recording taken during this study is shown in Figure 8.

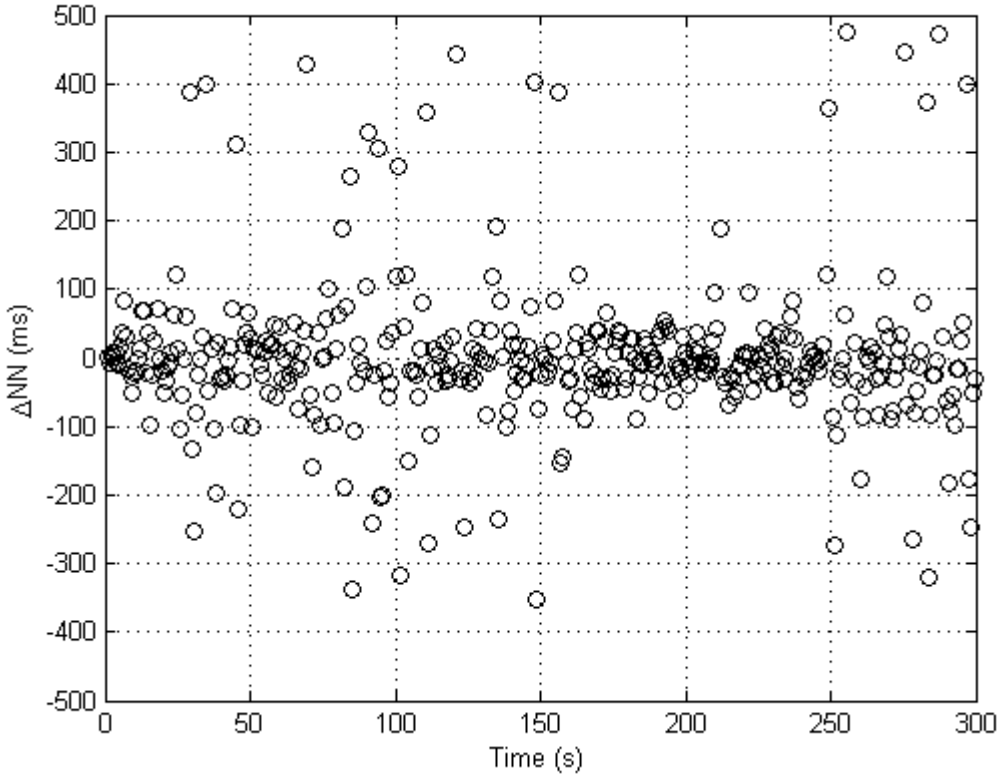

Figure 8. Sample ΔNN Intervals vs Time. The sample ΔNN intervals vs time was from a 5 minute ECG recording completed during the study.

3.2.3. Frequency Domain Analysis

Frequency domain analysis was calculated by first creating a cubic spline of the tachogram, re-sampling it, determining the PSD by FFT and AR modeling. The frequency domain indices from Table 2 were then determined from the two PSD plots.

3.2.3.1. Interpolated Tachogram (Cubic Spline)

A cubic spline was determined using the "not-a-knot" conditions. Appendix A discusses the details on how to calculate a cubic spline using such conditions. Once the cubic spline was determined, the interpolated tachogram was created by re-sampling the cubic spline at a frequency of 2 Hz (frequency is consistent with [2]). Finally, the mean of the interpolated

tachogram was subtracted from the interpolated tachogram to rid the signal power contribution from the 0 Hz frequency.

3.2.3.2. Non-Parametric Method: Fast Fourier Transform (FFT)

Once the cubic spline was re-sampled for each tachogram, a fast Fourier transform (FFT) was performed on the interpolated tachogram and modified to determine the power spectral density (PSD). Appendix B discusses the details on how to calculate a DFT and how to modify the DFT to determine the PSD. Each interpolated tachogram was zero padded to increase the frequency resolution (decrease Δf) to force Δf to be a common denominator of every endpoint of each frequency range of interest in frequency domain analysis (0, 0.04, 0.15, and 0.40 Hz), which results in no overlap or space between the bounds of integration for each respective frequency range of interest. A frequency spacing of $\Delta f = 1 * 10^{-5}\ Hz$ was used, so the interpolated tachogram was zero padded to $N = \frac{f_s}{\Delta f} = \frac{(2\ Hz)}{(1*10^{-5}\ Hz)} = 200{,}000\ points$. The PSD was then integrated using trapezoidal integration. The PSD is shown in Figure 9.

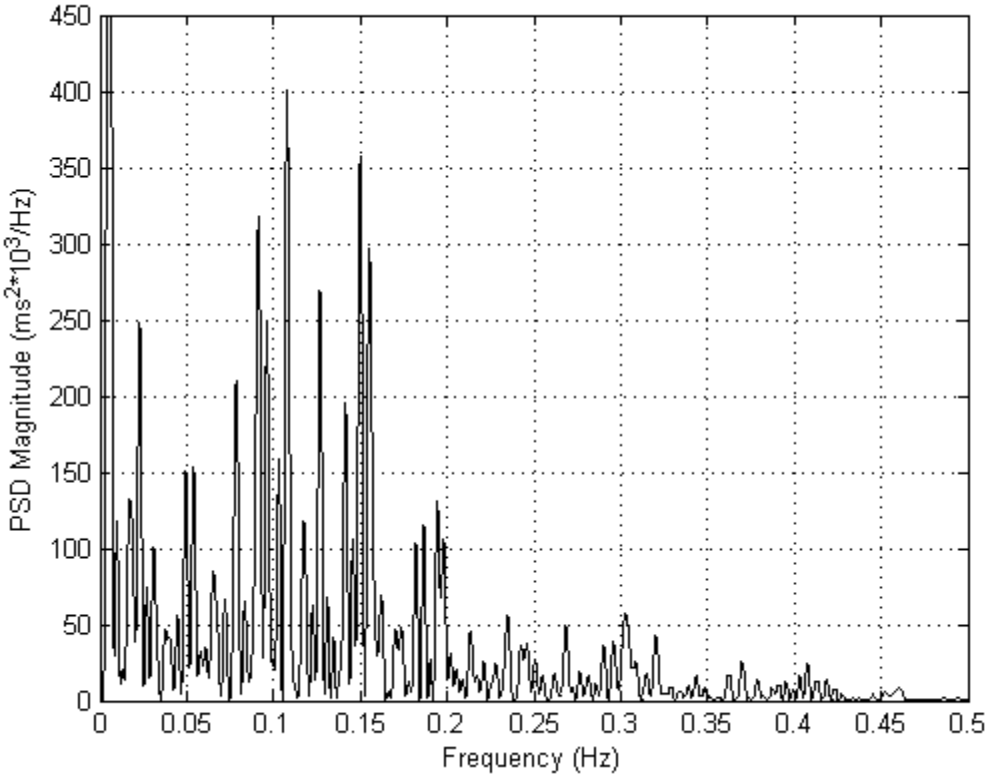

Figure 9. Sample Power Spectral Density (PSD) Using the Fast Fourier Transform (FFT) Method. The sample PSD was determined from a 5 minute ECG recording completed during this study.

3.2.3.3. Parametric Method: Autoregressive (AR) Modeling

Once the cubic spline was re-sampled for each tachogram, an autoregressive (AR) model was created for the interpolated tachogram and modified to determine the power spectral density (PSD). The AR model coefficients were determined by the Levinson-Durbin recursion algorithm. Appendix B discusses the details on how to create an AR model using the Levinson-Durbin recursion algorithm, and how to modify the AR model to determine the PSD. A 16th order AR model is sufficient for an interpolated tachogram, with a re-sampling frequency of 2 Hz, for a five minute ECG recording. The model order and re-sampling frequency were determined by investigation of previous HRV research [2]. The PSD amplitude was determined at points with a frequency spacing equal to $\Delta f = 1 * 10^{-5}\ Hz$, consistent with the spacing

created by the FFT method, and then integrated using trapezoidal integration. The PSD is shown in Figure 10.

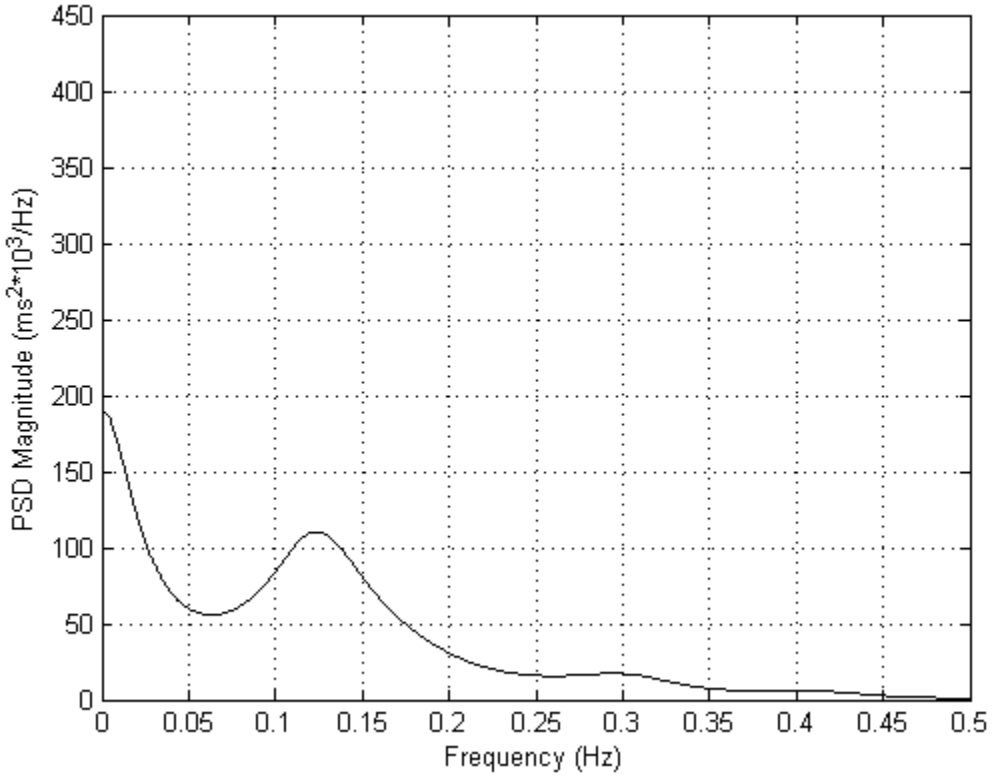

Figure 10. Sample Power Spectral Density (PSD) Using the Autoregressive (AR) Modeling Method. The sample PSD was determined from a 5 minute ECG recording completed during this study.

3.2.4. Correction for Mean Heart Rate (HR)

Because there is a non-linear relationship between NN intervals and the average heart rate, traditional HRV analysis of the interpolated tachogram was performed alongside HRV analysis of the interpolated tachogram that was corrected for average HR. The interpolated tachogram that was corrected for average HR is shown in Figure 11.

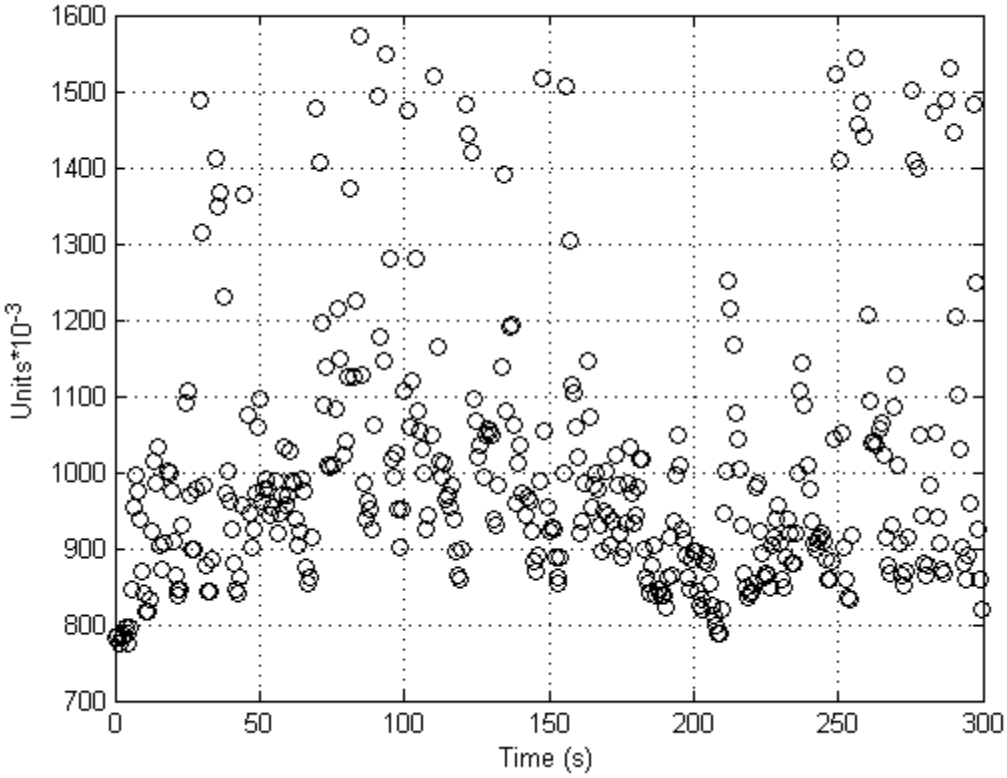

Figure 11. Sample Tachogram (NN Intervals vs Time) after Correction for Mean Heart Rate. The figure shows a tachogram after dividing all of the values in the tachogram by the mean NN interval.

3.3. Statistics

A 3x4 (supplement by time) analysis of variance (ANOVA) with repeated measures (time and supplement). The significance level was set at $p<0.05$. If there was a violation of Mauchly's Test of Sphericity, the Greenhouse-Geisser test was used to adjust p values as a result of the violation [13], [14], [15], [16]. When a supplement*time interaction was found, individual ANOVAs with repeated measures were performed at each time point (baseline, 30 minutes post consumption, post exercise, 60 minutes post consumption) to determine at which time point the interaction occurred. If a main effect was found, Bonferroni corrections were used to avoid type I error.

4. RESULTS

There were a few statistically significant supplement*time interaction effects found for the frequency domain indices determined from the original tachogram. Those were FFT LF n.u. (p=0.023), FFT HF n.u. (p=0.048), AR LF n.u. (p=0.022), and AR HF n.u. (p=0.037). There were also a few statistically significant supplement*time interaction effects found for the frequency domain indices determined from the original tachogram after correction for mean HR. Those were FFT LF n.u. (p=0.023), FFT HF n.u. (p=0.048), AR LF n.u. (p=0.022), and AR HF n.u. (p=0.037). The reason there were statistically significant supplement*time interaction effects for the same frequency domain indices in those determined by the original tachogram and those from the original tachogram after correction for mean HR is because the correction for mean HR does not affect these indices because they are ratios of the power present, not indicators of magnitudes of power. Because of this fact, only the statistically significant supplement*time interaction effects for the frequency domain indices as determined by the original tachogram was discussed to avoid redundancy. There were also numerous statistically significant time effects found for the frequency domain indices as determined from the original tachogram and frequency domain indices as determined from the original tachogram after correction for mean HR, however they were not investigated further.

The statistically significant supplement*time interaction effects found for the frequency domain indices determined from the original tachogram occurred immediately post exercise for the FFT LF n.u., FFT HF n.u., AR LF n.u., and AR HF n.u.. The information showing the statistically significant supplement*time effects for the FFT LF n.u., FFT HF n.u., AR LF n.u., and AR HF n.u. indices can be seen in Figure 12, Figure 13, Figure 14, and Figure 15 respectively.

