NOTE TO USERS

Comparison of respiratory sinus arrhythmia integration in athletes and non-athletes

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Abstract

A comparison of heart rate variability and respiratory sinus arrhythmia (RSA) characteristics was performed in 20 athletes and 12 age-matched sedentary controls (CTRL) (22 ± 2.4 yrs). More specifically, this study examined the role of regular physical activity on the breathing frequency (BF) – RSA amplitude response curve comparing varsity swimmers (SW) to endurance runners (RU) to test the hypothesis that a locomotor-respiratory entrainment resulting from the water-immersion breathing pattern of swimmers would alter their respiratory related cardiac vagal integrative response. Spectral power components of HRV were computed from R-R interval sequences. Five-minute recordings were performed with subjects breathing either at their spontaneous breathing rate, at four breathing cycles less (M4) and four cycles more (P4) than spontaneous. Amplitude and phase of RSA were computed from the sinusoid fitted to the instantaneous heart rate within each breath while the gain of the RSA response was obtained from the slope of the RSA amplitude versus BF. As expected both SW and RU had significantly lower resting heart rates (66±5, 60± 6 *vs* 75±9bts/min) and significantly higher VO_2 max ($p < 0.05$) than CTRL (66.5±7.8, 67.4±5.9, *vs* 40±7.3ml• kg^{-1}• min^{-1}) while spontaneous BF and tidal volume were comparable for all groups. Blood pressure values and HR remained stable throughout the recording for all breathing conditions. Spectral analysis of R-R intervals (ms^2) revealed similar total power, HF and Respiratory Centered Frequency power in all groups. Similarly, under baseline spontaneous breathing, RSA amplitude (fraction heart rate) and phase (fraction of breath cycle) was not different between RU (0.15±0.12, 0.42±0.1) SW (0.11±0.05, 0.5±0.2) or CTRL (0.10±0.04, 0.41±0.1). RSA amplitude increased at M4 and decreased at P4 in all groups ($p<0.05$) with no group differences being observed under any conditions. A negative relationship was observed between RSA amplitude and breathing frequency ($r = -0.66$; $p < 0.005$) was found in all groups. There was no relationship between BF and RSA phase in any of the groups. Results from this study indicate the heart rate variability and more specifically the respiratory-induced modulation to be the same in CTRL and athletes despite the resting bradycardia observed in athletes suggesting that HRV and RSA do not reflect tonic vagal activity.

Résumé

Cette étude présente les caractéristiques de la variabilité de la fréquence cardiaque (HRV) et de l'arythmie sinusale respiratoire (RSA) chez 20 athlètes d'endurance ainsi que des 12 sujets témoins (CTRL) de même âge (22 ± 2.4 ans). La courbe réponse de l'amplitude de RSA en fonction de la fréquence respiratoire (Bf) a été établi chez les CTRL nageurs (SW), des coureurs d'endurance (RU) afin de vérifier l'hypothèse d'une modification de l'intégration de la modulation vagale cardiaque par la respiration chez les nageurs en raison d'une adaptation de la synchronisation locomotion-respiration liée à la répétition du "pattern" respiratoire de la natation. Des enregistrements de l'ECG et du débit respiratoire de cinq minutes ont été obtenus chez tous les sujets pendant lesquels la respiration était libre (rythme spontané : SP) ou était imposée à un rythme de 4 respirations/minutes au-dessus (P4) ou en dessous (M4) de la respiration spontané. La variabilité sinusale a été obtenu par l'analyse spectrale des signaux de l'ECG tandis que l'amplitude et la phase de la RSA ont été obtenu par l'ajustement d'une fonctionnement sinusoïdale aux modifications de la fréquence cardiaque sur chacun des cycles respiratoires. Tel qu'attendu les résultats témoignent d'une fréquence cardiaque plus faible chez les SW et les RU que les CTRL (66±5, 60± 6 *vs* 75±9bts/min) ainsi qu'un VO_2 max plus élevé chez CTRL ($p< 0.05$) (66.5±7.8, 67.4±5.9, *vs* 40±7.3ml· kg^{-1}· min^{-1}) tandis que la Bf et le volume courant étaient similaires pour tous les groupes. Les valeurs de la pression artérielle ainsi que la fréquence cardiaque sont demeurées inchangées au cours des 3 enregistrements. Les résultats de l'analyse spectrale de la HRV (ms^2) ne révèlent aucune différence entre les groupes pour la puissance totale, les composantes HF ou RCF (Respiratory Centered Frequency). De la même façon, l'amplitude de la RSA (fraction de la fréquence cardiaque moyenne) ainsi que la phase de RSA (fraction du cycle respiratoire) n'étaient pas différents entre les RU (0.15±0.12, 0.42±0.1) les SW (0.11±0.05, 0.5±0.2) ou les CTRL (0.10±0.04, 0.41±0.1). Les résultats témoignent d'une augmentation de RSA en M4 et d'une diminution en P4 chez tous les groupes de sorte qu'une relation linéaire inverse a été observée ($r= - 0.66$; $p< 0.005$). Aucune relation n'a été observée entre la phase de RSA et la Bf pour l'ensemble ainsi que pour chacun des groupes. Les résultats de la présente étude suggèrent que les indices de HRV et de RSA ne témoignent pas de l'influence vagale tonique sur le nœud sinusale puisqu'ils sont les mêmes indépendamment de la fréquence cardiaque de base.

