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Spectral Analysis of Heart Rate Variability in Women

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1. Introduction

This chapter discusses heart rate variability (HRV) to understand autonomic mechanisms and the use of linear analysis tools for frequency domain measures of HRV and spectral analysis by fast Fourier transform (FFT), and describes some results found in women.

Heart activity is largely modulated by the autonomic nervous system (ANS), which promotes rapid adjustments in the cardiovascular system during different stimuli (i.e., physical exercise, mental stress and postural change) (Hainsworth, 1998). HRV is a non-invasive measure used to analyze the influence of the autonomic nervous system on the heart, providing information about both sympathetic and parasympathetic contributions to consecutive heart rate (HR) oscillations. It has been proposed that a decrease in HRV is a powerful predictor of morbidity and mortality resulting from arrhythmic complications. HRV decreases with age (Catai et al., 2002; Melo et al., 2005) as a consequence of parasympathetic reduction and predominance of sympathetic modulation (Lipsitz et al., 1990; Longo & Correia, 1995; Akselrod, 1995).

The tool most commonly used in the frequency domain is spectral analysis, which consists of decomposing the HR variation in a given period into its fundamental oscillatory components, defining them by their frequency and amplitude. One of the mathematical algorithms most commonly used to determine the number, frequency and amplitude of these components is the FFT. The sum of all the components constitutes the so-called total power spectral density. Spectral analysis involves three distinct spectral components: 1) very low-frequency (VLF) fluctuations related to the renin-angiotensin system and thermoregulation; 2) low-frequency (LF) fluctuations related to the sympathetic and parasympathetic nervous systems and to baroreflex activity; and 3) high-frequency (HF) fluctuations associated with vagal activity (Longo & Correia, 1995; Task Force, 1996). The sympathovagal balance can also be expressed by the LF/HF ratio. Based on this analysis, it is possible to observe the predominance of one component over the other and the relationship between them, reflecting the autonomic modulation of the heart in the control of HR.

Given the importance of the autonomic nervous system to cardiovascular health, several analytical measures, grouped into linear and non-linear methods, can be used to assess HRV. The ECG is recorded with the subject in a steady state (when rhythms are stationary) for a sufficiently long period to determine events occurring within the frequencies of interest. R-R interval spectral power is calculated from this series of intervals using an autoregressive algorithm, which yields center frequencies and absolute power of component fluctuations (Task Force, 1996).

Sympathovagal balance (in dimensionless units) is simply the ratio of absolute LF to absolute HF power, or the LF/HF ratio. The literature on sympathovagal balance is replete with disclaimers that spectral power reflects fluctuations, not absolute levels of autonomic nerve traffic (Akselrod 1995). If mathematical manipulation of R-R interval spectral power is to inspire confidence as a robust, reliable metric, it must be grounded solidly on physiological principles. It must stand on its own and calculations of sympathovagal balance may obscure rather than illuminate human physiology and pathophysiology (Eckberg 1997).

This chapter discusses the measurement and analysis of HRV, as well as results of data for women and the relationship between aging and hormonal changes (oral contraceptives and hormone replacement therapy), which contribute to modifications of the autonomic control of the heart. Each item will be discussed in a separate subitem of this chapter.

2. Measurement of heart rate variability

An electrocardiogram and HR data were obtained using a one-channel heart monitor (MINISCOPE II Instramed, Porto Alegre, RS, Brazil) and processed using a Lab. PC+ analog-to-digital converter (Lab PC + / National Instruments, Co., Austin, TX, USA) acting as an interface between the heart rate monitor and a microcomputer. The ECG signal was recorded in real time after analog-to-digital conversion at a sampling rate of 500 Hz and the R-R intervals (ms) were calculated on a beat-to-beat basis using specific software (Silva et al., 1994). To evaluate the effect of body position on the HR response and its variability, R-R intervals were recorded over a 15-min period under resting conditions with the subjects in the supine and sitting positions, respectively.