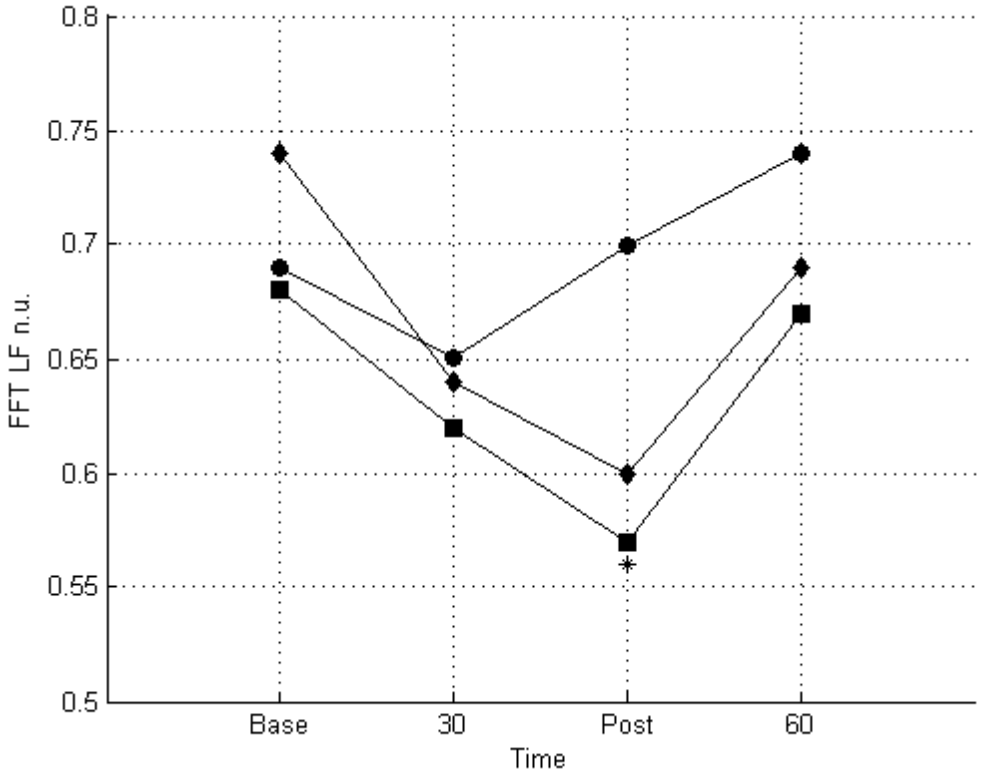

Figure 12. Fast Fourier Transform (FFT) Low Frequency (LF) Normalized Units (n.u.) before Correction for Mean Heart Rate. The figure shows the means of the placebo trial (closed circles), caffeine trial (closed squares), and preworkout trial (closed diamonds) at the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60). There was a statistically significant difference between the caffeine and placebo trials immediately post exercise ($p=0.005$), which is represented by the star. Although there was not a statistically significant difference between the preworkout and placebo trials, there was however a trend ($p=0.099$).

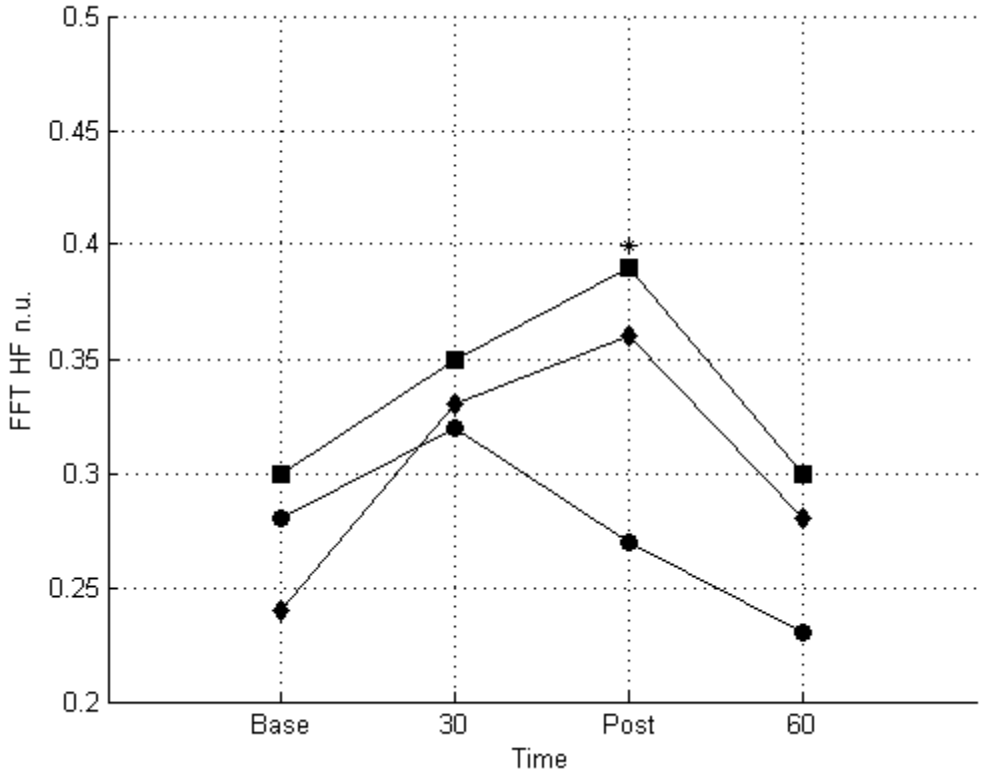

Figure 13. Fast Fourier Transform (FFT) High Frequency (HF) Normalized Units (n.u.) before Correction for Mean Heart Rate. The figure shows the means of the placebo trial (closed circles), caffeine trial (closed squares), and preworkout trial (closed diamonds) at the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60). There was a statistically significant difference between the caffeine and placebo trials immediately post exercise (p=0.010), which is represented by the star. Although there was not a statistically significant difference between the preworkout and placebo trials, there was however a trend (p=0.139).

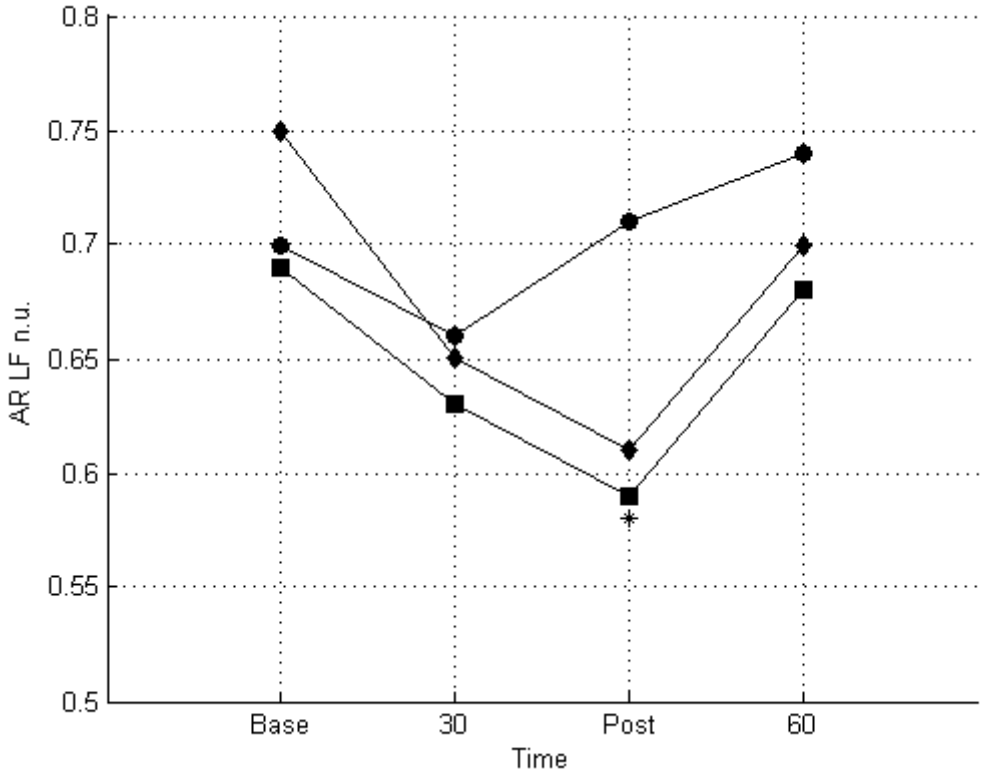

Figure 14. Autoregressive (AR) Low Frequency (LF) Normalized Units (n.u.) before Correction for Mean Heart Rate. The figure shows the means of the placebo trial (closed circles), caffeine trial (closed squares), and preworkout trial (closed diamonds) at the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60). There was a statistically significant difference between the caffeine and placebo trials immediately post exercise ($p=0.008$), which is represented by the star. Although there was not a statistically significant difference between the preworkout and placebo trials, there was however a trend ($p=0.092$).

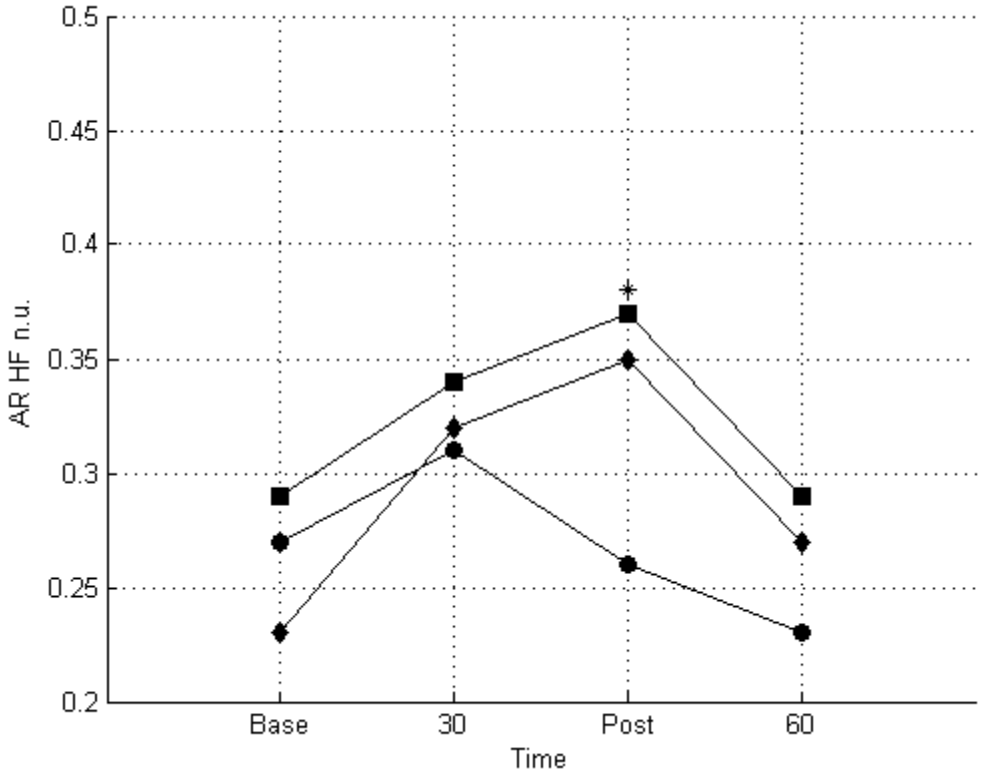

Figure 15. Autoregressive (AR) High Frequency (HF) Normalized Units (n.u.) before Correction for Mean Heart Rate. The figure shows the means of the placebo trial (closed circles), caffeine trial (closed squares), and preworkout trial (closed diamonds) at the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60). There was a statistically significant difference between the caffeine and placebo trials immediately post exercise ($p=0.014$), which is represented by the star. Although there was not a statistically significant difference between the preworkout and placebo trials, there was however a trend ($p=0.121$).

4.1. Summary of Statistics for Beats, NN, and ΔNN

A summary of the basic statistics for the # of beats, NN intervals, and ΔNN intervals used for HRV analysis of the 5 minute ECG recordings for the placebo, caffeine, and preworkout supplement trials is shown in Table 3, Table 4, and Table 5 respectively.

Table 3. Placebo Trial: Summary of the Basic Statistics for # of Beats, NN Intervals, and ΔNN Intervals Used for HRV Analysis. The table shows the values for the # of beats, # of NN intervals, and the # of ΔNN intervals used for short term HRV analysis during the placebo trial.

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
# of Beats	#	346.8	40.0	338.7	41.3	363.2	50.6	361.3	44.6
# of NN	#	345.4	40.1	336.3	42.1	361.3	51.1	359.1	44.7
# of ΔNN	#	344.1	40.2	334.1	43.2	359.7	51.6	357.3	44.9

Table 4. Caffeine Trial: Summary of the Basic Statistics for # of Beats, NN Intervals, and ΔNN Intervals Used for HRV Analysis. The table shows the values for the # of beats, # of NN intervals, and the # of ΔNN intervals used for short term HRV analysis during the caffeine trial.

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
# of Beats	#	332.3	34.3	319.4	40.6	359.0	55.1	352.2	38.1
# of NN	#	330.2	33.8	317.2	40.7	357.5	55.4	350.9	38.2
# of ΔNN	#	328.5	33.6	315.3	40.7	356.2	55.4	349.8	38.2

Table 5. Preworkout Trial: Summary of the Basic Statistics for # of Beats, NN Intervals, and ΔNN Intervals Used for HRV Analysis. The table shows the values for the # of beats, # of NN intervals, and the # of ΔNN intervals used for short term HRV analysis during the preworkout trial.

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
# of Beats	#	342.3	51.9	326.8	43.3	359.8	54.2	356.1	44.2
# of NN	#	341.3	51.9	325.3	43.6	358.2	54.2	354.4	44.4
# of ΔNN	#	340.3	51.9	324.0	43.8	356.6	54.1	353.0	44.5

4.2. Time Domain Indices

The time domain indices consisted of those determined from the original tachogram and those determined from the original tachogram after being corrected for mean HR. There were no statistically significant supplement*time interaction effects found for any of the time domain indices determined from the original tachogram or the time domain indices determined from the

original tachogram after correction for mean HR. There were numerous statistically significant time effects found for the time domain indices as determined from the original tachogram and time domain indices as determined from the original tachogram after correction for mean HR, however they were not investigated further.

4.2.1. Time Domain Indices from Original Tachogram

A summary of the time domain indices as determined from the original tachogram for the placebo, caffeine, and preworkout supplements are shown in Table 6, Table 7, and Table 8 respectively.

Table 6. Placebo Trial Time Domain Indices as Determined from the Original Tachogram. The table shows the mean and standard deviation for each time domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Min NN	ms	684.8	79.0	670.7	59.0	642.5	60.3	653.8	54.0
Max NN	ms	1177.5	112.4	1224.5	163.2	1090.8	141.5	1129.5	131.9
Mean NN	ms	874.5	98.2	892.8	105.3	837.8	108.7	840.2	102.7
SDNN	ms	89.5	27.9	94.9	27.0	84.8	26.7	81.1	18.6
RMSSD	ms	55.8	30.4	60.8	27.2	49.6	21.8	46.4	19.4
ln(RMSSD)	-	3.91	0.48	4.03	0.42	3.81	0.45	3.76	0.39
pNN50	%	23.3	15.6	27.3	14.1	23.9	16.7	19.4	13.6
pNN40	%	31.0	15.9	35.4	14.6	31.3	18.2	27.0	14.2
pNN30	%	40.9	15.4	46.2	14.2	41.3	18.6	38.1	14.8
pNN20	%	55.7	12.8	60.1	12.6	55.5	16.5	52.8	13.5
pNN10	%	74.2	8.6	77.3	7.5	74.2	10.6	73.8	8.9
Mean ΔNN	ms	-0.08	0.43	-0.14	0.65	-0.09	0.49	0.01	0.53
SD ΔNN	ms	55.8	30.4	60.8	27.2	49.6	21.8	46.4	19.4
Triangular Index	-	16.8	3.7	17.8	3.4	18.5	5.6	16.1	4.4

Table 7. Caffeine Trial Time Domain Indices as Determined from the Original Tachogram. The table shows the mean and standard deviation for each time domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Min NN	ms	692.7	69.1	663.7	88.8	652.5	101.3	657.7	62.0
Max NN	ms	1238.7	152.1	1272.8	159.1	1137.5	141.2	1133.7	177.8
Mean NN	ms	911.1	92.5	950.0	111.6	853.1	128.6	860.2	89.6
SDNN	ms	104.9	30.2	114.3	33.6	92.5	28.9	89.6	24.3
RMSSD	ms	66.5	37.7	76.9	33.8	62.0	30.4	55.7	30.3
ln(RMSSD)	-	4.07	0.53	4.26	0.43	4.03	0.47	3.90	0.50
pNN50	%	29.0	17.0	38.9	14.6	31.5	17.8	25.3	16.1
pNN40	%	36.9	17.0	47.7	14.4	39.2	19.0	33.7	17.8
pNN30	%	47.4	15.9	58.9	12.7	49.5	18.6	44.2	18.1
pNN20	%	61.6	12.7	71.4	10.4	63.2	16.6	58.8	16.0
pNN10	%	77.8	8.2	84.0	6.1	78.7	10.8	76.9	9.8
Mean ΔNN	ms	-0.09	0.71	-0.03	0.79	-0.07	0.65	-0.05	0.48
SD ΔNN	ms	66.5	37.7	76.9	33.8	62.0	30.4	55.7	30.3
Triangular Index	-	19.2	3.5	19.3	4.1	20.3	5.8	18.0	4.6

Table 8. Preworkout Trial Time Domain Indices as Determined from the Original Tachogram. The table shows the mean and standard deviation for each time domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Min NN	ms	676.0	92.3	692.5	91.2	645.5	80.3	652.5	76.2
Max NN	ms	1147.3	170.7	1211.2	144.0	1140.2	156.8	1109.5	160.2
Mean NN	ms	897.5	148.4	929.8	117.0	847.1	123.9	852.9	99.9
SDNN	ms	92.0	22.2	97.9	28.6	88.2	16.5	86.2	28.2
RMSSD	ms	57.1	29.9	72.1	34.5	59.6	26.8	54.8	32.0
ln(RMSSD)	-	3.91	0.57	4.18	0.44	3.98	0.50	3.87	0.53
pNN50	%	26.5	17.2	35.6	16.1	28.9	19.4	23.2	16.9
pNN40	%	33.8	18.3	43.8	15.0	37.3	20.0	31.1	17.6
pNN30	%	45.2	19.9	55.6	12.3	47.7	20.1	42.5	17.7
pNN20	%	57.4	19.8	67.8	8.8	59.8	17.6	56.4	16.2
pNN10	%	74.9	15.3	83.3	5.6	76.8	11.5	74.6	11.5
Mean ΔNN	ms	0.14	0.40	0.26	0.37	0.24	0.44	-0.02	0.64
SD ΔNN	ms	57.1	29.9	72.1	34.5	59.6	26.8	54.8	32.0
Triangular Index	-	18.6	4.7	17.8	3.9	18.2	3.9	16.4	3.8

4.2.2. Time Domain Indices from Original Tachogram after Correction for Mean HR

A summary of the time domain indices as determined from the original tachogram after correction for mean HR for the placebo, caffeine, and preworkout supplements are shown in Table 9, Table 10, and Table 11 respectively.