Part I

Review of Related Literature

1.0 Assessment and Interpretation of Heart Rate Variability

In healthy humans with normal sinus rhythm there exists a natural oscillation of the heart rate (HR) termed sinus arrhythmia. This oscillation is primarily but not essentially related to the movements of breathing (Donders, 1868, Davies et al., 1967, Hirsch and Bishop, 1981, Horner et al., 1995). This variability is a result of oscillations of a series of consecutive R-R intervals; demonstrating variability in their respective durations. Interest in cardiac rhythms first began with the observed fluctuations in arterial pressure by S. Hales in 1733 with direct sampling from the crural artery of a mare. The first observations of clinical relevance in humans were reported in the mid 1960's and incited the larger study of heart rate variability (HRV), its measurement and interpretation.

The clinical use of HRV commenced in the area of fetal monitoring. Hon and Lee in 1965 detected changes occurring in R-R interval lengths. The observation of diminished beat-to-beat variation in HR led to the identification of fetal heart distress reflecting diminished vagal influence on the sinus node. HRV has been used in a wide array of clinical studies on cardiac pathologies such as; sudden arrhythmic death (Huikuri et al., 1999, Pieper et al., 1995, Schwartz et al., 1992), hypertension (Guzetti et al., 1991, Brook and Julius, 2000), and chronic heart failure (Nolan et al., 1998). Its use as a prognostic tool has mainly confirmed its effectiveness as an independent factor for the prediction of mortality post myocardial infarction (Kleiger et al., 1987, Farell et al., 1991, Bigger et al., 1992, Stein & Kleiger 1999, Huikuri et al., 1999) in humans, and in canines (Schwartz et al., 1992). The non-cardiological condition known as diabetic neuropath, results in the depression of an individual's HRV has also initiated the use of HRV analysis as a valuable predictor of alterations in autonomic nervous system (ANS) functioning (Malpas et al., 1990, Luft, 1996, Howorka et al., 1997, Stein & Kleiger, 1999, Pagani, 2000). Identification of these alterations in ANS functioning in diabetic individuals could potentially allow for the prevention of the advancement of the disease to more severe states of neuropathy. Numerous studies have been published and reviewed by (Singer and Ori ,1995) to support the postulate that an imbalance of the parasympathetic and sympathetic divisions of the ANS can lead to symptoms increasing potential for lethal arrhythmias and sudden cardiac death.

1.1 Time Domain Analysis of HRV:

The recording of the HR through the use of electrocardiogram (ECG) is a common method for HRV assessment. The ECG allows for the collection of a series of consecutive R-R interval time changes and changes in instantaneous HR. Data can be presented in a tachogram; reflecting the change in R-R intervals in sec or the number of beats (see Figure 1). ECG recordings have traditionally been performed for durations of 2 minutes up to 48 hours; twenty-four hour recordings typically allow the fluctuations in HR to be assessed providing long-term data on night and day variability. The variability associated with twenty-four hour recordings is reflective of the influences over the entire circadian variations on HR (Burgess et al., 1997). Five-minute recordings of HR are commonly used to assess HRV and studies have shown that this recording length is stable and characteristic of an individual over time (Sinnreich et al., 1998). It should be noted that by limiting the recording length, there is preclusion to the study of slow rhythms wherein little is known.

