TRACKING R-R INTERVAL DYNAMICS BETWEEN MEN AND WOMEN DURING ORTHOSTASIS USING TIME-FREQUENCY DISTRIBUTION

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ABSTRACT OF THESIS

TRACKING R-R INTERVAL DYNAMICS BETWEEN MEN AND WOMEN DURING ORTHOSTASIS USING TIME-FREQUENCY DISTRIBUTION

To track evolution of autonomic responses during orthostasis in men and women, we used discrete pseudo-Wigner distribution based time-frequency analysis to compute dominant frequencies and spectral powers in RR intervals and Systolic Blood Pressure (SBP). Data were collected from 38 healthy volunteers (22 men, 16 women) during 10 min supine posture followed by 30 min of 70° head up tilt. The RR intervals were computed from ECG and systolic blood pressure was and spectral amplitudes of RR intervals were integrated in two regions viz., Low Frequency (LF) region defined between 0.05-0.15 Hz and High Frequency region (HF), sometimes referred to as respiratory frequency region, defined as mean breathing frequency of the individual +/- one standard deviation. Dominant frequencies of RR intervals in the LF region decreased in both men and women. There were no significant differences between men and women as far as the SBP data were concerned for the dominant frequencies, however women had higher values than men. Dominant frequencies of RR intervals in the HF region increased both in men and women from supine to tilt. No significant differences in dominant frequencies were found between men and women. Also there were no significant differences even for the SBP data, however men had higher values than women. Integrated powers within the auto spectra of RR showed that in the HF region, power decreased significantly for both men (p<0.005), and women (p<0.001) during tilt compared to supine. However, the HF power in women was significantly higher for men during both supine (p<0.001), and tilt (p<0.005). In LF region integrated power spectrum showed no significant difference between men and women although women showed a slight increase from supine to tilt. These results suggest that men have a higher sympathetic control while women have greater para-sympathetic influence.

KEYWORDS: Autonomic responses, Wigner distribution, dominant frequencies, spectral power, Para-sympathetic

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04/19/2007
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THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering in the Graduate School at the University of Kentucky

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Chapter One: Introduction

Variations in RR intervals, more frequently referred to as heart rate variability, have been extensively investigated as indicators of autonomic neural function in cardiovascular function. Many factors are thought to be involved in the variation of RR intervals such as gender, age, cardiovascular and neuro-pathological conditions, respiration, mental and physical stress[1],[7],[2],[3]. Spectral analysis of heart rate has been extensively used by several researchers to assess the control of autonomic nervous system on the heart. More widely used analyses such as spectral estimates do provide an average estimate of autonomic function, but do not include information about changes in frequencies and amplitudes as orthostasis proceeds. Time-frequency analysis has the flexibility of tracking the dynamics as it evolves in time during an experiment [4] [5]. Our objective in the present study was to track the dynamics of RR interval changes and investigate any differences in the dynamics between men and women in the low Frequency (LF) and respiratory frequency, also called High frequency (HF) region of the RR interval spectra[6]. We obtained time-frequency representations of RR intervals using a discrete pseudo-Wigner distribution algorithm [15]. We estimated dominant frequencies and dominant power spectral amplitudes of the RR interval in LF and HF regions. Dominant frequencies revealed the RR interval fluctuations in LF and HF regions[7]. Normalized dominant amplitudes and integrated spectral powers in LF and HF regions revealed the sympathetic and parasympathetic influences respectively which are in good agreement with previous studies by Pomeranz et al[8]. It has been shown that women have
higher average heart rate variability than men[9, 10] in supine and tilt. Therefore we wanted to explore whether the time-course of changes in RR interval variability was different between men and women. Differences between genders in terms of heart rate variability have been investigated for a shorter period of time [20], [9], [9] In the present study we investigated differences in RR interval variability throughout the orthostatic study which was about fifty minutes. The tilt-table test is considered a standard for assessment of autonomic function in the cardiovascular system[5]. Furthermore, it is widely accepted that HF power is an indicator of vagal control and LF power is a result of a complex interplay between vagal and sympathetic control of the heart[11]. As stated above, our objective in the present study was to compute the integrated spectral amplitudes and dominant frequencies of the RR intervals in LF and HF regions during the time course of a head up tilt study and to determine the differences observed in these variables between men and women. Our goal was to determine the influence of autonomic nervous system over the control of heart rate.
Chapter Two: Background