Table 9. Placebo Trial Time Domain Indices as Determined from the Original Tachogram after Correction for Mean HR. The table shows the mean and standard deviation for each time domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Min NN	10^{-3}	783.2	26.1	755.8	64.0	772.1	59.6	782.7	51.3
Max NN	10^{-3}	1357.0	166.4	1382.2	205.2	1304.5	82.3	1349.8	124.5
Mean NN	10^{-3}	1000.0	0.0	1000.0	0.0	1000.0	0.0	1000.0	0.0
SDNN	10^{-3}	103.5	37.4	106.7	30.0	100.2	24.1	96.0	14.3
RMSSD	10^{-3}	64.9	40.5	68.8	34.6	57.9	20.7	54.7	20.1
ln(RMSSD)	-	4.05	0.49	4.15	0.41	4.00	0.37	3.95	0.35
pNN50	%	28.2	15.0	32.6	13.0	31.0	15.0	26.4	12.4
pNN40	%	36.6	14.4	40.9	12.7	38.7	14.9	34.8	12.8
pNN30	%	48.6	12.8	52.4	11.1	50.4	13.4	46.6	12.1
pNN20	%	61.6	10.2	65.9	9.4	64.2	11.8	61.1	10.6
pNN10	%	79.9	6.3	82.0	5.4	80.8	7.6	79.7	6.4
Mean ΔNN	10^{-3}	-0.07	0.43	-0.14	0.72	-0.11	0.55	0.02	0.60
SD ΔNN	10^{-3}	64.9	40.5	68.8	34.6	57.9	20.7	54.7	20.1
Triangular Index	-	18.6	3.7	19.4	4.3	21.7	6.5	17.4	3.3

Table 10. Caffeine Trial Time Domain Indices as Determined from the Original Tachogram after Correction for Mean HR. The table shows the mean and standard deviation for each time domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Min NN	10^{-3}	762.5	58.9	703.9	95.4	765.3	43.3	766.2	35.8
Max NN	10^{-3}	1366.0	172.6	1345.2	142.1	1349.9	195.2	1323.4	210.4
Mean NN	10^{-3}	1000.0	0.0	1000.0	0.0	1000.0	0.0	1000.0	0.0
SDNN	10^{-3}	115.5	32.1	120.6	33.3	110.2	36.7	103.9	26.0
RMSSD	10^{-3}	73.4	44.0	81.8	38.6	72.3	31.4	64.7	34.8
ln(RMSSD)	-	4.16	0.52	4.31	0.44	4.20	0.43	4.06	0.48
pNN50	%	33.1	15.9	42.5	13.4	38.1	15.3	31.6	15.4
pNN40	%	40.9	15.6	51.7	13.2	46.9	15.0	40.2	15.7
pNN30	%	52.4	13.7	62.5	12.1	57.5	14.1	52.0	15.6
pNN20	%	65.5	11.3	73.8	9.9	70.2	11.0	66.0	12.3
pNN10	%	81.6	5.9	86.8	5.6	84.1	7.1	82.5	7.9
Mean ΔNN	10^{-3}	-0.11	0.78	-0.04	0.83	-0.06	0.74	-0.04	0.53
SD ΔNN	10^{-3}	73.4	44.0	81.8	38.6	72.3	31.4	64.7	34.8
Triangular Index	-	19.5	4.3	21.1	5.1	21.7	5.6	19.8	3.9

Table 11. Preworkout Trial Time Domain Indices as Determined from the Original Tachogram after Correction for Mean HR. The table shows the mean and standard deviation for each time domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Min NN	10^{-3}	756.9	35.7	745.4	46.7	765.3	41.7	766.1	45.4
Max NN	10^{-3}	1284.7	112.4	1310.3	138.6	1352.3	130.2	1305.4	154.3
Mean NN	10^{-3}	1000.0	0.0	1000.0	0.0	1000.0	0.0	1000.0	0.0
SDNN	10^{-3}	103.1	24.8	106.6	33.0	104.4	15.4	101.1	29.9
RMSSD	10^{-3}	62.9	32.1	78.1	37.5	69.4	28.3	63.9	35.9
ln(RMSSD)	-	4.03	0.50	4.26	0.44	4.16	0.43	4.03	0.51
pNN50	%	30.9	14.8	39.9	14.8	36.2	16.6	29.6	15.8
pNN40	%	39.3	16.0	48.7	14.0	44.3	15.9	38.0	16.1
pNN30	%	50.8	17.2	59.5	10.7	55.1	15.5	50.0	15.6
pNN20	%	64.2	16.4	71.0	7.9	68.2	11.7	63.5	14.1
pNN10	%	80.7	10.8	86.3	4.7	83.7	6.1	79.7	10.0
Mean ΔNN	10^{-3}	0.12	0.40	0.27	0.38	0.30	0.51	-0.06	0.70
SD ΔNN	10^{-3}	62.9	32.1	78.1	37.5	69.4	28.3	63.9	35.9
Triangular Index	-	19.4	3.5	19.9	5.0	20.1	3.2	18.2	3.7

4.3. Frequency Domain Indices

The frequency domain indices consisted of those determined from the original tachogram and those determined from the original tachogram after being corrected for mean HR. The statistically significant supplement*time interaction effects determined from the original tachogram and those determined from the original tachogram after correction for mean HR were discussed previously. There were numerous statistically significant time effects found for the frequency domain indices as determined from the original tachogram and frequency domain indices as determined from the original tachogram after correction for mean HR, however they were not investigated further.

4.3.1. Frequency Domain Indices from Original Tachogram

A summary of the frequency domain indices as determined from the original tachogram for the placebo, caffeine, and preworkout supplements are shown in Table 12, Table 13. And Table 14 respectively.

Table 12. Placebo Trial Frequency Domain Indices as Determined from the Original Tachogram. The table shows the mean and standard deviation for each frequency domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TP	ms^2	9307	7194	9682	5535	7752	4724	6885	2941
FFT VLF	ms^2	4799	3690	5403	3782	3996	3229	3580	1859
FFT LF	ms^2	2680	1415	2527	1149	2566	1507	2343	948
FFT HF	ms^2	1689	2698	1604	1794	1095	939	864	709
FFT LF:HF	-	3.94	3.07	2.70	1.54	4.04	4.50	4.88	4.74
FFT LF n.u.	-	0.69	0.18	0.65	0.17	0.70	0.11	0.74	0.12
FFT HF n.u.	-	0.28	0.17	0.32	0.16	0.27	0.11	0.23	0.11
AR VLF	ms^2	4729	3686	5274	3761	3960	3263	3524	1787
AR LF	ms^2	2791	1506	2666	1194	2638	1564	2396	1088
AR HF	ms^2	1645	2604	1598	1797	1060	882	866	717
AR LF:HF	-	4.10	3.25	2.87	1.71	4.09	4.13	5.28	5.83
AR LF n.u.	-	0.70	0.17	0.66	0.17	0.71	0.11	0.74	0.13
AR HF n.u.	-	0.27	0.16	0.31	0.16	0.26	0.10	0.23	0.12

Table 13. Caffeine Trial Frequency Domain Indices as Determined from the Original Tachogram. The table shows the mean and standard deviation for each frequency domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TP	ms^2	12022	7433	13750	7720	9419	6785	8688	4839
FFT VLF	ms^2	5978	4162	7477	5939	5144	4523	4469	2851
FFT LF	ms^2	3603	2470	3441	1351	2175	1090	2483	1502
FFT HF	ms^2	2304	3441	2650	2689	1951	2272	1601	2014
FFT LF:HF	-	3.03	1.98	2.23	1.36	3.06	4.72	3.90	3.97
FFT LF n.u.	-	0.68	0.15	0.62	0.15	0.57	0.18	0.67	0.18
FFT HF n.u.	-	0.30	0.15	0.35	0.15	0.39	0.17	0.30	0.17
AR VLF	ms^2	5877	4102	7351	5756	5025	4389	4396	2777
AR LF	ms^2	3739	2510	3603	1345	2323	1139	2551	1499
AR HF	ms^2	2266	3322	2612	2661	1922	2286	1606	2038
AR LF:HF	-	3.21	2.23	2.42	1.49	3.17	4.42	4.13	4.34
AR LF n.u.	-	0.69	0.15	0.63	0.16	0.59	0.18	0.68	0.18
AR HF n.u.	-	0.29	0.14	0.34	0.15	0.37	0.17	0.29	0.17

Table 14. Preworkout Trial Frequency Domain Indices as Determined from the Original Tachogram. The table shows the mean and standard deviation for each frequency domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TP	ms^2	8863	4556	10546	7010	7967	2707	8132	5803
FFT VLF	ms^2	3747	1768	4608	3155	4123	1668	3735	2325
FFT LF	ms^2	3534	2048	3510	2211	2158	1341	2772	2221
FFT HF	ms^2	1480	1655	2243	2220	1538	1315	1493	1954
FFT LF:HF	-	4.50	3.15	2.77	2.68	3.05	3.65	3.77	3.57
FFT LF n.u.	-	0.74	0.13	0.64	0.13	0.60	0.21	0.69	0.14
FFT HF n.u.	-	0.24	0.13	0.33	0.12	0.36	0.20	0.28	0.13
AR VLF	ms^2	3707	1743	4552	3155	4076	1567	3660	2246
AR LF	ms^2	3622	2094	3584	2222	2216	1285	2859	2286
AR HF	ms^2	1433	1589	2225	2239	1522	1296	1480	1940
AR LF:HF	-	4.86	3.40	2.84	2.56	3.18	3.88	3.90	3.56
AR LF n.u.	-	0.75	0.14	0.65	0.13	0.61	0.20	0.70	0.14
AR HF n.u.	-	0.23	0.14	0.32	0.12	0.35	0.19	0.27	0.13

4.3.2. Frequency Domain Indices from Original Tachogram after Correction for Mean HR

A summary of the time domain indices as determined from the original tachogram after correction for mean HR for the placebo, caffeine, and preworkout supplements are shown in Table 15, Table 16, Table 17 respectively.

Table 15. Placebo Trial Frequency Domain Indices as Determined from the Original Tachogram after Correction for Mean HR. The table shows the mean and standard deviation for each frequency domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TP	10^{-6}	13106	13825	12460	8451	10515	4781	9519	2819
FFT VLF	10^{-6}	6491	6161	6684	4240	5458	3765	4873	1901
FFT LF	10^{-6}	3781	2705	3373	1983	3461	1555	3313	1065
FFT HF	10^{-6}	2619	5165	2189	3071	1461	1093	1189	969
FFT LF:HF	-	3.94	3.07	2.70	1.54	4.04	4.50	4.88	4.74
FFT LF n.u.	-	0.69	0.18	0.65	0.17	0.70	0.11	0.74	0.12
FFT HF n.u.	-	0.28	0.17	0.32	0.16	0.27	0.11	0.23	0.11
AR VLF	10^{-6}	6398	6155	6537	4213	5404	3775	4807	1820
AR LF	10^{-6}	3941	2893	3528	2028	3561	1636	3370	1159
AR HF	10^{-6}	2547	4983	2186	3080	1416	1031	1197	1001
AR LF:HF	-	4.10	3.25	2.87	1.71	4.09	4.13	5.28	5.83
AR LF n.u.	-	0.70	0.17	0.66	0.17	0.71	0.11	0.74	0.13
AR HF n.u.	-	0.27	0.16	0.31	0.16	0.26	0.10	0.23	0.12

Table 16. Caffeine Trial Frequency Domain Indices as Determined from the Original Tachogram after Correction for Mean HR. The table shows the mean and standard deviation for each frequency domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TP	10^{-6}	14692	9543	15429	8967	13543	10169	11756	6898
FFT VLF	10^{-6}	6995	3860	8167	5614	7545	7814	5986	3898
FFT LF	10^{-6}	4474	3343	3878	1493	3169	1696	3366	1802
FFT HF	10^{-6}	3043	5310	3157	3722	2600	2585	2215	2852
FFT LF:HF	-	3.03	1.98	2.23	1.36	3.06	4.72	3.90	3.97
FFT LF n.u.	-	0.68	0.15	0.62	0.15	0.57	0.18	0.67	0.18
FFT HF n.u.	-	0.30	0.15	0.35	0.15	0.39	0.17	0.30	0.17
AR VLF	10^{-6}	6882	3826	8046	5552	7335	7482	5913	3898
AR LF	10^{-6}	4644	3425	4048	1432	3417	1954	3433	1759
AR HF	10^{-6}	2981	5116	3106	3657	2561	2603	2221	2870
AR LF:HF	-	3.21	2.23	2.42	1.49	3.17	4.42	4.13	4.34
AR LF n.u.	-	0.69	0.15	0.63	0.16	0.59	0.18	0.68	0.18
AR HF n.u.	-	0.29	0.14	0.34	0.15	0.37	0.17	0.29	0.17

Table 17. Preworkout Trial Frequency Domain Indices as Determined from the Original Tachogram after Correction for Mean HR. The table shows the mean and standard deviation for each frequency domain index at each the baseline (Base), 30 minutes post supplement consumption (30), immediately post exercise (Post), and 60 minutes post supplement consumption (60).

		Base		30		Post		60	
Index	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TP	10^{-6}	11316	7527	12567	8472	11203	3772	11094	7278
FFT VLF	10^{-6}	4592	2097	5348	3177	5793	2101	4931	2457
FFT LF	10^{-6}	4662	3573	4293	3031	3047	1691	3923	2964
FFT HF	10^{-6}	1916	2650	2681	2793	2149	2049	2056	2639
FFT LF:HF	-	4.50	3.15	2.77	2.68	3.05	3.65	3.77	3.57
FFT LF n.u.	-	0.74	0.13	0.64	0.13	0.60	0.21	0.69	0.14
FFT HF n.u.	-	0.24	0.13	0.33	0.12	0.36	0.20	0.28	0.13
AR VLF	10^{-6}	4550	2074	5288	3198	5737	2001	4843	2378
AR LF	10^{-6}	4787	3685	4366	3000	3120	1616	4022	3021
AR HF	10^{-6}	1833	2493	2669	2855	2124	2009	2044	2641
AR LF:HF	-	4.86	3.40	2.84	2.56	3.18	3.88	3.90	3.56
AR LF n.u.	-	0.75	0.14	0.65	0.13	0.61	0.20	0.70	0.14
AR HF n.u.	-	0.23	0.14	0.32	0.12	0.35	0.19	0.27	0.13

5. DISCUSSION

The first main finding of this thesis was that the consumption of a caffeine or preworkout supplement showed no statistically significant difference as compared to placebo for any HRV index measured (both time and frequency domain indices) at baseline or 30 minutes post consumption (both measurements during rest and prior to exercise). This suggests that there is no change in SNS/PNS activity prior to exercise or that the HRV indices used did not reflect changes in SNS/PNS activity because of a possible disconnect between the relationship between HRV and SNS/PNS activity.