HR and R-R intervals (RRI) can be obtained in real time, beat-by-beat, using the ECG and specific software (Silva et al., 1994). First, a visual inspection of RRI (ms) distribution obtained during 900s of collection at rest in the supine condition was carried out in order to eliminate the fragments containing spikes, which resulted in an interval with higher stability of ECG RRI tracing (Task Force, 1996).

3. Spectral analysis

Linear HRV can be assessed by frequency domains. For the frequency domain, a spectral analysis was performed by FFT applied to a single window after the subtraction of a linear trend, at the R-R intervals previously chosen. The power spectral components were obtained at low (LF: 0.04 to 0.15 Hz) and high (HF: 0.15 to 0.4 Hz) frequencies, in absolute units (ms^2), and the normalized units (nu) were computed by dividing the absolute power of a given LF or HF component (ms^2) by the total power minus very low frequency (0.003-0.04 Hz) power

and then multiplying this ratio by 100. Since the LF band is modulated by both sympathetic and parasympathetic activity and the HF band is correlated with vagal cardiac control, the LF/HF ratio was calculated to determine the sympathovagal balance (Task Force, 1996). Sympathovagal balance is the ratio between LF and respiratory-frequency powers. Based on this analysis, it is possible to determine the predominance of one component over the other and the relationship between them, reflecting the autonomic modulation of the heart in the control of heart rate.

Figure 1, which is based on an autoregressive model, illustrates the HRV power spectra at rest in the supine and sitting positions of a representative subject in different conditions.

Fig. 1. Power spectral density of heart rate variability of a representative subject from the groups of young women (A and B), and postmenopausal women undergoing (C and D) and not undergoing (E and F) estrogen therapy, obtained at rest in the supine and sitting positions, respectively. Spectral components are shown as LF (0.04 to 0.15 Hz), HF (0.15 to 0.4 Hz) and VLF (below 0.04 Hz). (adapted by Neves et al., 2007)

Figure 2 illustrates the analysis of the RRI (ms) of a volunteer at rest in the supine position, using the power spectrum of the autoregressive model for a better view of the spectral components. Three spectral frequency bands were obtained: 1) VLF, corresponding to frequencies varying from 0 to 0.04 Hz; LF, corresponding to the interval of 0.04 Hz to 0.15 Hz; and AF, corresponding to the interval of 0.15 Hz to 0.40 Hz. The LF and HF components are expressed in normalized units (UN) which correspond to the percentage of the total power spectrum subtracted from the VLF component. These components were also expressed as the ratio between the absolute areas of low and high frequency (LF/HF ratio), which is indicative of the vagosympathetic equilibrium. Figure 2 illustrates the temporal series of the RRI corresponding to the 256 values of analysis selected previously.

Fig. 2. Temporal series of 256 values of R-R intervals (ms) of a volunteer in the supine position

Because the HR presents fluctuations that are, in large part, periodic, a continuous electrocardiographic record over short or long periods (24 h) and a subsequent graphical representation of the normal R-R intervals over time (tachogram) produce a complex undulatory phenomenon that can be decomposed into simpler waves through mathematical algorithms, such as the FFT or the autoregressive model. This process, called spectral analysis, enables the electrocardiographic signal from the temporal series (tachogram) to be decomposed into its different frequency components, i.e., into so-called frequency bands. It should be noted that frequency refers to the number of times a given phenomenon (e.g., a

sound wave, electric current or any form of cyclic wave) occurs over time. Normally, the frequency unit employed is Hertz (Hz), which is equivalent to one cycle per second. Figure 3 shows the application of an autoregressive model to view the power spectrum of the analysis of heart rate variability corresponding to these values of a volunteer of this study.