2.1 Heart Rate Variability:
Heart rate variability (HRV) refers to the beat-to-beat alterations in heart rate. Under resting conditions, the ECG of healthy individuals shows a rhythmic variation in RR intervals. This rhythmic phenomenon is also sometimes referred to as respiratory sinus arrhythmia, which fluctuates with the phase or cycle of the respiration. Though respiratory sinus arrhythmia represents a major component in HRV, it has other rhythms as well.

Heart rate variability came to light during 1965 by Hon and Lee when they observed fetal interbeat intervals.[12] About two decades ago Sayers and others focused attention on the existence of physiological rhythms in the beat-by-beat heart rate signals.[13] Ewing et al during 1970 devised a number of simple bedside tests of short-term RR variability to detect autonomic pathology in diabetic patients.[14]. Askerold was the first one to introduce power spectral analysis of heart rate fluctuations to assess cardiac autonomic function[15]. These were referred to as frequency domain analysis since a spectral computation resulted in frequency values. The clinical significance of Heart rate heart rate variability was established in late 1980s where it was considered a predictor of cardiac mortality after myocardial infarction[16]. Studies pertaining to heart rate variability increased with advancement in data acquisition and analysis devices when collection of data and analysis of the same became easier for researchers.
2.2 Time-Frequency Analysis:
In the light of frequency domain analysis of cardiovascular variables an important technique called time-frequency analysis emerged the use of which for heart rate variability was pioneered by Vera Novak and Peter Novak in the early 1990s[5]. Spectral analysis is associated with the magnitudes of power at certain frequencies while observing these frequencies over a certain course in time yields time-frequency analysis. It soon became an important tool in assessing cardiac autonomic function since it provided the flexibility of establishing frequencies and spectral powers of RR interval fluctuations, systolic blood pressures and respiration with respect to time.
Several methods were tried and tested viz., Choi Williams transforms, short time Fourier transforms but Discrete Wigner distribution was one of the more widely used techniques for analysis of time frequency distributions in the area of heart rate variability.[17],[7],[5],[18].
Chapter Three: Methods

Data were collected from 38 normal subjects, mean age 26 +/- 6 years (range 19-42 years, 16 female, 22 male). The Institutional Review Board at the University of Kentucky approved all studies. Each subject gave written informed consent. To elicit orthostasis we used passive 70 degrees head up tilt and thus investigate the changes in cardiac autonomic function. Figure 1 shows the study protocol, which consisted of 10 minutes of supine followed by 30 minutes of tilt which was followed by 10 minutes of supine rest. The moment any of the subjects developed symptoms such as dizziness, nausea, sweating or any other discomfort, which are also called pre-syncopal symptoms, they were asked to inform us about the same. A medical monitor also monitored ECG, BP and subject’s condition throughout the tilt test to determine onset of pre-syncopal symptoms even if the subject did not report development of these symptoms. The protocol included immediate termination of tilt if a subject developed pre-syncopal symptoms; however none of the subjects developed any presyncopal symptoms.

Non-invasive continuous blood pressure measured at the finger level using Finapress and lead II electrocardiogram were used to compute systolic blood pressures (SBP) and RR intervals as described previously [19]. We used an arm sling to keep the finger probe at the heart level.
Figure 1. Schematic of the experimental protocol. The figure shows the three sections of the study: Supine Control; Tilt and Supine Rest.