During the placebo trial, it was found that LF power n.u. increased by 0.06 ± 0.03 (mean ± standard error (SE)) and HF power n.u. decreased 0.05 ± 0.03 (mean ± SE) from prior to exercise (30 minutes post consumption) to immediately post exercise. Again, LF Power n.u. increased 0.02 ± 0.02 (mean ± SE) and the HF power n.u. decreased 0.02 ± 0.02 (mean ± SE) from immediately post exercise to 60 minutes post consumption in the placebo trial. These results are consistent with [12]. One of the test groups in [12] performed 3 sets of 20 repetitions of six different exercises (bench press, 70° angle leg press, lat pull down, leg curl, biceps curl, and 40° leg press) at 40% of a 1 rep max (RM) with 45 seconds between sets and 90 seconds between exercises. They also measured LF Power n.u. and HF Power n.u. using an AR model. The baseline LF Power n.u. and HF Power n.u. were 0.64 ± 0.03 and 0.36 ± 0.03 (mean ± SE) (n=17) respectively for the control trial in [12] as compared to 0.66 ± 0.05 and 0.31 ± 0.05 (mean ± SE) (n=12) just prior to exercise for the placebo trial (30 minutes post supplement consumption) in this study. The LF Power n.u. increased by 0.23 ± 0.04 (mean ± SE) and HF Power n.u. decreased by 0.01 ± 0.00 (mean ± SE) in [12] from pre exercise to 20-30 minutes post exercise as compared to this study where LF Power n.u. increased by 0.08 ± 0.02 (mean ± SE)

and the HF Power n.u. decreased by 0.08 ± 0.02 (mean ± SE) from pre exercise (30 minutes post consumption) to approximately 10 minutes post exercise (60 minute post supplement consumption).

The conclusion in [12] states that the respective changes in LF Power n.u. and HF Power n.u. suggests an increase in SNS activity and decrease in PNS activity. This agrees with what is already known about SNS/PNS activity during exercise, providing additional evidence that the measures used (LF Power n.u. and HF Power n.u.) can provide information about SNS/PNS activity.

During the caffeine trial, contrasting to the placebo trial, it was found that the LF Power n.u. decreased 0.04 ± 0.04 (mean ± SE) and the HF Power n.u. increased 0.03 ± 0.04 (mean ± SE) from prior to exercise (30 minutes post consumption) to immediately post exercise. Using the same rational as used in [12], this result suggests a decrease in SNS activity and an increase in PNS activity. This does not agree with the known SNS effects of both exercise and caffeine. In addition to this result, there was a statistically significant difference between caffeine and placebo at the same time point (post exercise), adding to the significance of this finding.

The main result that has been found among other caffeine studies is an increase in HF power caused by consumption of caffeine [11], which was not found in this study, however it did show in increase in HF power n.u.. What was made clear though in [11] is that the impact of caffeine on HRV is inconclusive, and this study adds to the body of research already completed on caffeine as well as leads research into the effects of preworkout supplements on HRV.

During the preworkout supplement trial, the LF Power n.u. and HF Power n.u. followed the exact same trend as the caffeine trial, however there was no statistically significant difference between preworkout and placebo post exercise as in the caffeine trial case (n=12). There was

also no statistically significant difference between the preworkout and caffeine trials at any time point (baseline, 30 minutes post consumption, post exercise, and 60 post consumption) (n=12).

The results of this study provide information for an important discussion. The study reveals which HRV indices may be sensitive enough to detect statistically significant differences between the supplements. The only indices that were shown to be sensitive enough (if differences existed) were LF Power n.u. and HF Power n.u. while all of the other frequency domain indices as well as all of the time domain indices were not. This suggests that when performing studies dealing with caffeine and exercise, and potentially preworkout supplements and exercise, LF Power n.u. and HF Power n.u. should be used to detect changes in HRV.

The second point which needs to be discussed is how LF Power n.u. and HF Power n.u. seemed to reflect SNS/PNS activity in agreement with what is expected with a placebo and exercise, however the same indices reflect the opposite of what is expected with SNS/PNS activity during the caffeine and preworkout trials. This suggests that these indices do not reflect the SNS/PNS activity accurately as they have previously been thought to.

The third point which needs to be discussed is the variability within HRV among subjects. Through inspection of the results tables, large variations (leading to large standard deviations) in all HRV indices among subjects may be a large contributing factor to why there may have been so few time*supplement interaction effects found. There may be multiple reasons for this variability, however that doesn't take away from the fact that this variability should be investigated. Along with the variability in measurements, the trends shown by the mean changes from baseline, 30 minutes post consumption, post exercise, and 60 minutes post consumption did not represent the changes seen for each subject (the changes were inconsistent).

The main points of concern are the variability in each HRV index among subjects along with the other unpredicted results in this study including decreased SNS activity and increased PNS activity based on HRV measurements post exercise during the caffeine trial and inconsistency in trends among each subject as compared to the mean changes throughout all three trials.

A proposed reason for these issues is that, even though HRV may reflect some kind of autonomic cardiovascular regulation, the interpretation of what these HRV indices reflects is a more complex relationship between the autonomic cardiovascular control system and HRV than previously thought. Similar statements on the topic have also been made by [17], specifically discussing how the LF:HF ratio cannot be used to reflect the SNS/PNS balance. These types of discussion force researchers to rethink conclusions made by previous research. It is always important to question the validity of conclusions made during early research in any field, including early research in HRV analysis, as they are always used as the building blocks for future research. In this study there were statistically significant findings for the LF Power n.u. and HF Power n.u. which represented unexplainable physiological effects (changes in SNS/PNS activity) in the caffeine trial as compared to the placebo trial post exercise. This suggests there was not a physiologically significant effect (as opposed to statistically significant) of caffeine post exercise as compared to placebo, and that the validity of the relationship between HRV measures and SNS/PNS activity should be investigated further.

6. LIMITATIONS

Although the study was performed with much attention to detail, there were however some limitations that came along with it. One of the limitations dealing with the calculations of HRV was that there was not a stationarity test performed on each ECG recording. A stationarity test would have brought forth any ECG recording that is not suited to be analyzed as is without any type of de-trending.

One of the limitations dealing with the preworkout procedure is the lack of time the subjects were able to relax prior to the baseline ECG recording. The HRV results showed that, even during the placebo trial, many of the HRV indices, both time and frequency domain, had changed (visually, but was not investigated statistically) from the baseline measurement to the 30 minute measurement. This causes reason to believe that although the heart rates seemed to be at a baseline level, the HRV values were not at a baseline level (because of the previous discussion, it is possible that the changes seen were just general variability in measurements, not that there was a physiological change happening).

Another limitation of the study was the number of subjects. Observing the figures in the results section of this thesis, it can be seen that the preworkout supplement had a similar trend (in its mean at each time measurement) to the caffeine supplement, however the preworkout supplement did not show statistical significance between it and the placebo at any time. It is possible that if there were more subjects, the preworkout supplement would have also shown a significant difference between it and placebo, similar to that in the case of the caffeine supplement. Again, some of the results from this study suggest that the reason that we did not see this is because of the large variability among subjects.

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APPENDIX A. CUBIC SPLINE INTERPOLATION EXAMPLE

A cubic spline consists of $n = (\#\ of\ points\ of\ data) - 1$ continuous piecewise function that passes through each data point where all n equations are continuous, even at each "knot" (any original data point, which represents the end points for each function in the piecewise function). The n equations also have first and second derivatives that are continuous, even at the "knots". Typically with real NN interval data, the data points do not occur at equal intervals, and the spline is used to create a continuous piecewise function that represents the data, which can be re-sampled for frequency domain analysis. The information for the process of creating a cubic spline can be found in [19].

To demonstrate the concept, the following example shows how to determine a cubic spline for a 10-point, aperiodic signal that was originally sampled at equal intervals (unlike a tachogram that was sampled an unequal intervals), for simplicity of demonstration, at 1 Hz.

Let $x[n] = [1 \quad 3 \quad 8 \quad 4 \quad 9 \quad 2 \quad 5 \quad 6 \quad 2 \quad 7]$.

The cubic spline of the signal $x[n]$ is represented by a piecewise function consisting of $n = (10) - 1 = 9$ cubic functions. Note that the cubic spline consists of $4n = 4(9) = 36$ unknowns. To solve for 36 unknowns, there must be 36 equations. x in each of the following functions represents the time at which the points of $x[n]$ occurred. Because $x[n]$ in this example was sampled at equal intervals, the time at which each points occurred started at $t = 0$ and increased at intervals $\Delta T = \frac{1}{f_s} = 1\ s$ up to $\frac{\#\ of\ points\ in\ x}{f_s} - \frac{1}{f_s} = \frac{(10)}{(1\ Hz)} - \frac{1}{(1\ Hz)} = 9\ s$. Equation 2 shows the 9 cubic functions that make up the cubic spline.

$$S_{3,9} = \begin{Bmatrix} p_1(x) = a_1 + b_1x + c_1x^2 + d_1x^3, & x \in [x_0, x_1] \\ p_2(x) = a_2 + b_2x + c_2x^2 + d_2x^3, & x \in [x_1, x_2] \\ p_3(x) = a_3 + b_3x + c_3x^2 + d_3x^3, & x \in [x_2, x_3] \\ p_4(x) = a_4 + b_4x + c_4x^2 + d_4x^3, & x \in [x_3, x_4] \\ p_5(x) = a_5 + b_5x + c_5x^2 + d_5x^3, & x \in [x_4, x_5] \\ p_6(x) = a_6 + b_6x + c_6x^2 + d_6x^3, & x \in [x_5, x_6] \\ p_7(x) = a_7 + b_7x + c_7x^2 + d_7x^3, & x \in [x_6, x_7] \\ p_8(x) = a_8 + b_8x + c_8x^2 + d_8x^3, & x \in [x_7, x_8] \\ p_9(x) = a_9 + b_9x + c_9x^2 + d_9x^3, & x \in [x_8, x_9] \end{Bmatrix} \quad (2)$$

Interpolation creates $n + 1 = (9) + 1 = 10$ equations. Interpolation is defined by Equation 3.

$$p_i(x_{i-1}) = f_{i-1} \text{ and } p_i(x_i) = f_i \text{ for } i = 1, 2, \dots, n \quad (3)$$

There were 26 additional equations that had to be determined. Using the fact that the piecewise function is continuous at the "interior knots" creates $n - 1 = (9) - 1 = 8$ equations. Continuity at the "interior knots" is defined by Equation 4.

$$p_i(x_i) = p_{i+1}(x_i) \text{ for } i = 1, 2, \dots, n - 1 \quad (4)$$

There were 18 additional equations that had to be determined. Using the fact that the first derivative of the piecewise function is continuous at the "interior knots" creates $n - 1 = (9) - 1 = 8$ equations. Continuity of the first derivative at the "interior knots" is defined by Equation 5.

$$p_i'(x_i) = p_{i+1}'(x_i) \; for \; i = 1, 2, \dots, n - 1 \quad (5)$$

There were 10 additional equations that had to be determined. Using the fact that the second derivative of the piecewise function is continuous at the "interior knots" creates $n - 1 = (9) - 1 = 8$ equations. Continuity of the second derivative at the "interior knots" is defined by Equation 6.

$$p_i''(x_i) = p_{i+1}''(x_i) \; for \; i = 1, 2, \dots, n - 1 \quad (6)$$

There were only 2 additional equations that had to be determined. There are a few methods that can be used to achieve the last 2 equations. A typical method is to apply the "not-a-knot" conditions. The "not-a-knot" conditions declare that the third derivative of the function on each side of the first and last "interior knot" are equal. The "not-a-knot" conditions are defined by Equation 7.

$$p_1'''(x_1) = p_1'''(x_1) \text{ and } p_{n-1}'''(x_{n-1}) = p_n'''(x_{n-1}) \tag{7}$$

There were then 36 equations to solve for 36 unknowns. The MATLAB *spline* command solves the system of equations, creates the cubic spline, and re-samples it at a specified frequency very efficiently. Figure A1 shows the original signal plotted vs time, Figure A2 shows the cubic spline of the signal plotted vs time, and Figure A3 shows the re-sampled cubic spline of the original signal at a frequency of $f_s = 2\ Hz$.

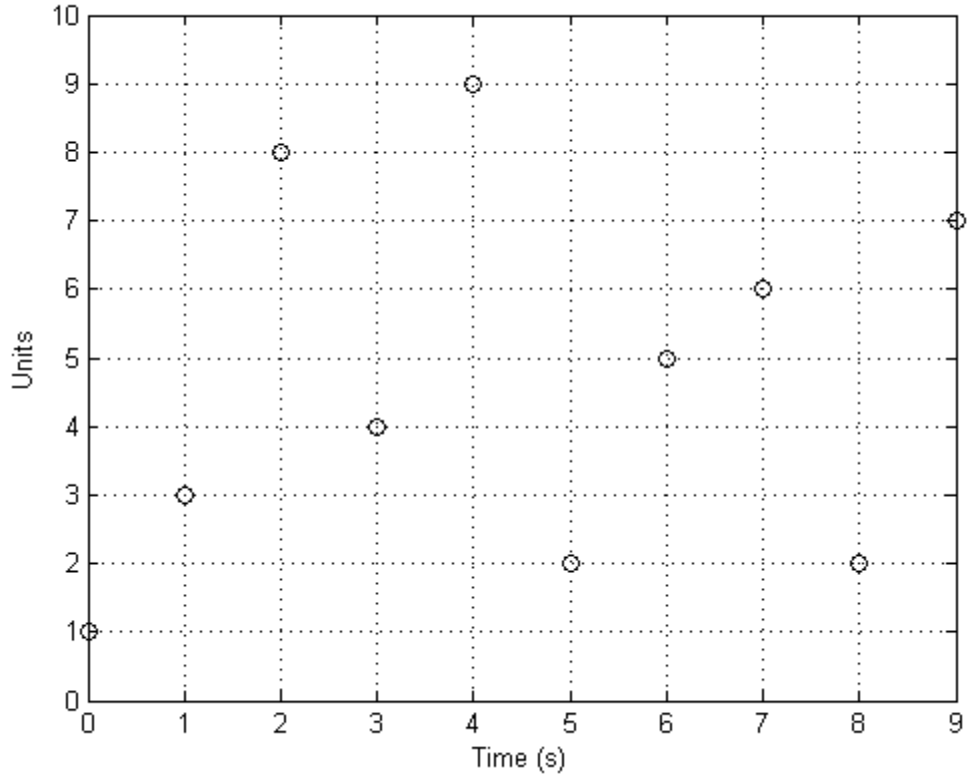

Figure A1. The Sample Signal x vs Time.

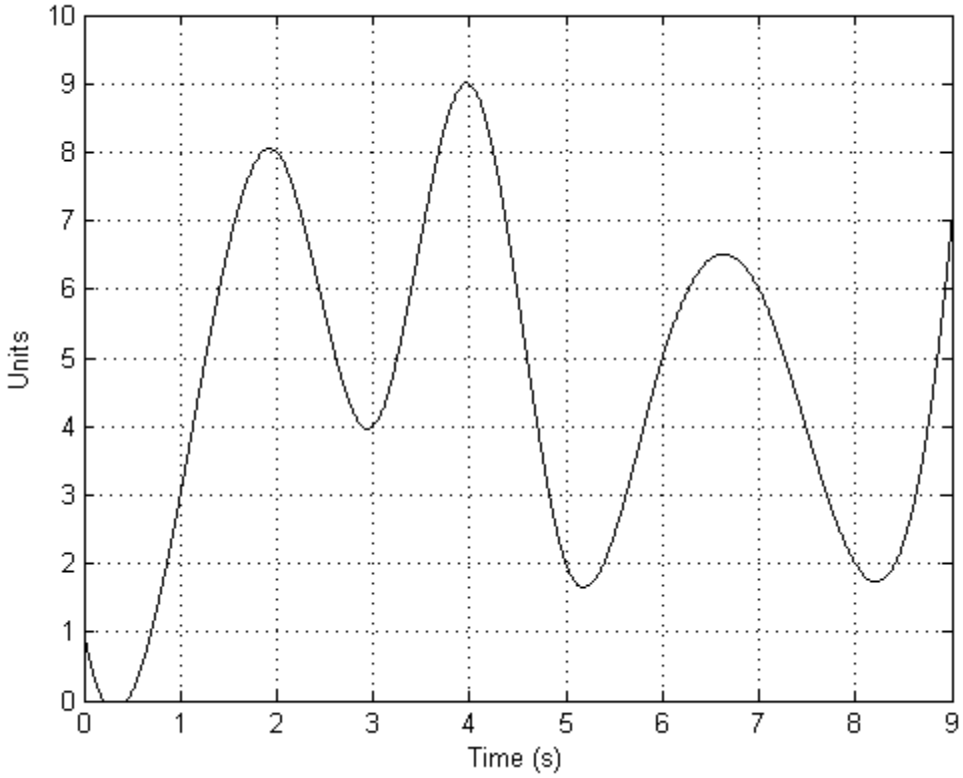

Figure A2. The Cubic Spline of the Sample Signal x vs Time. The figure shows the cubic spline (continuous waveform) of the sample signal x before it has been resampled.