The treatment of HRV data focuses primarily on two methods: time and spectral domain analyses. Pertinent values can be calculated in the time domain and consist of simple calculations of mean and standard deviation of the R-R intervals and the mean of the instantaneous HR. Calculations can be applied to both short and long-term recording lengths as well as a segment of an entire recording. Time-domain analysis is considered more useful for long-term recordings and spectral analysis more informative for short-term ECG recordings; time-domain analysis provides highly ambiguous information regarding underlying autonomic nerve traffic to the heart (Eckberg, 2000)

1.2 Frequency Domain Analysis of Heart Rate Variability

The procedure of spectral analysis involves the collection of a series of HR or R-R intervals. The data is generally treated with a second order statistical method such as an autocorrelation function or Fast Fourier Transform (FFT) algorithm producing a power spectral density curve. The FFT is effective in producing a reliable spectral estimation based on a short-term HR recording such as 5 minutes, which must be considered a stationary condition. The frequency resolution will be compromised on longer recordings of HR as in a twenty-four hour recording. The use of spectral analysis in the field of HRV research has created the necessity for standards for analytical protocol and interpretation. These standards have been reviewed in the joint paper on standards of HRV measurement and interpretation (Task Force, 1996).

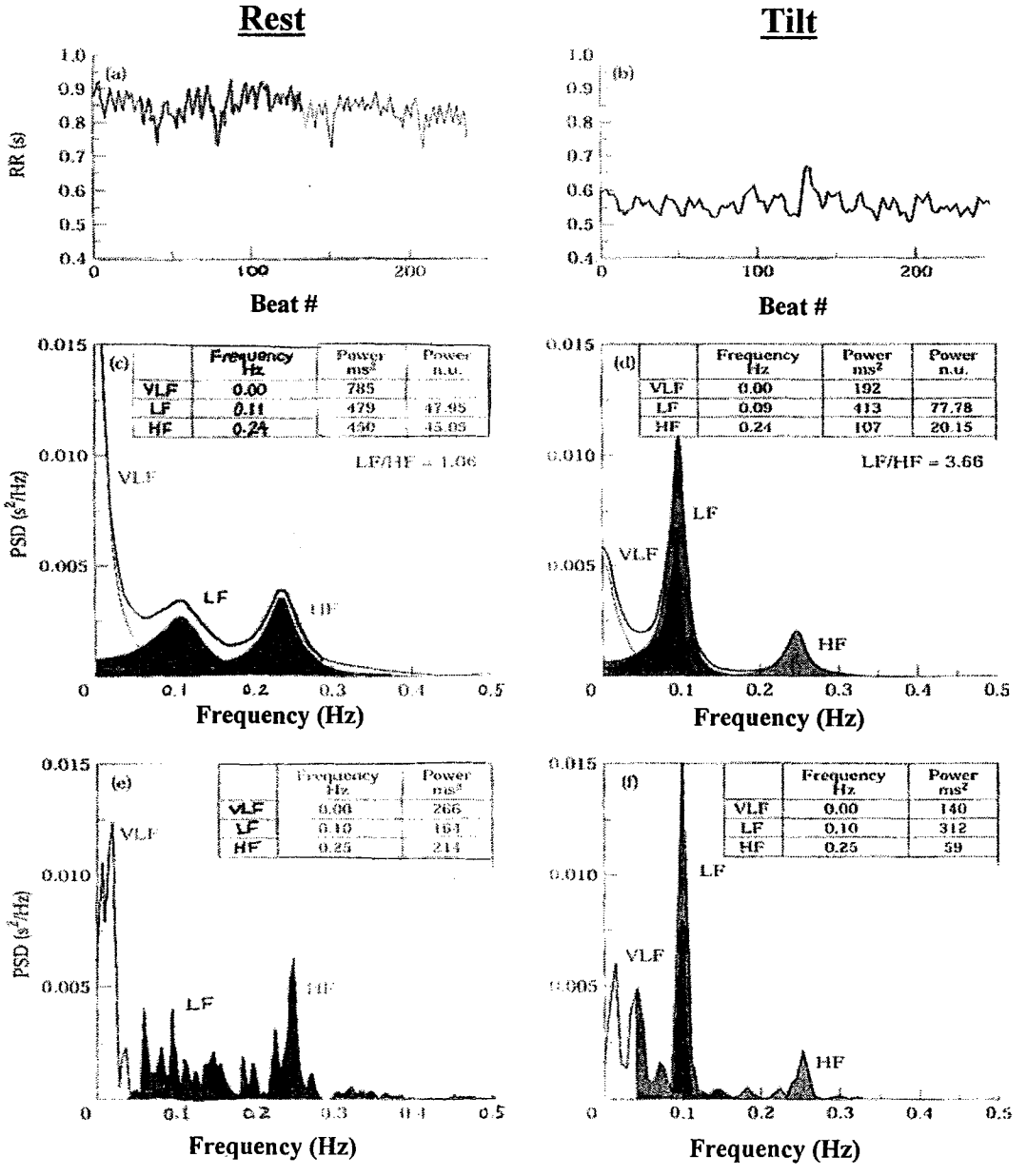
The paper outlines the standards for a frequency domain method based on the power spectral distribution (PSD) of variance as a function of frequency. This technique has been applied to HR to decompose the variability of the signal into variabilities at different frequencies. PSD analysis can provide the delineation of several physiological rhythms, which may be occurring simultaneously, as the result of modulations in respiration, arterial pressure, thermoregulation and other humoral factors. The frequency ranges of interest have been evaluated, determined and interpreted based on experimental evidence. The evidence has been provided from analyses on five minute to twenty-four hour R-R interval recordings.