Figure 2. An example segment of a time series of simulated data. The thick black line indicates the mid-point of the segment where it is folded and multiplied.
Figure 3. Resulting signal after point-by-point multiplication of the first half of the segment in Figure 2 with the mirror image of the second half of the same segment.
Chapter Four: Analysis

All data were digitized at 500 Hz. A threshold detection algorithm was used to compute RR intervals from the ECG. In order to compute time frequency estimates the resulting piecewise RR interval data was resampled at 5Hz. Also the peaks of the blood pressure data within each cardiac cycle were stored to get values for SBP. We used Discrete Wigner distribution algorithm to compute time frequency estimates. The following equation describes a Discrete Wigner distribution.

\[
DWD (r, f) = \sum h(r) e^{-j2\pi fr} s^*(t-1/2r) s (t+1/2r) \, dr
\]

\(r, f\) are time and frequency indices respectively and \(s(r)\) is the analytic signal which we want to analyze. The analytic signal is obtained from the original time series data by using the Hilbert transform. This signal will then have no negative frequency components in the Fourier domain while using the DWD. Thus, DWD of this analytic signal will reduce the interference between positive and negative frequencies[17], [18].

In the procedure used in this study, a long time series was divided into smaller segments of length 100 sec and each segment was detrended and windowed with 50 sec hamming window. After windowing, the segment was divided into two halves, the first half was multiplied by the mirror image of the second one. After applying Fourier transform and taking the absolute value, the index (dominant frequency) and the corresponding peak (dominant amplitude) of the Fourier Transform of the signal were stored in an array. For the integrated auto-spectral computations we used Welch method in which the data were divided into 100 sec
segments with 50% overlap using a Hamming window of the same length. Hence, dominant frequency and the corresponding dominant amplitudes were plotted against time. In order to verify the method we generated simulated signals with known frequencies and applied the method described above to check whether the detected frequencies were in agreement to that in the simulated signals. Figure 2 and figure 3 illustrates the technique employed in DWD.

To generate a simulated test signal we explored the area of frequency modulation. So, a signal whose frequency was modulating in a sinusoidal manner was generated and tested. Therefore in order to generate such a signal, consider a sinusoidal modulated sinusoidal modulating wave defined by

\[ m(t) = A_m \cos(2\pi f_m t); \quad \text{where } f_m \text{ is the modulating frequency (FM)} \]

Now the argument of the FM wave

\[ \theta(t) = 2\pi f_c t + \beta \sin(2\pi f_m t) \quad \ldots \ldots \ldots \ldots \ldots \ldots (I) \]

\[ f_c \text{ is the frequency of the carrier wave} \quad \beta \text{ also called modulation index}. \quad [29] \]

Equation (I) is the argument of the FM wave. The signal that has a sinusoidal variation in frequency is obtained by taking cosine of (I). Now our FM signal for sinusoidal modulation will be \( \cos(\theta(t)) \) i.e. \( \cos(2\pi f_c t + \beta \sin(2\pi f_m t)) \) Here, in the above signal a \( f_c \) Hz carrier is oscillating about the modulating index value \( \beta \). \( \beta \) is a scaling factor and the frequency of the above signal varies as \( f_c +/- \beta \). Therefore if we generate a signal with known values of carrier frequency and
the scaling factor $\beta$, we can easily predict the frequency of the generated wave before doing any analysis. The dominant amplitudes were determined as the values of magnitude of spectral power occurring at the dominant frequencies. As described earlier, in this study we were interested in dominant frequencies and dominant amplitudes of the RR intervals in LF and HF regions, so we decided to generate and test two simulated signals in LF and HF regions. First, LF test signal with frequency varying from a minimum of 0.05 Hz to 0.15 Hz was generated using the method described above. Now, the generated test signal was divided into smaller segments and each segment was analyzed employing DWD technique. 100 sec data segments with 50% overlap were used in this analysis. After removing mean of the entire segment from each point in the segment a Hamming window of length 50 sec was used for spectral smoothing. The dominant frequencies were expected to oscillate from 0.05 Hz to 0.15 Hz in a sinusoidal waveform since the simulated LF test signal was derived from a sinusoidal modulating waveform.