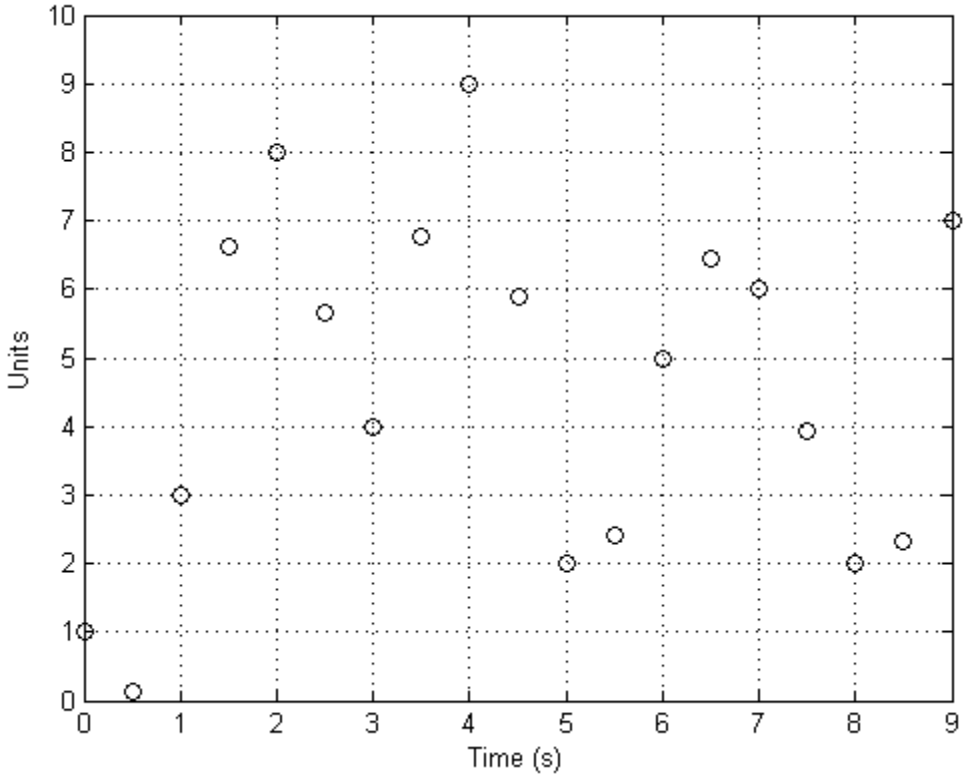

Figure A3. The Signal x Plotted vs Time after Resampling of Cubic Spline. The cubic spline of x was resampled at 2 Hz.

APPENDIX B. FAST FOURIER TRANSFORM (FFT) EXAMPLE

For HRV analysis, an FFT is performed to determine the PSD to gain information about the frequency components in a tachogram. An FFT is a more efficient method (typically present in computer programs such as MATLAB) of calculating the Discrete Fourier Transform (DFT). The values calculated by each are equivalent. The DFT is defined by Equation 8. Note that the DFT results in a series of discrete points (not a continuous waveform).

$$X[r] = \sum_{k=0}^{N-1} x[k] e^{-j(2\pi kr/M)} \tag{8}$$

where $x[k]$ is a discrete time signal, M is the number of DFT points desired, and N is the number of points in $x[k]$. Typically, $M = N$, so if $M > \#\ of\ points\ in\ x[k]$, then $x[k]$ is "zero padded" (completed by adding trailing zeros to the signal) so that N, the length of $x[k]$, is still equal to M. Zero padding the signal increases the resolution of the DFT and is commonly performed with frequency domain analysis on a tachogram.

The DFT consists of complex values (values with real and imaginary parts), however in this case, only the magnitude of each complex value is important (the phase can be ignored). The magnitude can be calculated by squaring the real and imaginary parts, adding them together, and then taking the square root. The magnitude values can then be manipulated to determine the PSD. The following example shows how to calculate the DFT of a signal and determine its PSD. The information describing this process was found in [4] and [18]. Also, the MATLAB *fft* command can calculate an N point DFT very efficiently, but be aware that the output must be modified in the same manner as in the following example.

B.1. Discrete Fourier Transform (DFT)

Determine the power spectral density (PSD) of a 10-point aperiodic signal that was sampled at a rate, f_s, of 2 Hz:

Let $x[n] = [1 \quad 3 \quad 8 \quad 4 \quad 9 \quad 2 \quad 5 \quad 6 \quad 2 \quad 7]$.

The original signal plotted vs time is shown in Figure B1.

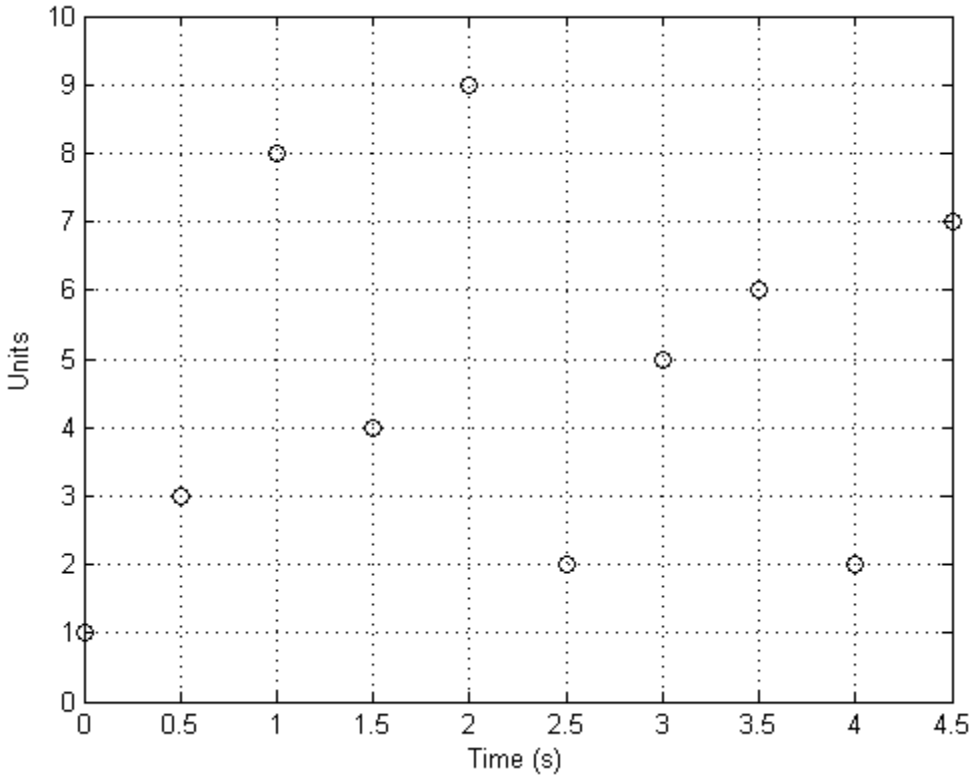

Figure B1. The Sample Signal x vs Time.

Before calculating the DFT, subtract the mean of the signal from the signal as in HRV analysis. The signal with its mean subtracted is shown in Figure B2.

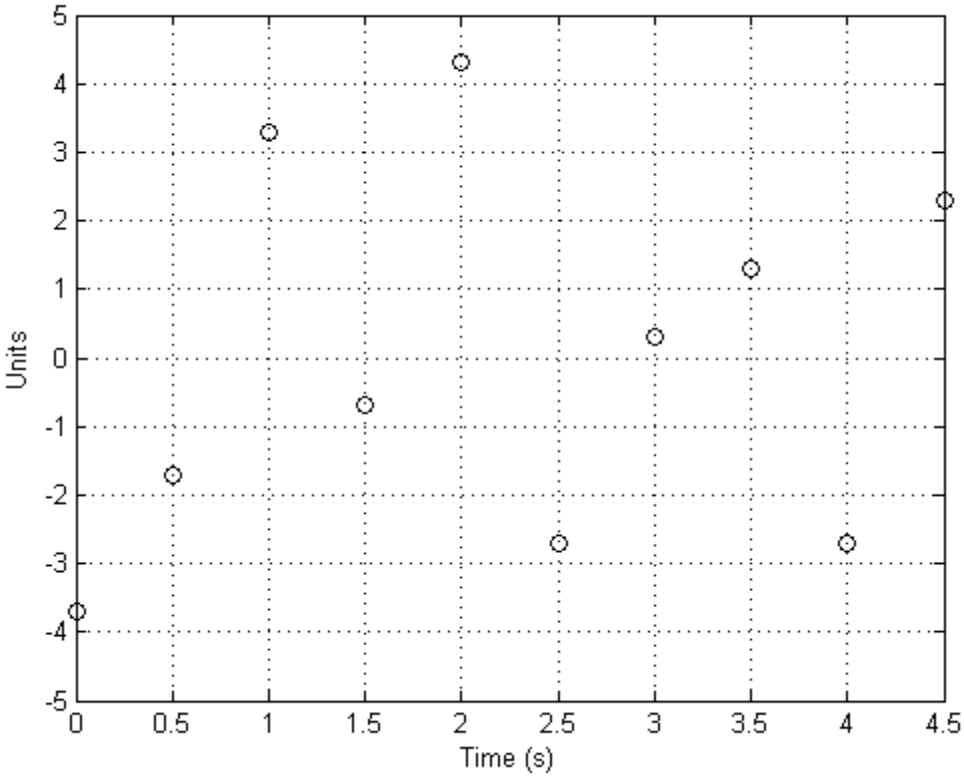

Figure B2. Sample Signal x vs Time after Subtracting its Mean.

Using Equation 8, the 10-point DFT was calculated and is shown in Figure B3.

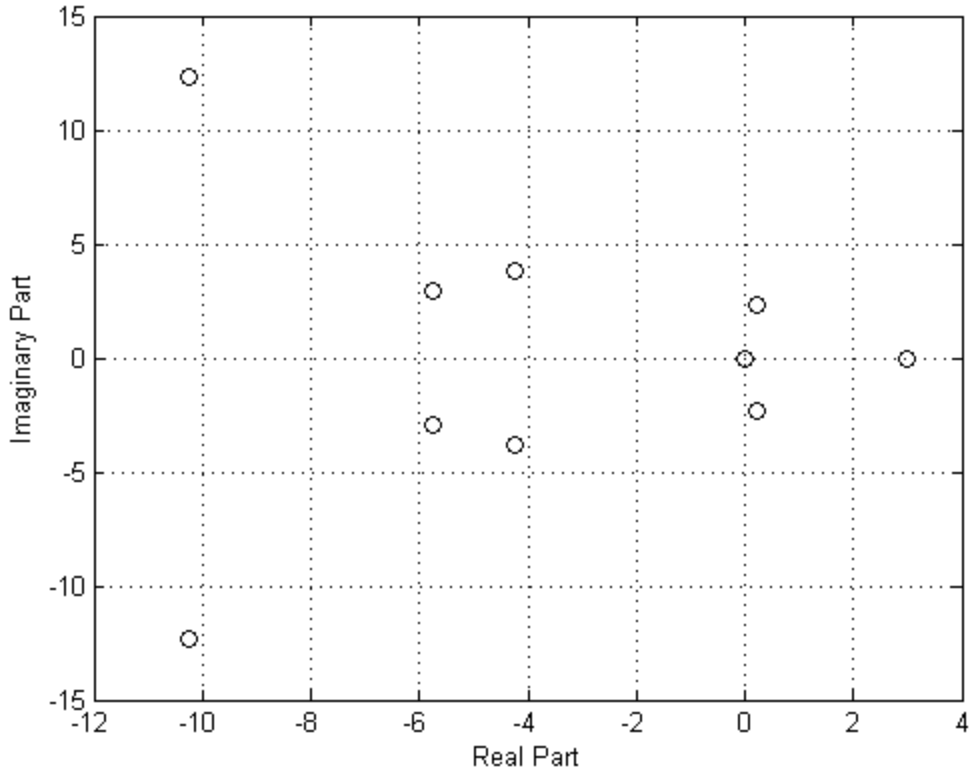

Figure B3. Discrete Fourier Transform (DFT) of Sample Signal x. The figure shows the DFT of Sample Signal x on a complex plane (real vs imaginary).

B.2. Unscaled Amplitude (Peak), Two Sided Frequency Spectrum

The conversion from the DFT to an unscaled amplitude (peak), two sided frequency spectrum is described by Equation 9.

$$X_{Unscaled\ Amplitude\ (Peak),Two\ Sided}[r] = \sqrt{(Real\ Part\ of\ X[r])^2 + (Imaginary\ Part\ of\ X[r])^2} \quad (9)$$

The unscaled amplitude (peak), two sided frequency spectrum of x is shown in Figure B4.

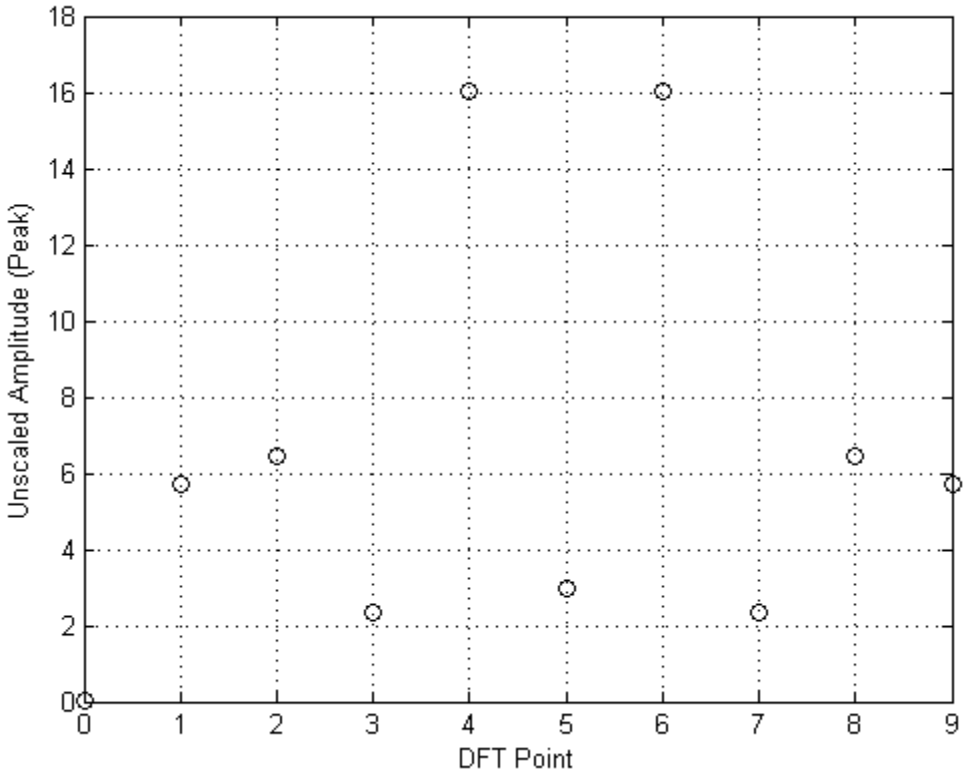

Figure B4. The Unscaled Amplitude (Peak), Two Sided Frequency Spectrum of Sample Signal x. The figure shows the unscaled amplitude (peak), two sided frequency spectrum of sample signal x as a function of DFT points (0-9).

B.3. Scaled Amplitude (Peak), Two Sided Frequency Spectrum

The conversion from the unscaled amplitude (peak), two sided frequency spectrum to the scaled amplitude (peak), two-sided frequency spectrum is done by scaling each amplitude by dividing by the number of points in x and is shown in Equation 10.

$$X_{Scaled\ Amplitude\ (Peak),Two\ Sided}[r] = \frac{X_{Unscaled\ Amplitude\ (Peak),Two\ Sided}[r]}{\#\ of\ points\ in\ x} \qquad (10)$$

The scaled amplitude (peak), two sided frequency spectrum of x is shown in Figure B5.