In long records (24 h), the total power is decomposed into four distinct bands: 1) high frequency band (HF), oscillating at a frequency of 0.15 a 0.40 Hz, i.e., 9-24 cycles/min, corresponding to the heart rate variations related to the respiratory cycle (respiratory sinus arrhythmia), which are typically modulated by parasympathetic activity; 2) low frequency or LF band (0.04 to 0.15 Hz or 2.4 to 9 cycles/min), modulated by both sympathetic and parasympathetic activities, with a predominance of sympathetic in some specific situations, and which reflects the oscillations of the baroreceptors system; 3) very low frequency or VLF band (0.003 to 0.04 Hz or 0.2 to 2.4 cycles/min), depending on the thermoregulatory mechanisms and the renin-angiotensin system, which is also regulated by sympathetic and parasympathetic activities; and 4) ultra low frequency or ULF band (< 0.003 Hz or < 0.2 cycles/min), which corresponds to most of the total variance, but whose physiological significance is not yet well defined. This band is influenced by the parasympathetic and sympathetic systems and is obviously absent from short duration records. It appears to be related with the neuroendocrine system, circadian rhythm, and other systems (Task Force, 1996).

A high frequency component equivalent to 0.25 Hz (15 cycles/min = 15 cycles/60 s = 0.25 cycles/s = 0.25 Hz), a low frequency component equivalent to 0.1 Hz (6 cycles/min) and a very low frequency component of 0.016 Hz (1 cycle/min). The combination of these three sine waves generates a complex wave signal that can be compared to the signal obtained when the heart rate is expressed on a temporal graph (tachogram). Moreover, the calculation of the area covered by each frequency band (which is proportional to the square of the amplitude of the original signal and hence, in this case, is expressed in ms^2) enables one to separate the amount of variance (power) ascribed to each frequency. This allows for a more detailed study of the individual participation of each of the divisions of the ANS (sympathetic and parasympathetic) in different physiological and pathological situations, as well as its relationship with the main systems that interfere with HRV (respiratory, vasomotor, thermoregulatory, renin-angiotensin and central nervous systems). In fact, this is the main difference between spectral analysis and time domain analysis, since the latter generally fails to distinguish the dominant rhythms or oscillations that give the heart rate its variability (Task Force, 1996).

Spectral components are usually measured in absolute values of power (ms^2). However, the values of LF and HF can also be expressed in normalized units (nu), which represent the value of each of these components in relation to the total power (TP) minus the VLF component. These values are calculated by means of the following formulas: $\text{HF (nu)} = \text{HF}/(\text{TP} - \text{VLF}) \times 100$ and $\text{LF (nu)} = \text{LF}/(\text{TP} - \text{VLF}) \times 100$. This minimizes the effects of changes in the VLF range on the other two components with faster frequencies (LF and HF). Another frequently used measure is the LF/HF ratio, which can provide useful information about the balance between the sympathetic and parasympathetic systems. It should also be noted that, because absolute values in ms^2 are highly variable and distributed asymmetrically, they usually require logarithmic transformation (Task Force, 1996).

Fig. 3. Power spectrum of the analysis of HRV obtained by applying an autoregressive model to a dataset of 256 values of R-R intervals in the supine position from one of the volunteers of this study, showing the VLF (light gray), LF (medium gray) and HF (dark gray) bands

4. Heart rate variability and oral contraceptives

Third-generation combined oral contraceptives (COCs) containing desogestrel and gestodene (GEST) were introduced to reduce adverse effects such as fluid retention, nausea, headaches, and weight changes (Arangino et al., 1998; Read, 2010). The balance of risks and benefits of COC use varies, depending on patterns of usage and background risk of disease (Hannaforde et al., 2010). The repercussions of COCs on cardiac autonomic modulation have not yet been thoroughly investigated. Studies reveal that female sex hormones influence cardiovascular autonomic function (Minson et al., 2000; Neves et al., 2007; Carter et al., 2009). Leicht et al. (2003) reported a positive correlation between circulating estrogen levels and HRV.