Using (I) we can derive an LF test signal whose equation is $\cos(2\pi 0.1 t + 0.05 \cos(2\pi 0.001 t))$. We can expect that frequencies of this signal will vary from 0.05 Hz to 0.15 Hz. The LF test signal is shown in figure 4.

After applying the Discrete Wigner Distribution, as expected the dominant frequencies changed from a minimum of 0.05 Hz to a maximum of 0.15 Hz in a sinusoidal manner shown in figure 5.

Similarly, using (I) we can derive an HF test signal whose equation is $\cos(2\pi 0.27^t + 0.07 \cos(2\pi 0.001 t))$. Again, here we can expect that frequencies of this signal will vary from 0.2 Hz to 0.35 Hz. The HF test signal is shown in figure 6.
Again, after applying Discrete Wigner Distribution on the HF test signal, as expected the dominant frequencies changed from a minimum of 0.2 Hz to a maximum of 0.35 Hz as shown in figure 7.

RR intervals were then used to compute the dominant frequencies and amplitudes. As discussed earlier analytic signal was used from the original time series data. Again 100 sec data segments with 50% overlap and Hamming window were used in the algorithm. Since we are interested in tracking the RR interval fluctuations in LF and HF regions, we used a low pass filter with a cut off at 0.12 Hz for LF DWD detection. For HF DWD detection we first low pass filtered the data at 0.5 Hz and high passed the resulting data series with a cut off at 0.12 Hz. Since we used the data resulting from filtering operation and ‘forced’ them to be in the LF and HF regions we did not expect any interference or cross terms effect in using the DWD technique.

In an effort to further validate our results with the method we used we computed auto spectral amplitudes in each segment viz., supine control and tilt. These data were used to compare the average of the spectral amplitudes measured by the present analysis method. The auto spectral values showed a similar trend observed by taking the average of the values in each segment of dominant amplitudes computed along the entire experiment.

All the analysis was performed using MATLAB 7.0.1. Statistical significance was established using two factor ANOVA. One factor was tilt and the other was gender. Wherever there was significance it was tested using t-test. Since we were interested in gender differences, probabilities were computed at each time
instant the dominant frequencies and amplitudes were estimated between all men and women using t-test. Significance was accepted at a level of $P<0.05$.

Figure 4. An example of Low Frequency Test Signal
Figure 5. Contour plot of the resulting DWD of the LF test signal *-Dominant Frequencies

Figure 6. An example of HF test Signal
Figure 7. Contour plot of the resulting DWD of the HF test signal - Dominant Frequencies
Chapter Five: Results

FREQUENCIES:
Dominant frequencies in the LF region in RR intervals decreased both in men and women from supine to tilt and further decreased from tilt to supine control, although women had higher frequencies than men (P>0.05). Men had an average dominant frequency of 0.066+/-0.001 Hz in supine, which decreased to 0.06+/-0.001 Hz while women had 0.073+/-0.002 Hz in supine, which decreased to 0.07+/-0.002 Hz in tilt. The average dominant frequencies were calculated for each segment in the experiment. For example in supine control all the values of dominant frequencies were averaged to get an average dominant frequency in supine control and so forth. In the HF region, dominant frequencies increased both in men and women from supine to tilt. Men showed an average dominant frequency of 0.219+/-0.008 Hz in supine that increased to 0.224+/-0.009 Hz while women had an average dominant frequency of 0.214+/-0.009 Hz during supine, which increased to 0.222+/-0.007 Hz in tilt. The change in HF dominant frequencies was not statistically significant. Probabilities were calculated along the duration of the experiment to assess the significance between men and women during orthostasis. We found that the dominant frequencies in the LF region were not significantly different between men and women during the supine segment but during the first ten minutes of tilt there was a significant difference between men and women. And the significance was not clearly established afterwards due to the fluctuation of P values later in the experiment. In the HF region dominant frequencies were not significantly different between men and
women except for occasionally P value being less than 0.05. Both men and women had similar values of dominant frequencies in the HF region with no clear difference. For SBP values, Dominant Frequencies in the LF region were not significant between men and women in any part of the experiment. However, women had higher values than men. Dominant Frequencies in HF region again showed no significant differences between men and women however, men had higher values than women