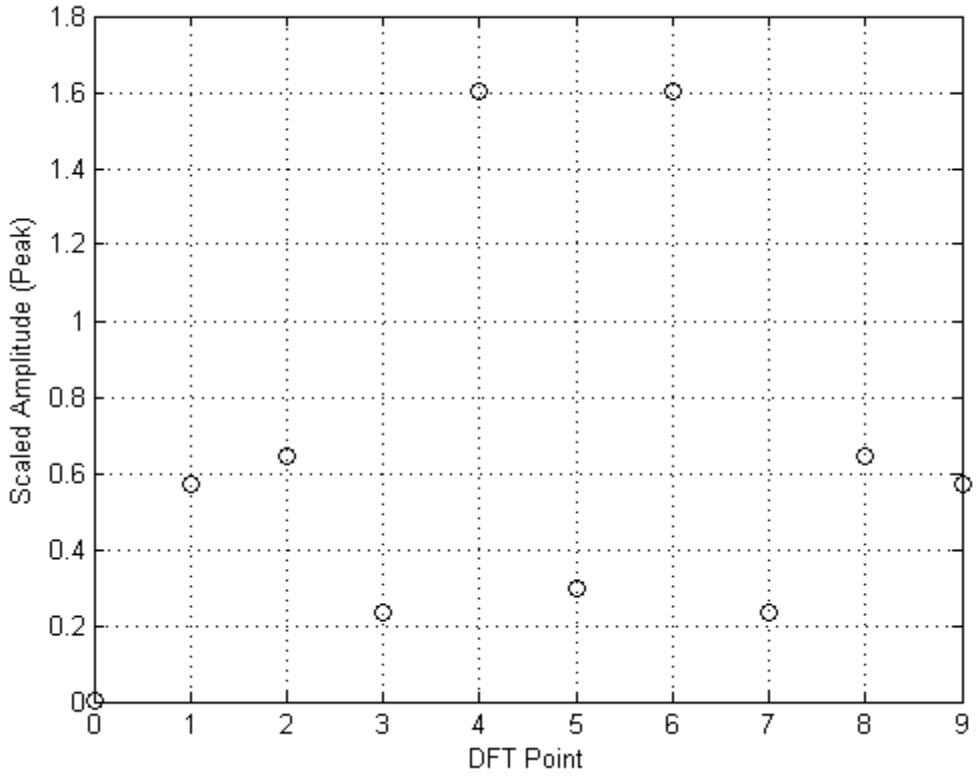

Figure B5. The Scaled Amplitude (Peak), Two Sided Frequency Spectrum of Sample Signal x. The figure shows the scaled amplitude (peak), two sided frequency spectrum of sample signal x as a function of DFT points (0-9).

B.4. Scaled Amplitude (Peak), One Sided Frequency Spectrum

The conversion from the scaled amplitude (peak), two sided frequency spectrum of x to the scaled amplitude (peak), one-sided frequency spectrum is described here. The first element of the DFT corresponds to the zero frequency. The midpoint of the DFT (or the point just to the right of the midpoint if the length is even), corresponding to half the sampling frequency of the data, is the Nyquist Point. For the 10-point DFT, the midpoint is at $\frac{N-1}{2} = \frac{(10)-1}{2} = 4.5$. Because the 10-point DFT is even in length (10), the midpoint is actually the point to the right of the calculated midpoint. The calculated midpoint is therefore 5. To determine the unscaled, one sided frequency spectrum, multiply all of the DFT elements non-zero frequencies ($1 \leq r \leq 5$)

components by 2. The scaled amplitude (peak), one sided frequency spectrum of x is shown in Figure B6.

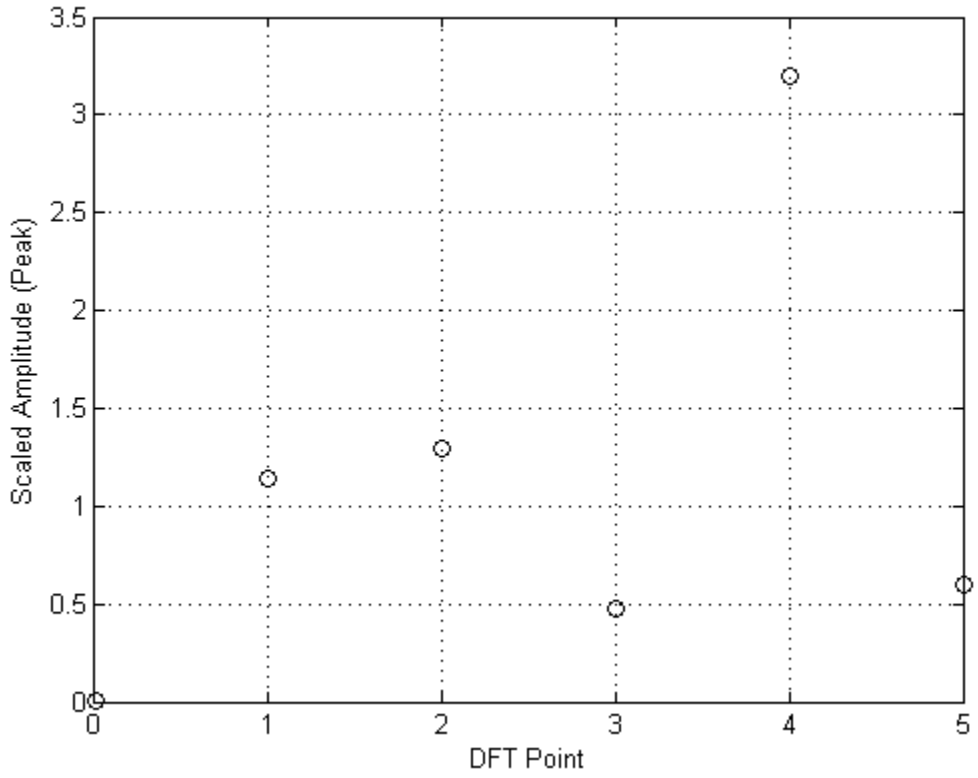

Figure B6. The Scaled Amplitude (Peak), One Sided Frequency Spectrum of Sample Signal x. The figure shows the scaled amplitude (peak), one sided frequency spectrum of sample signal x as a function of DFT points (0-5)

B.5. Scaled Amplitude (Root Mean Square (RMS)), One Sided Frequency Spectrum

The conversion from the scaled amplitude (peak), one sided frequency spectrum of x to the scaled amplitude (root mean square (RMS)), one sided frequency spectrum is done by dividing each non-zero frequency value by $\sqrt{2}$. The scaled amplitude (RMS), one sided frequency spectrum of x is shown in Figure B7.

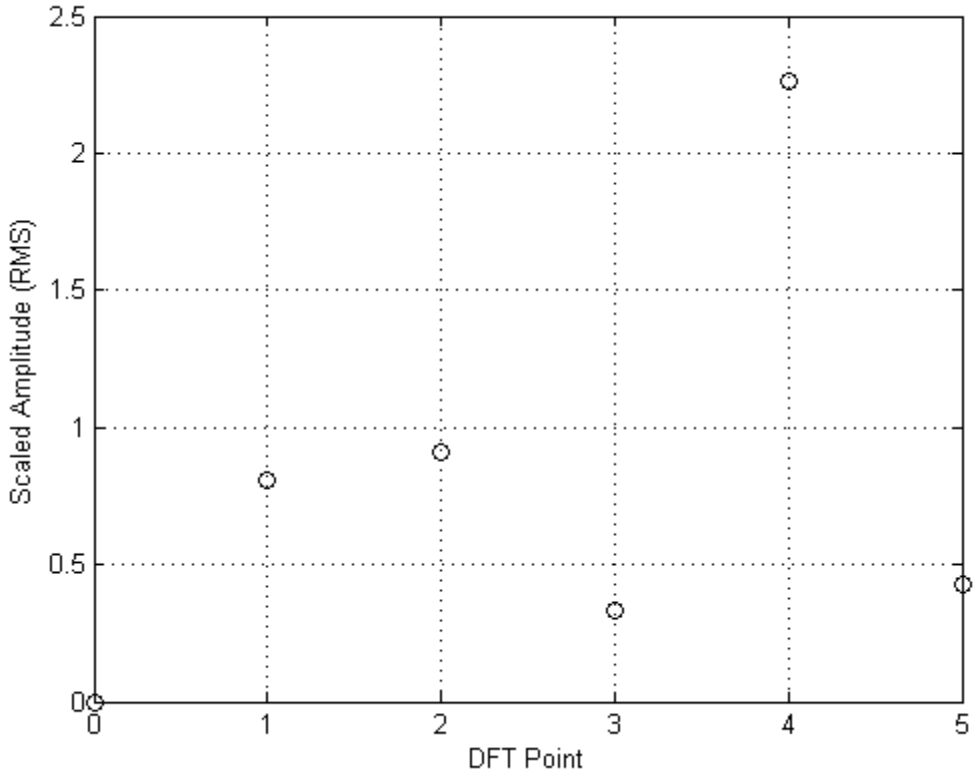

Figure B7. The Scaled Amplitude (RMS), One Sided Frequency Spectrum of Sample Signal x. The figure shows the scaled amplitude (RMS), one sided frequency spectrum of sample signal x as a function of DFT points (0-5).

B.6. Power Spectrum

The conversion from the scaled amplitude (RMS), one sided frequency spectrum of x to the Power Spectrum is done by squaring each value of the scaled amplitude (RMS), one sided frequency spectrum. The power spectrum is x is shown in Figure B8.

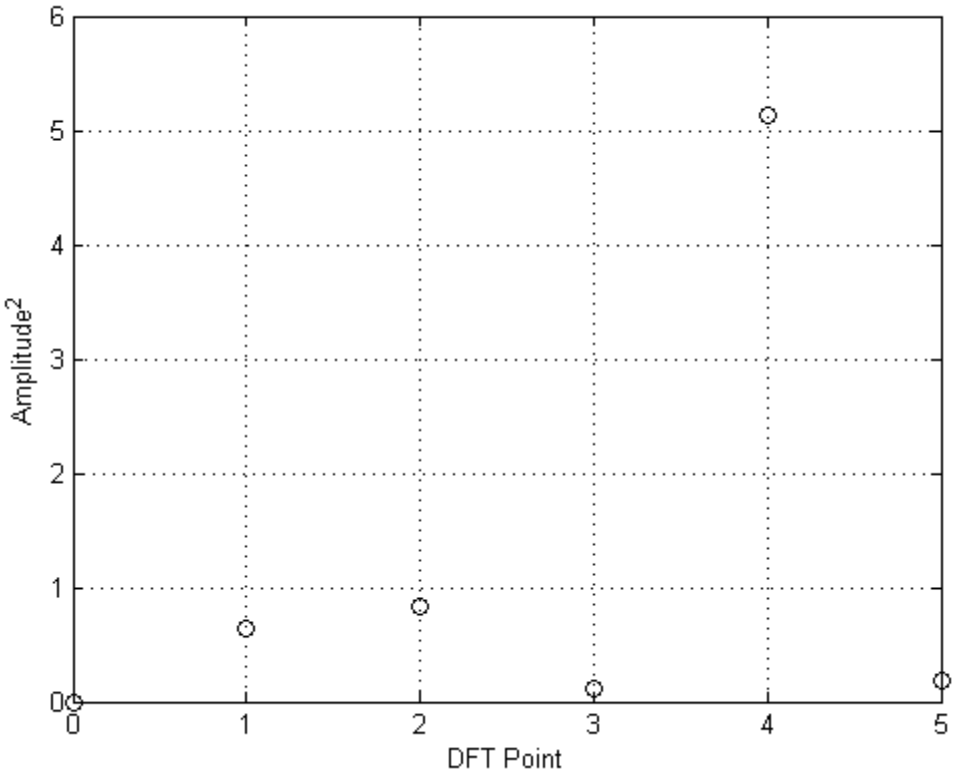

Figure B8. The Power Spectrum of Sample Signal x. The figure shows the power spectrum of sample signal x as a function of DFT points (0-5).

B.7. Power Spectral Density (PSD)

The conversion from Power Spectrum to the PSD is done by dividing by the Δf spacing as shown in Equation 11.

$$X_{PSD}[r] = \frac{X_{Power\ Spectrum}[r]}{\Delta f} = \frac{X_{Power\ Spectrum}[r]}{F_s/_{\#\ of\ points\ in\ x}} \tag{11}$$

Because the PSD's independent variable is data points, it was necessary to convert the independent variable to frequency for better interpretation of the PSD. The first PSD element corresponds to the zero frequency and the last PSD element corresponds to the frequency $f = \frac{f_s}{2}$. The remaining PSD elements correspond to frequencies equally spaced between zero and $f = \frac{f_s}{2}$.

The increment is equal to $\Delta f = \frac{f_s}{N} = \frac{(2\ Hz)}{(10)} = 0.2\ Hz$. Therefore the six PSD elements correspond to the frequencies $0, 0.2, 0.4, 0.6, 0.8, and\ 1\ Hz$. The PSD of x using an FFT is shown in Figure B9.

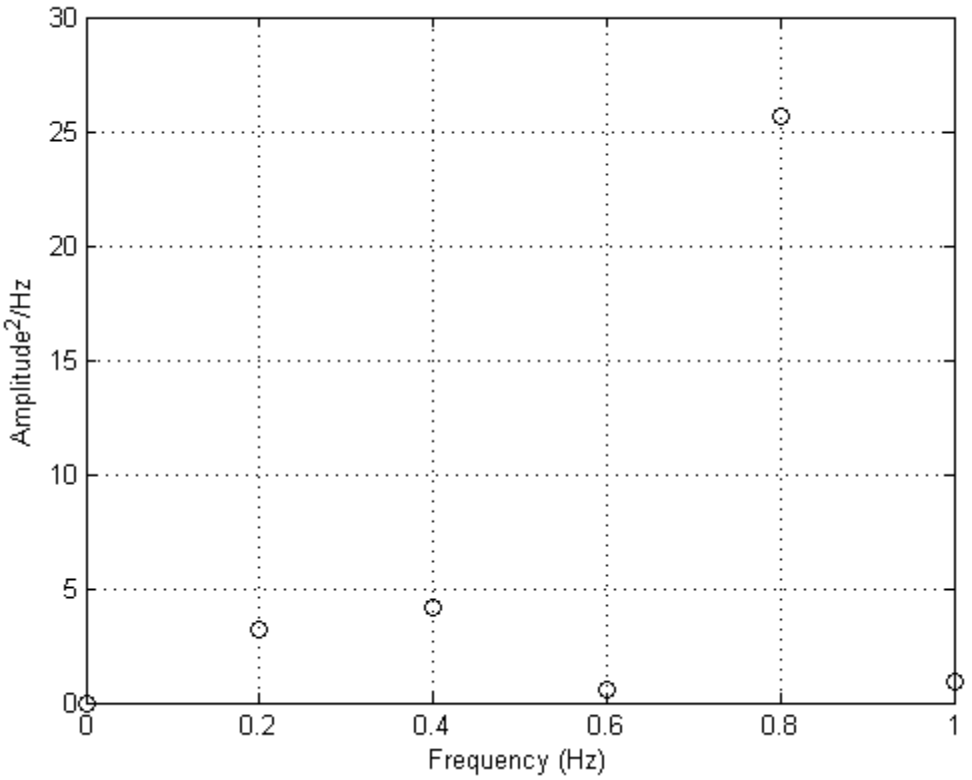

Figure B9. The Power Spectral Density (PSD) of Sample Signal x Using an FFT. The figure shows the PSD of sample signal x using an FFT as a function of frequency.

APPENDIX C. AUTOREGRESSIVE (AR) MODEL EXAMPLE

An autoregressive (AR) model is a model that predicts each data point of a data set based on a specific number of previous data points that are each weighted. With each prediction comes an error from the actual data point. The number of previous data points used for the prediction is the model order. The AR model is defined by Equation 12.

$$x[n] = \sum_{i=0}^{M} a_i x[n-i] + \varepsilon[n] \tag{12}$$

Where M is the model order and a_i represents the coefficients for the AR model. It can be seen that any set of coefficients can be used for prediction using a particular model order, but a certain set of coefficients can lead to a lot of error for each data prediction. There are many ways to describe the error based on a certain set of coefficients, but the most typical way is to determine the error is by the sum squared error (SSE). The goal then is to choose a set of coefficients that minimizes the SSE for a chosen model order. One method of choosing the coefficients to minimize this sum squared error is the Levinson-Durbin recursion algorithm. Information on AR models and the Levinson-Durbin algorithm derivation can be found in [20] and [21].