Furthermore, the cardioprotective effects of endogenous estrogen through vasodilation and inhibition of blood vessel injuries have been reported (Mendelsohn & Karas, 1999). Low levels of estrogen are associated with a reduction of cardiac autonomic modulation (Moodithaya 2009). Large clinical trials have shown that the long-term use of estrogen in combination with a progestogen may not be beneficial, and could even compromise the efficiency of autonomic HR modulation. Minson et al. (2000) confirmed that COC use can modify baroreflex sensitivity and sympathetic activity. However, Santos et al. (2008) and Schueller et al. (2006) found that COC users and non-users showed similar HRV indices.

Carter et al. (2009) observed no effects of OC use on the sympathetic modulation of the heart during orthostatic stress, nor differences in that regard between the phase of intake of active pills and that of intake of inactive pills. Women with greater physical activity, both users and non-users of OCs, showed a predominance of parasympathetic modulation and presented a greater complexity of pattern distribution and less regularity and predictability of sequential patterns than sedentary groups. Wenner et al. (2006) evaluated amenorrheic and eumenorrheic athletes who were users and non-users of OCs, and observed no influence on cardiac autonomic function. However, other studies suggest that there is a relationship between OC use and autonomic HR modulation, which the authors attribute to changes in vagal peripheral modulation caused by high levels of circulating estrogen (Minson, 2000; Leicht et al., 2003).

Santos et al. (2008) analyzed the autonomic modulation of HR based on frequency domain (LF, HF and LF/HF) indices and found that the use of contraceptives did not affect the results, since they detected no difference among the groups under study. This finding may be attributed to the pharmacological properties of low estrogen/progesterone dosages, as well as to the maintenance of the integrity of the autonomic modulation of HR, since the values found here fall within the range of normality. The results of this study suggest that low estrogen/progesterone dosages do not impair autonomic modulation in the age group under study.

5. Heart rate variability and hormonal therapy

The aging process causes changes in the autonomic modulation of the cardiovascular system, and particularly in HR. The literature reports that parasympathetic activity in the sinus node decreases with age, leading to a reduction in HRV and a greater risk for cardiovascular events (Lipsitz et al., 1990). Structural and functional changes in the blood vessels, in the cardiac conduction system and in the sensitivity of baroreceptors, as well as increased myocardial stiffness, leading to greater force of contraction and reduced ventricular filling, contribute to reduce the functional capacity of the cardiovascular and hemodynamic system (Walsh, 1987). In addition, with increasing age, submaximal physical activity and decline in functional capacity lead to increased physiological stress (Perini et al., 2002).

The incidence of cardiovascular diseases among premenopausal women is low when compared to that of men in the same age group, but increases significantly after this period (Gensini et al., 1996). In several countries, cardiovascular diseases are the major cause of morbidity and mortality among postmenopausal women, representing an important public health problem (Mosca et al., 1997). The increase in the incidence of cardiovascular events among middle-aged women has been associated with the hypoestrogenism typical of this period of women's lives (Greendale et al., 1999).

With regard to autonomic heart function, some studies have demonstrated the harmful effects of hypoestrogenism on HRV. Mercuro et al. (2000) found a reduction in HRV indices, analyzed in the time and frequency domains, after bilateral oophorectomy, i.e., through the interruption of estrogen production, as occurs in menopause. Liu et al. (2003) demonstrated higher values of HRV, analyzed in the time domain, in premenopausal

women than in postmenopausal women and men in the same age group, illustrating the importance of estrogens in the autonomic differences brought about by menopause. Similar findings, also analyzed in the time domain, were reported by Brockbank et al. (2000) for premenopausal women compared to women after more than one year of menopause. Davy et al. (1998) reported that young women have higher HRV than menopausal women, and that HRV in both active and sedentary women tends to decline with advancing age. In earlier studies conducted in our laboratory (Ribeiro et al., 2001; Neves et al., 2007), lower levels of HRV in menopausal women compared to young women were also recorded.