1.2.3 Components of the Power Spectrum and their Physiological Interpretation:

Spectral analyses of R-R interval recordings in healthy humans for a twenty-four-hour period have resulted in a basic pattern of power distribution. The spectral power distribution consists of 3 distinguishable peaks. One peak is found to occur at a very low frequency of 0 to 0.03 Hz which captures the long period rhythms; a second peak is found to occur between 0.03 to 0.15 Hz and is generally centred around 0.1 Hz (Malik & Camm, 1995) and a third peak will occur at the average breathing frequency. It has been well documented that resting breathing frequencies in adult humans generally vary between six and thirty-one breaths per minute, which translates into a frequency range of 0.15 to 4.0 Hz. This frequency range is referred to as the high frequency band and the breathing related peak or respiratory peak is usually found within this band. In order to ensure this peak is in the appropriate band range, a common procedure is to provide subjects with and auditory or visual signal for paced breathing at a minimum frequency of 0.20 Hz approximately twelve breaths per minute. The HF band is thought to reflect the HR variance related to respiratory changes mediated by modulations in the parasympathetic and sympathetic influences on the sinoatrial node.

The occurrence of very low frequency (VLF) peak is generally thought to be related to circadian rhythmic events such as night and day differences in cortisol secretion and thermoregulatory responses. To effectively determine the physiological importance of this

Figure 1. R-R interval tachogram at rest (a) and head-up-tilt (b) HRV power spectra parametric autoregressive modelling (c & d) and Fast Fourier Transform non-parametric algorithm (e & f), (Malik & Camm, 1995)



frequency band ECG, recordings must be prolonged up to forty-eight hours to capture physiological events, which occur every twenty-four to twenty-six hours .

Finally, according to the Task Force standards (1996) the low frequency (LF) peak is commonly found to occur in a band range between 0.04 to 0.15 Hz. The area within each band provides an index of the extent of variance that distributes as a function to that underlying frequency range (see Figure 1). The sum of the spectral power within each band represents total spectral density or power. The extent of total spectral power and its distribution will be modulated and subject to shifts depending on a person's health, environmental condition and body position. For example, total spectral power may be increased during night versus day or conversely decrease upon standing compared to lying or sitting. The change in total spectral power may in turn be associated to one of the major frequency bands or spectral components of interest. This has been demonstrated to be the case using manipulations such as supine-to-standing or body tilting. These orthostatic challenges when performed on healthy individuals generally result in decreases in the HF component with concomitant increases in the LF power component. The physiological significance of these power spectral density changes has been linked to modulations in cardiac vagal and sympathetic activity.

1.2.4 Physiological Significance of R-R Frequency Spectrum

Pharmacological approaches have been used to confirm the postulations concerning the contribution of parasympathetic and sympathetic influences on HRV, as suggested by results of spectral analysis. These studies have been performed in both animal and human models.

In general autonomic blockade of the cardiac vagal nerve with substances such as atropine or glycopyrrolate results in the abolishment of the HF component and a reduction in the LF component. This has been observed in both humans (Elghozi et ., 2001, Médigue et al., 2001, Langewitz et al., 1991, Tapp et al, 1990, Kim et al., 1997, Alcalay et al., 1992, Montano et al., 1998, Pomeranz et al., 1985, Jokkel, 1995, Goldberger et al., 1999, Selman et al., 1982, Hayano et al., 1991, Taylor et al, 1998) and animals (Akselrod et al., 1981, Ferrari et al., 1987, Jansen et al, 1989, Zwiener et al 1990, Japundzic et al., 1990, Murphy, 1991, Kuwahara et al., 1992, Clement & Barrey, 1995).

In a study by Akselrod (1981) on 8 unanaesthetized, healthy, conscious dogs power spectrum analysis was performed on heart rate fluctuations after the administration of intravenous

bolus of 0.01 mg/kg of glycopyrrolate. The R-R power spectrum data revealed that the mid and high HF peaks had been abolished while the magnitude of the LF component was reduced. Combined β-sympathetic (0.01 mg/kg propranalol) and parasympathetic blockade abolished all HR fluctuations. Sympathetic blockade alone caused a reduction in LF a result not consistently observed because of the low tonic level of sympathetic activity seen in the resting dog (Akselrod et al, 1981).