AMPLITUDES:
Integrated powers within the auto spectra of RR showed that in the HF region, power decreased significantly for both men (p<0.005), and women (p<0.001) during tilt compared to supine. The HF region was defined for each subject individually as mean breathing frequencies for the individual subjects +/- one standard deviation. A two-way ANOVA was used to assess significance. However, the HF power in women was significantly higher than men during both supine (p<0.001), and during tilt (p<0.005). Results from the integrated power spectrum shows that in the HF region integrated spectra decreased significantly for both men (p<0.005) and women (p<0.001) from supine to tilt. However women had significantly higher average spectra in the HF region than men in supine (p<0.001) and tilt (p<0.005). In the LF region integrated power spectrum showed no significant difference between men and women although women showed a slight increase from supine to tilt.
Table 1. Dominant frequencies (Hz) in LF region. Data represents average of 22 men and 16 women with standard deviations

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<th></th>
<th>Supine</th>
<th>Tilt</th>
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<tr>
<td>Men</td>
<td>0.066 +/- 0.001</td>
<td>0.06 +/- 0.001</td>
</tr>
<tr>
<td>Women</td>
<td>0.073 +/- 0.002</td>
<td>0.07 +/- 0.002</td>
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Table 2. Dominant frequencies (Hz) in HF region. Data represents average of 22 men and 16 women with standard deviations

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<td>Women</td>
<td>0.214 +/- 0.009</td>
<td>0.222 +/- 0.007</td>
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**Normalized Amplitudes:**

Normalized amplitudes were considered for both LF and HF regions. For example dividing LF amplitudes by the combined sum of LF and HF amplitudes would give normalized LF amplitudes and vice versa. Normalized Dominant Amplitudes in the LF region were greater in men than women during supine control while there was no significant difference between them during tilt and supine recovery after tilt. Normalized Dominant Amplitudes in HF region were greater in women (0.04688 +/- 0.0151) than in men (0.0225 +/- 0.0121) during
supine control and during first 10 min of tilt. (P<0.05). During supine control women had higher HF amplitudes than men with significance observed during first two minutes (P<0.05). As described above, P values were calculated along the entire duration of the experiment to establish significance. As far as Normalized LF amplitude values are concerned, during the first three minutes of supine there was no significance observed. Later in supine until the tilt, men showed significantly higher amplitudes than women. During tilt there were no significant differences between men and women except in the last three minutes. Normalized HF amplitudes were higher in women than men throughout the experiment. Except for the first minute during supine, women had significantly higher HF amplitudes than men. During tilt, women had significantly higher dominant HF values than men for the first ten minutes. As far as SBP is concerned dominant Amplitudes in LF region were greater in men than women after 20 min of the tilt and until the end of the tilt. Dominant Amplitudes in the HF region were greater in women than in men after 20 min of tilt till the end of the experiment with significant difference between them (p<0.05).
Figure 8. Comparison of Dominant Frequencies in LF region between Men and Women. Women had higher frequencies than men during supine control (P>0.05)

Figure 9. Comparison of Dominant Frequencies in HF region between Men and Women
Figure 10. Comparison of Normalized LF Amplitudes between Men and Women

Figure 11. Comparison of normalized dominant HF amplitudes between men and women
Figure 12. Average Autospectra of RR intervals between 22 men and 16 women during supine.

Figure 13. Average Autospectra of RR intervals between 22 men and 16 women during tilt.
Chapter Six: Discussion