To ascertain if a physical training program could promote physiological adaptations and improved sympathovagal balance of the heart, attenuating the deleterious effects of menopause on the cardiovascular system, Sakabe (2007) evaluated 18 sedentary women divided into two groups: Control Group – 10 postmenopausal women (50 to 60 years old) without hormone therapy (HT); and HT Group – 8 postmenopausal women (50 to 60 years old) undergoing HT (estradiol plus levonorgestrel). Both groups were assessed at two different times: before (assessment) and after (reassessment) a 3-month physical training program (PTP). Protocol 1 – to evaluate the autonomic modulation of the HR, the HR was recorded under resting conditions, supine and sitting positions, for 15 minutes in each position. The indices evaluated in Protocol 1 were: mean HR and R-R intervals (RRI), RMSSD index of the RRI, low (LF) and high (HF) frequency bands of the spectral analysis, in normalized units, and LF/HF ratio. It was concluded that hormone replacement therapy did not have a significant effect on HRV.

6. Heart rate variability and menopause

Postmenopausal women have greater sympathetic and less parasympathetic activity than premenopausal women (Brockbank et al., 2000; Earnest et al., 2010). Moreover, Mercurio et al.'s study (2000) reveals the harmful effects of hypoestrogenism on the autonomic modulation of the HR, while other studies have demonstrated numerous evidences that endogenous hormones (estrogen and progesterone) contribute to a cardioprotective phenotype in women (Vitale et al., 2009).

Parasympathetic modulation shifts to a lower range with normal aging. Although parasympathetic modulation is generally higher in women than men, aging reduces the difference between genders, with changes in HRV beginning approximately at menopause (Earnest et al., 2010). Boettger (2010) examined changes in cardiovascular autonomic parameters obtained from short-term recordings over time. The data he collected indicated a lifelong shift in autonomic balance toward sympathetic predominance, starting at the age of 30 years.

Zuttin (2009) evaluated and compared autonomic modulation of the HR at rest in healthy young, premenopausal and postmenopausal women leading a sedentary lifestyle, to verify cardiovascular adjustment in response to postural changes. This investigation involved 113 healthy sedentary women, who were divided into a young group (YG) with an average age of 23 ± 3.4 years ($n=40$), a premenopausal group (PreMG) aged 36 ± 3.1 years ($n= 39$), and a postmenopausal group (PostMG) with an average age of 55 ± 4.5 years ($n=34$).

In the supine position, it was found that the YG presented significantly higher values of the HF index in absolute units (ms^2) and lower LF values (ms^2) and ratio than the PostMG. In addition, the YG and PostMG showed a statistical difference in all the evaluated indices ($p < 0.05$), while no difference was found between the PreMG and PostMG groups ($p > 0.05$). In a comparison of the YG vs. PreMG and YG vs. PostMG groups in the sitting position, the YG presented significantly higher values for the ratio ($p < 0.05$).

With regard to the effect of postural adjustment on the autonomic HR modulation, a comparison of the indices obtained in the supine and sitting positions revealed significant differences ($p < 0.05$) in all the indices. On the other hand, the PreMG groups showed a difference in the LF/HF index ($p < 0.05$), while the PostMG group showed no significant difference ($p < 0.05$).

Having calculated the regression coefficients, it was found that the straight line of the adjusted regression indicates that, as the age of the subjects increases, it is possible to estimate the reduction of the HF index (ms^2). The parameters indicate mainly a reduction of the postural change in parasympathetic modulation. With aging, the adjustment capacity diminishes, as indicated by the delta between the supine and sitting positions.

7. Conclusions

This chapter discussed the measurement and analysis of HRV, as well as results of data for women and the relationship between aging, hormonal changes (oral contraceptives and hormone replacement therapy) which contribute to modifications in the autonomic control of heart rate.

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