In humans, several studies have administered atropine under resting conditions to examine effects on R-R interval PSD (Selman et al., 1982, Pomeranz et al., 1985, Alcalay et al., 1992, Izraeli et al., 1991, Montano et al., Tappet al, 1990). These pharmacological studies performed between 1982 and 1999, included healthy subjects aged between twenty-one and fifty-two years of age. In general the data resulted in an overall decreases in total R-R interval spectral power to as little as 6% compared to drug free levels These decreases in total power are comprised of decreases in HF component from 74 to 92% with concomitant decreases in LF component of between 17 and 84% as compared to baseline conditions. Some studies have performed these pharmacological blockade protocols from a standing position to evaluate the effect of the drug on the cardiac or vascular sympathetic nervous system (Elghozi et al., 2001). In a by Pomeranz (1985) the standing versus the supine position under muscarinic receptor blockade using atropine caused an attenuated decrease in LF component from 84±4.6 to 72±4.4% and HF component decreased from 92±4.2 to 95±1.8 percent from supine to standing respectively.

Spectral analysis of HRV under varying doses of atropine have revealed a bi-phasic response inducing both vagomimetic and vagolytic effects (Akselrod, 1981, Alcalay et al., 1992, Ali-Melkkila et al., 1991, Medigue et al., 2001, Yuasa et al., 2000). In general, administration of clinical doses of atropine between 0.1 and 0.2 mg intravenously, will induce bradycardia. Higher doses between 0.5 and 1.0 mg paradoxically will induce an initial bradycardia and then sustained tachycardia (Kamath, 1993). It has also been observed that at low doses the vagomimetic action of glycopyrrolate is less pronounced compared to atropine (Ali-melkkila et al, 1991).

Spectral analysis of R-R intervals using cardiac vagal enhancing agents such as phenylephrine have also been employed to provide more information on cardiac vagal activity as determined and interpreted by data on HF power (Goldberger et al, 1994, Bloomfield et al., 1998). Findings from the study by Bloomfield et al (1998) demonstrated that increased dosage of phenylephrine from 0.4 to 1.2 $\mu g/kg^{-1}min^{-1}$ caused a progressive augmentation in HF component

up to 568% with a range of 293 to 1036% in 10 healthy human subjects (mean29±3 yrs.). An increase in the LF power was also observed of 77% but the LF:HF was reduced by 74% reflecting a dominant HF component.

Overall the findings from pharmacological muscarinic receptor blockade, and stimulation support the premise that HF R-R spectral power can be used as a marker of cardiac parasympathetic activity

Although, LF spectral power has been considered to be a marker of sympathetic activity, spectral analysis from pharmacological studies has not provided enough evidence to clearly support this assumption. Beta-adrenoreceptor blockade studies using such agents as propranolol and atenolol have provided incongruous data making it more difficult to predict its effects on HRV and power spectrum distribution in both animal (Akselrod, 1981, Japundzic et al, 1990, Kuwahara et al., 1994 and human models (Akselrod, 1981, Goldberger, 1999, Ahmed et al, 1994). Fluctuations in the low frequency range appear to be jointly mediated by β-sympathetic blockade and parasympathetic activities (Saul et al., 1990, Jokkel, 1995, Pomeranz et al., 1985, Ahmed et al, 1994). Thus, while there is general agreement that under normal conditions HF power reflects vagal modulation there is no clear evidence for the power of the LF band to represent the isolated sympathetic influences on heart rate. This has led to the use of a LF:HF ratio to describe the interaction and balance of both the sympathetic and parasympathetic influences on the HR referred to as the sympathovagal balance.

In order to gain insight into the significance of the R-R spectrum pattern, investigators have also used several experimental strategies known to affect the sympathetic and parasympathetic influences on the sinoatrial node. In humans, head-up tilting, standing from supine or sitting, as well as exercise have been used to mimic conditions that result in enhanced sympathetic and diminished cardiac vagal flow (Pagani et al., 1986,1991, 1992, Vybiral et al., 1989, Montano et al., 1994, Hayano et al., 1994, Goldberger, 1999, Bloomfield & Sweibel, 1998, Strano et al., 1998, Freitas, 2000).