C.1. Power Spectral Density (PSD)

To determine the PSD using an AR model, first, the AR model coefficients must be determined (Levinson-Durbin Algorithm). Once the coefficients have been determined, the transfer function of the AR model must be determined. The transfer function of the AR model is defined by Equation 13. Note that the transfer function of the AR model is a continuous function (unlike the discrete function in the DFT case).

$$H(z) = \frac{1}{\sum_{i=0}^{M} a_i z^{-i}} \tag{13}$$

Once the AR synthesis filter is determined, the AR synthesis filter can be modified to determine the transfer function for power spectral density. The transfer function for power spectral density is defined by Equation 14.

$$PSD(z) = \frac{2E_M}{\left|\sum_{i=0}^{M} a_i z^{-i}\right|^2} * \frac{1}{f_s * (\#\ of\ points\ in\ x)} \tag{14}$$

Where E_M is the prediction error variance and f_s is the sampling frequency of the data in $x[n]$.

C.2. Levinson-Durbin Algorithm

The Levinson-Durbin recursion algorithm is used to determine the coefficients for an AR model while minimizing the mean squared error (MSE). The algorithm is a recursion algorithm, meaning that if a 3rd order model is desired, the coefficients must first be determined for the 1st order model, the 2nd order model, and finally the 3rd order model. The Levinson-Durbin algorithm is defined by these steps from [20] with slight modifications in syntax:

1. Compute R_j for $0 \leq j \leq M$ where $R_l = \sum_{n=-\infty}^{\infty} y_n y_{n+l}$
2. Calculate $A_1 = \begin{bmatrix} a_0^1 \\ a_1^1 \end{bmatrix} = \begin{bmatrix} 1 \\ -\frac{R_1}{R_0} \end{bmatrix}$
3. Calculate $E_1 = R_0 + R_1 a_1^1$
4. For $1 \leq k \leq M - 1$:
    a. $\lambda_{k+1} = \frac{-\sum_{j=0}^{k} a_j^k R_{k+1-j}}{E_k}$
    b. $A_{k+1} = U_{k+1} + \lambda_{k+1} V_{k+1}$
    c. $E_{k+1} = (1 - {\lambda_{k+1}}^2) E_k$

Where $A_{k+1} = \begin{bmatrix} 1 \\ a_1^{k+1} \\ a_2^{k+1} \\ \vdots \\ a_{k+1}^{k+1} \end{bmatrix}$, $U_{k+1} = \begin{bmatrix} 0 \\ a_1^k \\ a_2^k \\ \vdots \\ a_k^k \\ 1 \end{bmatrix}$, and $V_{k+1} = \begin{bmatrix} 1 \\ a_k^k \\ \vdots \\ a_2^k \\ a_1^k \\ 0 \end{bmatrix}$.

A_1 and a^k represent all of the coefficients for the model order k. For example, A_3 includes the coefficients a_0^3, a_1^2, a_2^3, and a_3^3. a_2^3 is the coefficient that corresponds to the second lag value (the weight placed on the second preceding data point already known) of the for a 3th order model. E_k is the prediction error variance and λ_k is the reflection coefficient for model order k. It is known that the prediction error variance will keep decreasing as the model order increases, however increasing the model order increases the complexity of the model. There are methods that can be used, which are not discussed here, to determine the optimal model order.

The following example shows how to create an AR model using the Levinson-Durbin recursion algorithm, and how to determine the PSD from it. Also, the MATLAB *levinson* command can easily solve for the AR model coefficients, reflection coefficient, and prediction error variance for any model order very efficiently. Be aware that the output of the command must be modified in the same fashion as the example below to determine the PSD.

Calculate the power spectral density (PSD) of an aperiodic signal that was sampled at a rate, f_s, of 2 Hz using a 3rd order autoregressive (AR) model:

Let $x[n] = [1 \quad 3 \quad 8 \quad 4 \quad 9 \quad 2 \quad 5 \quad 6 \quad 2 \quad 7]$.

The original signal vs time is shown in Figure C1.

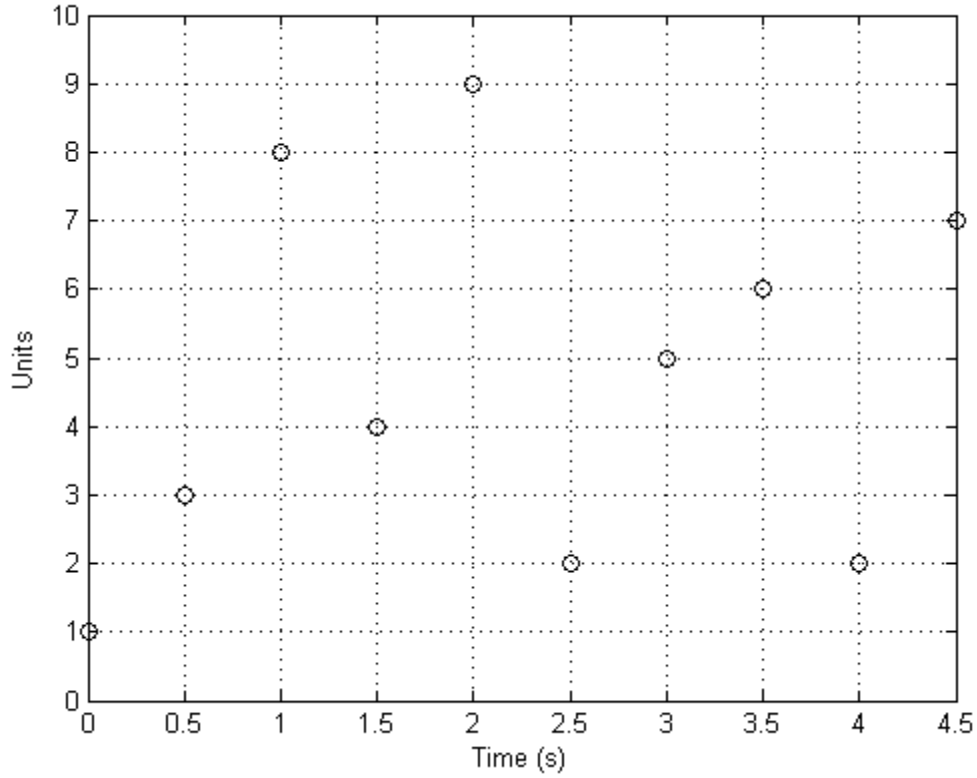

Figure C1. The Sample Signal x vs Time.

Before determining the AR model, mean of the signal was subtracted from the signal as in HRV analysis. The signal with its mean subtracted is shown in Figure C2.

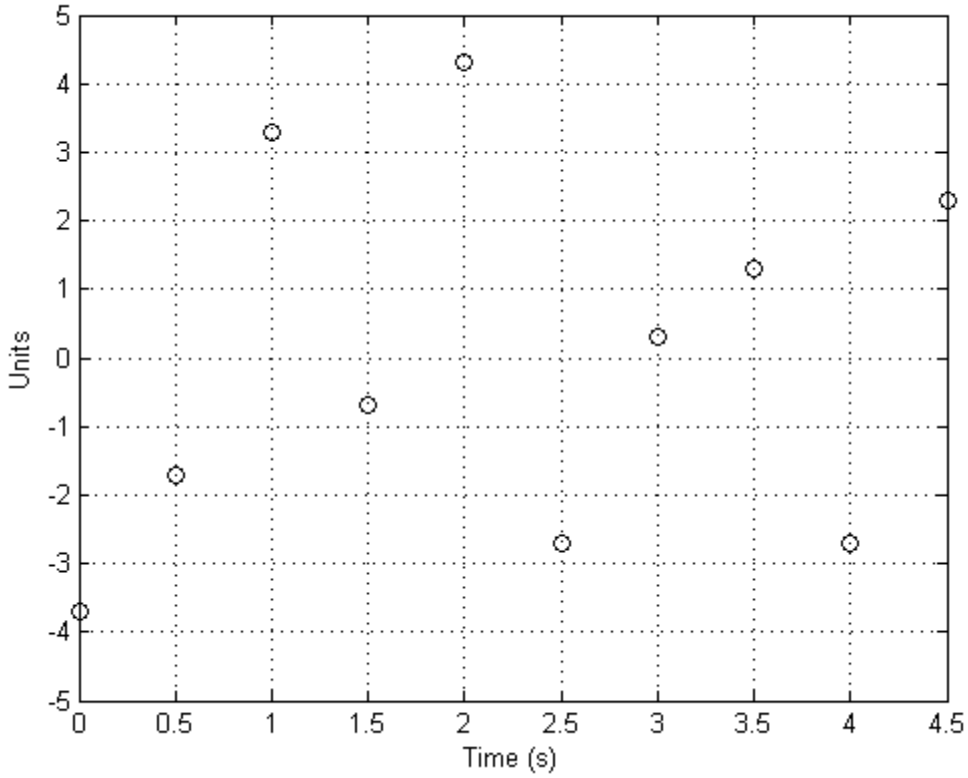

Figure C2. The Sample Signal x with its Mean Removed.

C.3. Third Order AR Model

To determine a 3nd order model, the Levinson-Durbin Algorithm was followed with a model order $M = 3$.

C.3.1. Power Spectral Density (Z Domain)

Once the autoregressive model coefficients and prediction error variance were determined and the corresponding AR synthesis filter, is could then be converted to create the PSD. The equation for the PSD of x (z domain) using a 3rd order AR model is defined by Equation 15. The equation is defined in the z domain which is fine, but is more useful when it is defined in the absolute frequency domain.

$$H_{3,PSD}(z) = \frac{2(57.40)}{|1 + (0.423)z^{-1} + (0.094)z^{-2} + (0.009)z^{-3}|^2} * \frac{1}{(2\ Hz) * (10)} \qquad (15)$$

C.3.2. Power Spectral Density (in Terms of Real Frequency)

The PSD in the z domain was converted to the absolute frequency domain. The conversion from the z domain to the absolute frequency domain is described by Equation 16.

$$z = e^{j\omega T} = e^{j2\pi fT} = e^{j2\pi f/f_s} \tag{16}$$

The conversion was completed and the PSD of x (absolute frequency domain) using a 3rd order AR model is defined by Equation 17.

$$H_{3,PSD}(f) = \frac{2(57.40)}{\left|1 + (0.423)e^{-j2\pi f/(2\ Hz)} + (0.094)e^{-j4\pi f/(2\ Hz)} + (0.009)e^{-j6\pi f/(2\ Hz)}\right|^2} * \frac{1}{(2\ Hz) * (10)} \tag{17}$$

Because the sampling frequency was 2 Hz, by Nyquist's Theorem, the highest frequency that can be plotted is 1 Hz. The resulting PSD vs absolute frequency plotted over a continuous range of frequencies is shown in Figure C3.

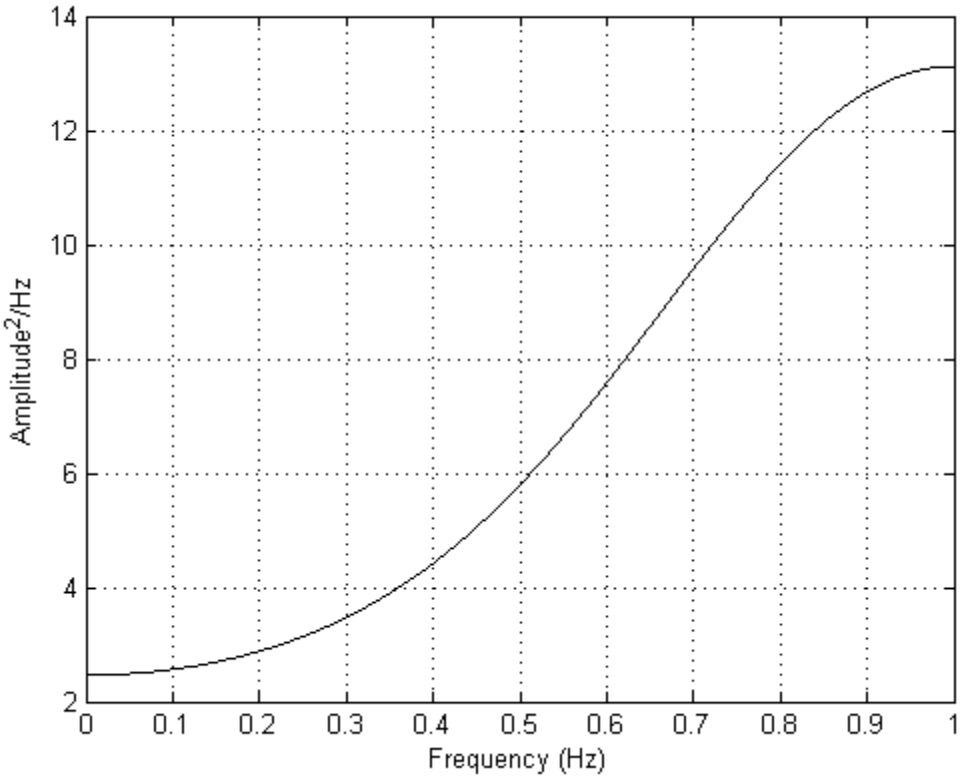

Figure C3. The Power Spectral Density (PSD) of x Using a 3rd Order Autoregressive (AR) Model. The figure shows the PSD of x using a 3rd order AR model as a function of frequency.

APPENDIX D. VALIDATION OF MATLAB SOFTWARE PACKAGE

Before using MATLAB commands to determine time and frequency domain indices of HRV for a real tachogram, it was necessary to validate the commands by inputting a simple known discrete signal, which was used to simulate a real tachogram, into the system and compare the results with those performed by hand calculations. The following example shows how the time and frequency domain indices of HRV were calculated by hand for a simulated tachogram. It is understood that a real tachogram has data points that are spaced at unequal intervals, but to simplify the hand calculations for time and frequency domain indices of the simulated tachogram, the simulated tachogram is completely periodic and has data points that are all spaced at equal intervals.

D.1. Simulate Tachogram (Create Discrete Waveform)

Let $x(t) = A_1 \sin(2\pi f_1 t) + A_2 \sin(2\pi f_2 t) + A_3\ ms$ for $0 \leq t \leq t_{end}$.

If the continuous signal $x(t)$ is sampled at f_s, the continuous signal becomes the discrete signal

$x[n] = A_1 \sin(2\pi f_1 n\Delta t) + A_2 \sin(2\pi f_2 n\Delta t) + A_3\ ms$ where $\Delta t = \frac{1}{f_s}$ and $0 \leq n \leq \frac{t_{end}}{\Delta t}$.

Let $A_1 = 10$, $A_2 = 20$, $A_3 = 1000$, $f_1 = 0.1\ Hz$, $f_2 = 0.2\ Hz$, $f_s = 0.8\ Hz$, and $t_{end} = 300\ s$. Therefore, $\Delta t = \frac{1}{f_s} = \frac{1}{(0.8\ Hz)} = 1.25\ s$ and $0 \leq n \leq N = \frac{t_{end}}{\Delta t} = \frac{(300\ s)}{(1.25\ s)} = 240$.

D.2. HRV Measures Using Original Tachogram

Once the simulated tachogram was created, it was possible to perform time and frequency analysis on the tachogram, before and after the tachogram was corrected for average heart rate.

D.2.1. Time Domain Indices

To determine the time domain indices, it was necessary to know every data point. Because the waveform is periodic, it is easier to describe all of the points by gaining information

from a single period. To determine the length of one period of the signal, the greatest common denominator of the frequencies in the waveform had to be determined. The greatest common denominator of the two frequencies in the waveform is $f_{GCD} = f_1 = 0.1\ Hz$, therefore one period of the signal is $t_{period} = \frac{1}{f_{GCD}} = \frac{1}{(0.1\ Hz)} = 10\ s.\ 0 \leq n \leq \frac{t_{period}}{\Delta t} = \frac{(10\ s)}{(1.25\ s)} = 8$. The period is 8 units and there are $\frac{241}{8} = \frac{240}{8} + \frac{1}{8} = 30\frac{1}{8}\ periods = 30\ periods + 1\ extra\ point$. The data for the first period is shown in Figure D1.