There has been no index which could be described as a gold standard for the autonomic balance and what dynamics of the RR interval can be the best reflection of sympathovagal balance [20]. Therefore heart rate variability has been accepted as the closest measure to assess the autonomic control of the heart rate. It is generally accepted that power in the high frequency region also called the respiratory frequency region is a marker of vagal modulation.[9] Quantification of parasympathetic control is achieved by calculating spectral power in the HF region, while that of spectral power in LF region is influenced by both sympathetic and parasympathetic controls. Therefore the ratio of LF and LF plus HF power is also referred to as the normalized LF power which indicates sympathovagal balance [21]. The primary findings of our study revealed that along the course of the experiment, normalized HF power i.e. HF/LF ratio was higher in women than men and the normalized LF power i.e. LF/HF power ratio was higher in men. Dominant frequencies in LF region were higher in women while in HF region there was no significant difference between men and women. Novak et al observed that in the respiratory frequency region there was a rapid decrease in the frequency of the RR intervals by using the DWD analysis[5]. On the contrary we observed a slight increase in the frequency of the RR intervals both in men and women during tilt though the increase was not found to be significant. This might be because of the tilt duration analyzed by them, which was less (approximately five min) compared to us that were approximately thirty
minutes. Also the data we show represents an average of at least sixteen subjects. They observed that the frequency below 0.2 Hz was approximately stable at 0.05 Hz. Consistent with their studies we observed a frequency of about 0.06 Hz in men and 0.07 Hz in women in the LF region. The fact that the frequencies did not change as indicated by small changes in frequency around a constant value indicates that in the LF region frequency of the RR intervals was more or less stable.

Pikkujamsa et al observed that women have lower LF to HF ratios than men, which is in very good agreement with our studies. We observed similar results in terms of LF to HF ratios which were observed in previous studies as well[21-23]. Recent studies concluded that women have higher parasympathetic control on the heart rate based on higher ratios of HF to LF power than men which is again in good agreement with our studies [23]. These studies used auto spectral estimates to compute the normalized LF and HF powers. Greater LF to HF power in men suggests a shift of dominance towards sympathetic influence in the sympathovagal balance as reported in the previous studies [23]. Consistent with our study, Ryan et al concluded that women have higher vagal high frequency power than men using spectral analysis and approximate entropy methods of 8 minute ECG segments during quiet and metronomic breathing in 27 women and 40 men and that these differences are pronounced in young and middle-aged subjects [21]. Bigger et al showed that men had significantly higher LF/HF power than women[24]. Although the previous studies did not observe these differences
between men and women during orthostasis, it gives a general idea as to how gender difference could affect the heart rate variability. Freitas et al, during their study involving 70-degree head-up tilt to investigate gender differences, observed that women had higher vagal activity than men without any regard to the posture, which is in agreement with our study. The ratio of HF to LF power was high both in men and women during supine and it decreased after tilt with a simultaneous increase in ratio of LF to HF power indicating a compensatory mechanism in the face of stress i.e. orthostasis. Though this increase was not significantly higher in men than women, it indicates sympathetic dominance. Recent unpublished studies by us involved estimating coherencies and transfer functions estimates between systolic blood pressures and RR intervals to investigate gender differences. These studies revealed that women did not have sufficient baroreflex reserve in terms of engagement as indicated by no further increase in coherence during tilt from supine. Transfer function estimates revealed that both men and women had higher baroreflex sensitivity in tilt however the degree of engagement did not change in women. Whether the dominance of parasympathetic regulation in women or lesser baroreflex engagement in women is the reason as to why young women are being more susceptible to conditions like syncope needs to be explored [25]. In all the observations we made, there was no significant difference between men and women for the first two minutes or so. This might indicate anxiety/nervousness in all the subjects before the tilt started.
Our study cannot be generalized to all men and women because of the following factors. We included subjects in the age range of 19 to 42, which represents young subjects. Effects could be different for subjects aged greater than 42 especially women since they attain menopause during late forties.[26] The production of estrogen may play a significant role in the heart rate variability in women and declining secretions of estrogen after menopause could have different effects on our study. Another limitation is that the recruitment of women subjects in our study was random and independent of their menstrual cycles. Ryan et al and others observed that the heart rate dynamics reduces with age though their sample size was small.[10, 27, 28]. As stated in the introduction other factors such as physical and mental stress, physical fitness of the individuals could affect the results. Though the subjects were healthy without any pathological condition we cannot assess the level of stress they might have undergone and each individual might have his/her own fitness levels that could influence the results.
References


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