In general, orthostatic challenge results in a decrease in total spectral power and more specifically, significant decreases in the HF component with concomitant increases in the LF component compared to baseline conditions. A typical spectral profile after orthostatic challenge is depicted in Figure 1. part (c and d). Head-up tilt resulted in a decrease in total spectral power from 1714 to 712 $msec^2$ with a shift in LF, HF balance towards the LF from 47.96 to 77.78 with a

concomitant shift in the HF component 45.05 to 20.15 expressed in normalized units (Malik, 1996). The extent of augmentation in LF and fall in the spectral power of HF has been observed to range between 15 and 105% and 63 and 71% respectively (Pomeranz et al., 1985, Fallen et al., 1988, Pagani et al., 1986, 1991, Vybiral et al., 1989, Hayano et al., 1994, Goldberger, 1999, Bloomfield & Sweibel, 1998, Strano et al., 1998). The LF component in normalized units (LF_{nu}) has been shown to range between 37 and 58 at rest with increases from 71 to 89 during 90 degree tilting (see Table 1.). It appears that passive tilting results in greater increases in LF than standing as reflected by the larger LF:HF ratios. This could be explained by the understanding that tilting creates greater orthostatic challenge while standing helps facilitate venous return and maintaining blood pressure. A study by Montano (1994) examining the correlation of tilt angle to LF_{nu} , HF_{nu} and LF:HF resulted in r=0.78, r=-0.72 and r=0.68 respectively.

Other factors such as mental stress have been observed to have effects on HRV resulting in diminished HF and augmented LF power (Pagani et al., 1989, Lucini, 1997, Pagani et al., 1991, Pagani et al., 1991). The use of spectral analysis to examine the response of stress on HRV in humans has resulted in observed decreases in HF power between 17 and 28 percent while LF increased by 10 t o 30 percent (Pagani et al., 1991, Pagani et al., 1991).

Finally, dynamic and static exercise has been used to examine autonomic nervous system effects on HRV (Dixon et al., 1992, Furlan et al., 1993, Bernardi et al, 1990, Yamamoto et al., 1991, Casadei et al., 1995, Iellamo, 1999a, Gonzàlez-Camarena et al., 2000). Typically during exercise HR increase, thus reducing the variability and is the result of decreased parasympathetic influences and increased sympathetic activity on the sinoatrial (SA) node. With increased levels of exercise intensity vagal tone is almost completely abolished. The spectral analyses of HRV during exercise has resulted in an overall decrease in total spectral power with concomitant decreases in the absolute values of LF and HF components. The use of LF and HF in normalized units is more informative when presented with overall changes in total power, which demonstrates the shifts that

Table 1. Spectral Analysis of R-R interval during orthostatic challenge in healthy subjects during rest and tilt.

Study	N	Age yrs	Posture	LF (nu)	HF (nu)	LF:HF
Pagani et al (1986)	30	20-30	S	58.2(3.3)	24(2.0)	3.6(0.7)
			90°	89.7(1.4)	7.5(0.9)	20.8(3.68)
Pagani et al (1991)	14	28.5±4.8	S	58(5)	28(3)	3.9(1)
			90°	81(4)	8(1)	17(4)
Lombardi et al (1987)	26	54±2	S	53(3)	45(3)	
			90°	78(3)	14(2)	14(3.3)
Furlan et al (1993)	29	16±0.4	S	37.4(2.9)	53.1(2.59)	0.8(0.1)
			90°	71.7(2.7)	21.5(1.9)	4.5(0.6)
Dixon et al (1992)	14	27±0.7	S	69(19)[1]	43(22)[1]	1.6(0.8)
			ST	83(17)[1]	24(11)[1]	3.2(1.7)
Pomeranz et al (1985)	8	22-36	S	3.3(0.8)[2]	6.5(2)[2]	
			ST	33(9.6)[2]	2.1(0.5)[2]	
Bloomfield et al (1998)	10	29±3	S			
			60°		-63%	105%
Hayano et (1994)	9	23±2	S	23.5(3.0)[3]	42.4(8.2)[3]	1.0(0.2)
			70°	34.0(5.1)[3]	15.2(4.2)[3]	2.3(0.6)

S=supine, ST=standing, 90°=degree of tilt, nu=normalized units, ( ) =standard deviation,
( )[1]=beats/min^{-1} *Hz $^{-1}$, ( )[2]=beats/min^{-2} X 10^{-2}, ( )[3]=ms/Hz ½, % change from supine.