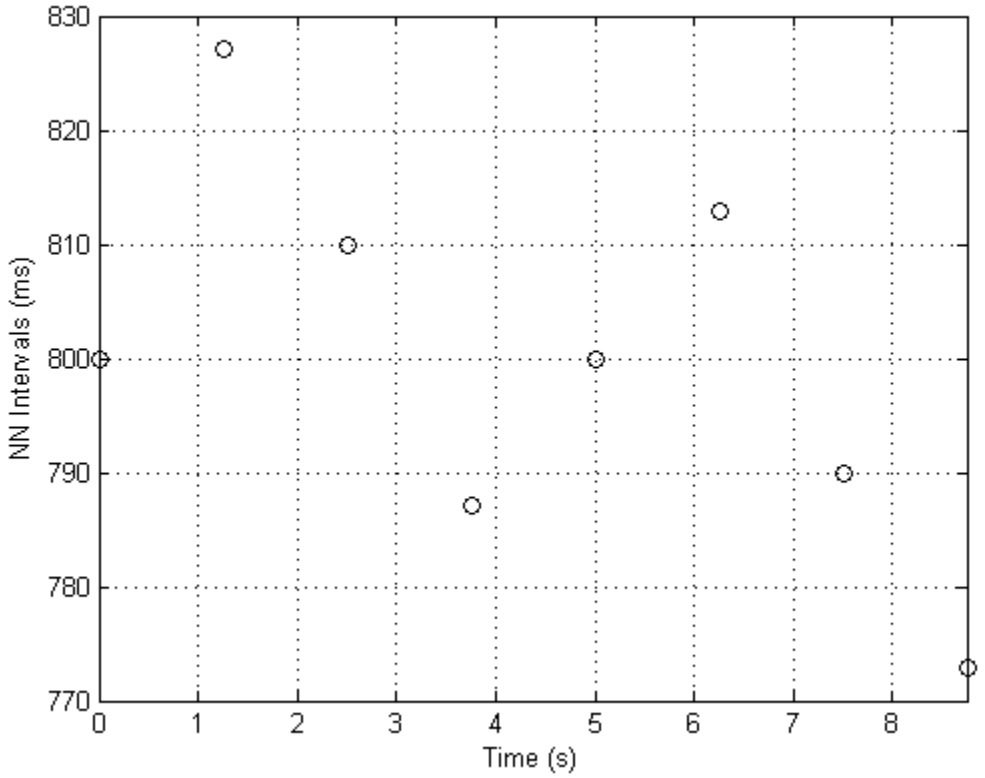

Figure D1. The First 8 Points of the Simulated Tachogram (NN Intervals vs Time). The figure shows the first period (8 points) of the simulated tachogram.

For some of the time domain indices, it was necessary to determine the ΔNN intervals. Much like the tachogram, the ΔNN vs time data is also periodic. The period is still 8 units,

however the number of periods is different because there is less data points. There are $\frac{240}{8} = 30\ periods$. The data for the first period of the ΔNN intervals vs time is shown in Figure D2.

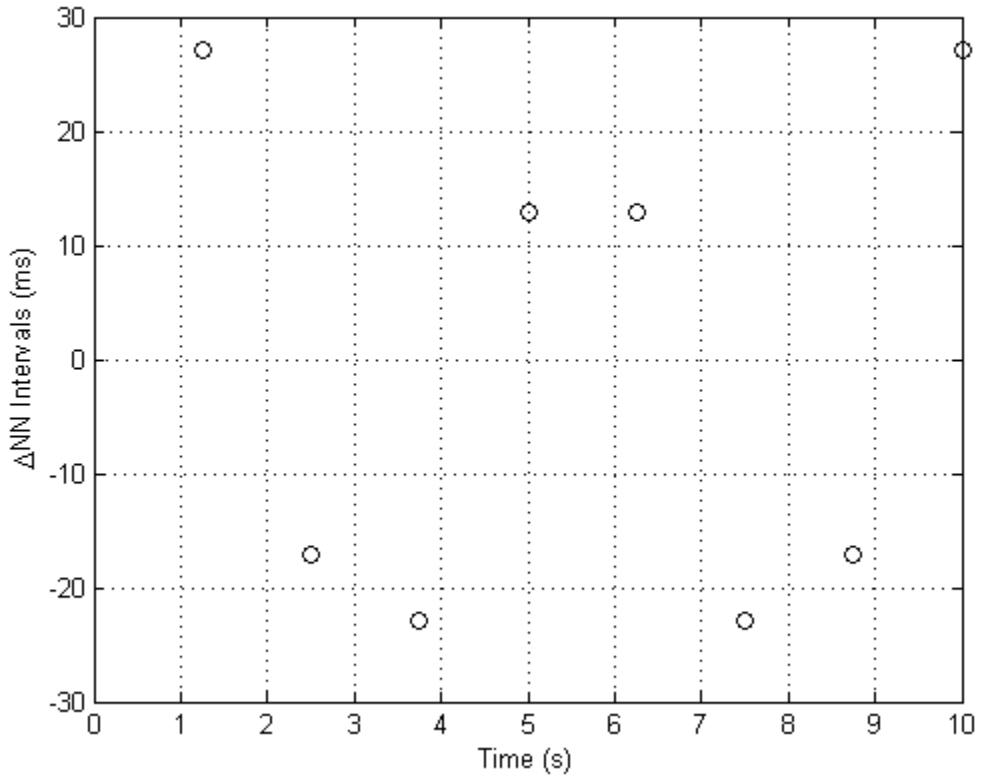

Figure D2. The ΔNN Intervals vs Time of the Simulated Signal.

D.2.2. Frequency Domain Indices

The total power was calculated by determining the variance of all the data points. The VLF, LF, and HF power were determined by looking at the frequencies present in the signal (0, 0.1 and 0.2 Hz as declared when the simulated signal was created) and determining the corresponding power present at each frequency. The power at 0 Hz is always zero because the mean is removed before performing frequency domain analysis. The power at the nonzero frequencies is defined by Equation 18.

$$Power_f = \left(\frac{Magnitude\ of\ Sinusoidal\ Component\ at\ f}{\sqrt{2}}\right)^2 \quad (18)$$

Once the power at each frequency was determined, it was placed into the corresponding frequency range (VLF, LF, or HF) that it belonged.

D.3. HRV Measures Using Tachogram Corrected for Average Heart Rate

HRV measures as determined by tachogram corrected for mean HR was performed the same way as those determined by the original tachogram. To determine the tachogram that was corrected for mean HR, all of the data points in the original tachogram were divided by the mean NN interval.

D.3.1. Time Domain Indices

The time domain indices were calculated in the same manner as those calculated for the original tachogram. The tachogram that was corrected for mean HR is shown in Figure D3, and the ΔNN intervals vs time from the tachogram that was corrected for mean HR is shown in Figure D4.

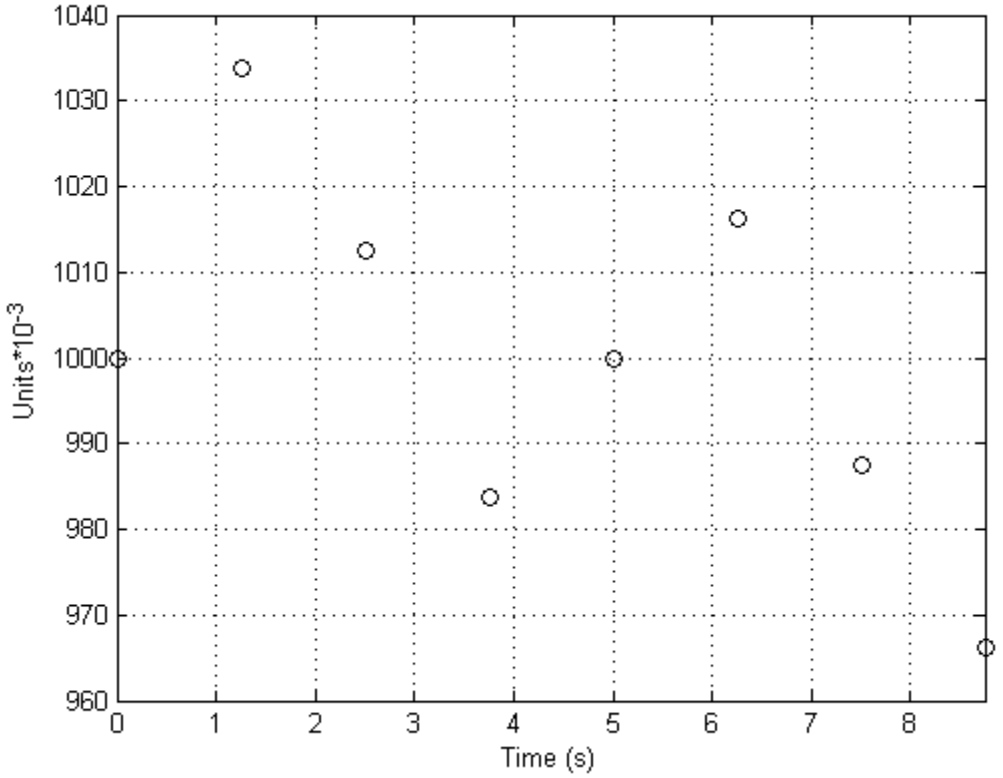

Figure D3. The Simulated Tachogram (NN Intervals vs Time) after Correction for Mean Heart Rate. The figure shows the simulated tachogram after dividing all NN intervals by the mean NN interval.

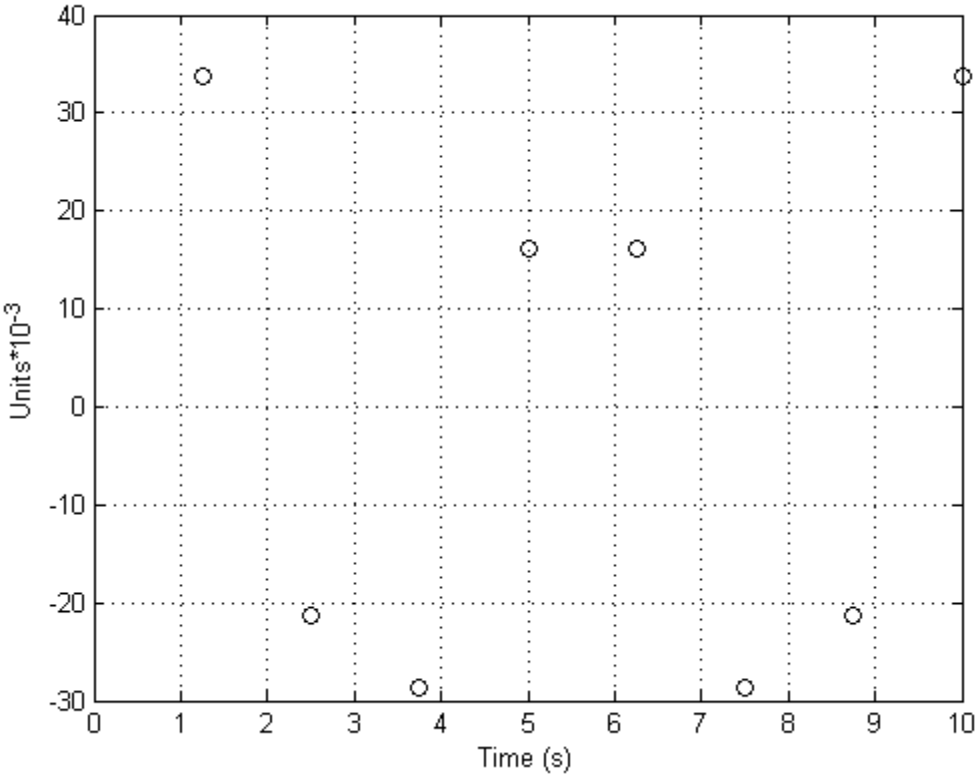

Figure D4. The ΔNN Intervals vs Time after Correction for Mean Heart Rate.

D.3.2. Frequency Domain Indices

Frequency domain analysis for the original tachogram after correction for mean HR was performed in the same manner as frequency domain analysis for the original tachogram.

D.4. Validation

Comparisons between HRV analysis performed by hand to the calculations performed by MATLAB functions were completed for both HRV analysis performed on the original simulated tachogram and the original simulated tachogram after correction for mean HR. Table D1 shows the summary of the amount of data (gained from simulated signal) used for the HRV analysis. Table D2 shows the comparison for HRV analysis performed on the original simulated tachogram and Table D3 shows the comparison for the HRV analysis performed on the original simulated tachogram after correction for mean HR.

Table D1. Comparison of the Amount of Data Used for HRV Analysis of Simulated Tachogram. The star represents an absolute error in cases where the program value was compared to a theoretical value of zero.

Index	Units	Theoretical Value	Program	Percent Error (%)
# of NN	#	241	241	0
# of ΔNN	#	240	240	0

Table D2. The Comparison of HRV Analysis Performed on Original Simulated Tachogram. The star represents an absolute error in cases where the program value was compared to a theoretical value of zero.

Index	Units	Theoretical Value	Program	Percent Error (%)
Min NN	ms	772.9	772.9	0
Max NN	ms	827.1	827.1	0
Mean NN	ms	800	800	0
SDNN	ms	15.811	15.811	0
rMSSD	ms	20.763	20.763	0
Ln(rMSSD)	–	3.03	3.03	0
pNN50	%	0	0	*0
pNN40	%	0	0	*0
pNN30	%	0	0	*0
pNN20	%	50	50	0
pNN10	%	100	100	0
Mean Delta NN	ms	0	0	*0
SD of Delta NN	ms	20.763	20.763	0
Triangular Index	–	3.951	3.951	0
FFT TP	ms^2	250	244.136	-2.3
FFT VLF	ms^2	0	0.568	*0.6
FFT LF	ms^2	50	50.513	1.0
FFT HF	ms^2	200	192.811	-3.6
FFT LF:HF	–	0.25	0.262	4.8
AR TP	ms^2	250	244.136	-2.3
AR VLF	ms^2	0	0.479	*0.479
AR LF	ms^2	50	50.999	2.0
AR HF	ms^2	200	192.422	-3.8
AR LF:HF	ms^2	0.25	0.265	6

Table D3. The Comparison of HRV Analysis Performed on Original Simulated Tachogram after Correction for Mean HR. The star represents an absolute error in cases where the program value was compared to a theoretical value of zero.

Index	Units	Theoretical Value	Program	Percent Error (%)
Min NN	$10^{-3}\ Units$	966.2	966.2	0
Max NN	$10^{-3}\ Units$	1033.8	1033.8	0
Mean NN	$10^{-3}\ Units$	1000	1000	0
SDNN	$10^{-3}\ Units$	19.764	19.764	0
rMSSD	$10^{-3}\ Units$	25.953	25.953	0
Ln(rMSSD)	–	3.26	3.26	0
pNN50	%	0	0	*0
pNN40	%	0	0	*0
pNN30	%	25	25	0
pNN20	%	75	75	0
pNN10	%	100	100	0
Mean Delta NN	$10^{-3}\ Units$	0	0	*0
SD of Delta NN	$10^{-3}\ Units$	25.953	25.953	0
Triangular Index	–	3.951		
FFT TP	$10^{-6}\ Units^2$	390.625	381.46	-2.3
FFT VLF	$10^{-6}\ Units^2$	0	0.888	*0.888
FFT LF	$10^{-6}\ Units^2$	78.125	78.927	1.0
FFT HF	$10^{-6}\ Units^2$	312.5	301.27	-3.6
FFT LF:HF	–	0.25	0.262	4.8
AR TP	$10^{-6}\ Units^2$	390.625	381.46	-2.3
AR VLF	$10^{-6}\ Units^2$	0	0.748	*0.748
AR LF	$10^{-6}\ Units^2$	78.125	79.686	2.0
AR HF	$10^{-6}\ Units^2$	312.5	300.66	-3.8
AR LF:HF	–	0.25	0.265	6

There was no error present in the time domain indices, however there was some nonzero error in all of the frequency domain indices. The following discussion explains why it was expected that there was a small, nonzero error in the frequency domain indices. The total power

of the signal, when performing hand calculations, was calculated by determining the variance of the signal, because the total power of a signal is equal to the variance of a signal. The total power of the signal, when using MATLAB, was calculated by performing an interpolation by creating a cubic spline and re-sampling it at 2 Hz, and then integrating the corresponding PSD from 0 to 1 Hz. The total power, as determined by the integration from 0 to 1 Hz should be approximately equal to the integration over all frequencies, which is known to be equal to the variance of the interpolated signal. It makes sense that the variance of the original signal and the interpolated signal will be close, but not equal, because of the interpolated signal does not consist of exactly the same data as the original signal since it is a different representation of the original signal, hence the error.