occur when HRV is reduced by exercise. The results from six studies shown in Table 2 (a) from dynamic exercise experiments using primarily cycle ergometers with and without incremental protocols were comprised of subjects between the ages of 19 and 33 years. Exercise intensities ranged from 20-120 watts, 30 to 221% of peak oxygen consumption, and exhaustion. Overall the study findings indicate that the spectral power balance shifts more towards the LF as depicted by the increases in LF nu and the LF:HF ratios from rest to exercise(Furlan et al., 1993, Dixon et al., 1992, Bernardi et al., 1990). The absolute values for LF $msec^2$ decreased from resting levels with increases in LF:HF ratio under five of six exercise intensities ranging between 1.68 and 6.19 (Yamamoto et al., 1991). In the study by Bernardi et al (1990) higher levels of exercise intensity above 60 W resulted in decreases in LF_{nu} and stabilizing of HF_{nu}. At severe levels of exercise such as 221% of VO_2 max abolishment of LF component has been observed while HF has remained relatively stable (Casadei et al., 1995). The absolute values for LF, HF and total R-R interval spectral power reported in Table 2 (a) demonstrate that total power in $msec^2$ decreases with increased exercise intensity (Yamamoto et al., 1991, Casadei et al., 1995, Gregoire et al., 1996). Some studies have reported that HF increased at exercise levels when cardiac vagal activity is expected to be minimal. The observation that the resulting HF power shifted to a frequency band reflective of the exercise breathing rate led to the suggestion that the large intrapleural swings typical of exercise breathing could exert an influence on the rhythmic activity of the R-R intervals such that the phenomenon would reflect mechanical factors rather than vagal influences. The recent use of spectral analysis for the assessment of the autonomic nervous system functioning during dynamic exercise has proven controversial in terms of physiological interpretation of data and lack of uniformity of study protocols (Casadei et al, 1995).

Examination of HRV during static exercise however suggests a different pattern of response than that during dynamic exercise. While a decrease in total power in all band has been reported in Table 2 (b) with a decrease in the HF component expressed in normalized units of between 28 to 50% and an increase in the LF component of 14 to 23% (Iellamo, 1999a, Pagani et al., 1988), an increase in total power and middle frequency components of HRV expressed in absolute unites have been reported during static leg exercise performed at 30% of maximal voluntary contraction. Power of the HF component was not significantly increased from rest but

Table 2 (a) Spectral Analysis of R-R interval during dynamic exercise in healthy subjects.

Study	N	Age	Ex	Position	Watt	LF (nu)	HF (nu)	LF :HF
Furlan (1993)	10	22±3	TR	UP	R	41.3	50.9	1.3
					Exh	79	13.7	9
Dixon (1992)	14	28±3.5	CE	UP	R	69(19)	44(22)	1.6(0.8)
					50%	68(21)	22(10)	3.2(1.6)
Bernardi (1990)	9	23±0.7	CE-I	UP	R	56(4)	44(4)	
				UP	30W	63(4)	37(6.3)	
					60W	61(8)	38(8)	
					90W	28(12)	71(12)	
					120W	11(6)	89(6)	
						(ms^2)	(ms^2)	
Yamamoto	8	22±3	CE-I	UP	R	2285.7	4230.2	0.58
(1991)					20W	467.1	855.1	1.68
					30%	322.8	387.1	1.58
					60%	32	125.5	0.85
					90%	8.7	21.5	1.2
					100%	8.4	4.7	1.77
					110%	5.5	1.1	6.19
Casadei (1995)	11	21±0.4	CE-I	UP	R	2141(378)	3105(1571)	
					110%	258(72)	202(113)	
					147%	77(35)	131(100)	
					184%	9.2(3.5)	132(124)	
					221%	0	128(124)	
						TotPower*		
Gregoire (1996)	10	18-30	CE-I	UP	R	2607(597)		
					50W	250(78)		
					100w	74(23)		

EX=exercise, W= watts, LF=low frequency, HF=high frequency, nu=normalized units, CE=cycle ergometer, CE-I=cycle ergometer incremental, TR=treadmill, UP=upright, %=percent of VO_2max, W=watts, R=rest, ( ) =standard deviation, Exh=exhaustion, TotPower*= total power (ms^2)

remained higher during static that during dynamic exercise. (Gonzalez-Camarena, 2000). Because cardiovascular responses to static exercise are related both to the extent of muscle mass involved in the exercise and the intensity of exercise, discrepancies in findings between the current few studies may be associated with differences in experimental designs and protocols.

2.0 The Contribution of Respiratory Sinus Arrhythmia to Heart Rate Variability

It is well established that respiratory sinus arrhythmia is the primary if not the essential component of HRV under resting conditions. Respiratory sinus arrhythmia (RSA) is defined as the change in cardiac interval length associated with the inspiratory and expiratory phases of the respiratory cycle. Typically, HR may be seen to accelerate during inspiration and to decelerate during expiration. The extent of such changes in HR throughout a breathing cycle has been commonly used as an index of the magnitude of RSA. Investigations of RSA have helped to provide more information on the role of the vagal influences in the integration of the cardio-respiratory interactions. While the assessment of heart rate variability using the frequency spectrum approach enables to approximately define a frequency band where breathing rate is thought to be found, it does not enable to clearly quantify the variability in heart rate specifically related to the breathing cycle changes. An approach used to specifically assess RSA consists of examining the changes in spontaneous heart rate throughout the breathing cycle. An example of such a method is the breath-by-breath fractional cardiac cycle count (Pham Dinh et al., 1999). The quantification of the amplitude of RSA is based on the fitting of a sinusoidal curve on one respiratory cycle and the changes in instantaneous relative HR within that breath cycle. The phase of the RSA phenomenon may also be identified as the timing of the respiratory cycle at which maximal amplitude of heart rate change is observed. This method presents the advantage of allowing for a selective detection and quantification of the respiratory component of heart rate variability

Figure 2. illustrates the primary factors influencing the modulation of heart rate via the cardiac vagal center. The integrative model suggests some explanations for the phenomenon of respiratory sinus arrhythmia. The cycle of respiration as it is understood is initiated by phrenic nerve stimulation via the respiratory medullary centre causing the diaphragm and external intercostals to contract. These events in turn may trigger inter-central communication between the respiratory centre and the cardiac vagal centre, leading to an inhibition of vagal flow. As a result of

Table 2 (b) Spectral Analysis of R-R interval during static exercise in healthy subjects.

Study	N	Age	Position	Ex	W	LF(nu)	HF(nu)
Iellamo (1999a)	11	26±2.4	S	SLE	R	63.8(6.1)	28.9(6.2)
					30%	82.8(4.5)	14(3.9)
Gonzàlez-Camarena	10	20-27	S	BLE	R	60[1]	38[1]
(2000)					30%	70[1]	28[1]

EX=exercise, W= watts, LF=low frequency, HF=high frequency, nu=normalized units, SLE=static leg extension, BLE=bilateral leg extension, S=seated, R=rest, %=percent of VO_2max, mean (standard deviation), ( )[1]=$bt^2/min^2 * Hz^{-1}$.

inspiratory muscle contraction, changes in intrathoracic pressure are observed resulting in an increase in lung volume. In turn, lung inflation may stimulate pulmonary stretch receptors leading with an afferent input to inhibit cardiac vagal flow. Finally, respiration while causing changes in intrathoracic pressure, indirectly causes changes in venous return leading to fluctuations in arterial pressure. These pressure fluctuations lead to baroreceptor reflex stimulation increasing cardiac vagal influences or decreasing efferent sympathetic influences on the sinus node.

2.1 Influences of Breathing Pattern on HRV and RSA

Typical investigation methods for the determination of RSA in animals consist of anaesthesia, vagotomy (surgical, thermal or pharmacological) and mechanical ventilation. Some methods include both parasympathetic and sympathetic blockade as well as arterial pressure manipulation to isolate and study the resulting effects of breathing frequency on RSA. Data analysis is obtained using mathematical approaches such as: a) the linear model for identification of maximum and minimum R-R interval throughout a respiratory cycle, and more specifically during the inspiratory and expiratory phases of the breathing cycle (time-domain) b) sine wave function of the breathing frequency fitted to heart rate data, or the autoregressive power spectral analysis of heart rate variability at the respiratory centred frequency. Results have generally indicated RSA amplitude to be directly related to tidal volume such that a decrease in the rate of ventilation or an increase in tidal volume results in an increase in RSA. Spectral power analysis of R-R intervals has been an approach used to investigate the influences of breathing frequency on HRV (Brown et al., 1993, De Meersman et al.,1995, Cooke et al., 1998, Stark et al., 2000, Eckberg, 1995). These studies were performed using imposed breathing protocols between 3 and 24 breaths a minute on a total of 90 healthy humans between the ages of 20 and 31 years. Results from these studies typically indicate a 70 to 90 percent reduction in total spectral power of R-R intervals with increasing breathing frequency to 24 breaths/minute. More specifically the HF component power was reduced by 15 to 77 percent. These changes in HRV in concert with changes in the heart rate variability of the HF band may be taken to suggest that the extent of RSA might be more directly related to modulations in breathing. Indeed, both in humans and in animals the extent of RSA appears to be related to change in either depth or frequency of breathing or a combination of both. Results from animals studies using anaesthesia, vagotomy (surgical, thermal or pharmacological) and mechanical ventilation generally indicate the extent of RSA to be proportional to changes in both tidal volume and rate of ventilation (Katona and Jih., 1975, Horner et al., 1995, Perlini et al.,1995). There exists

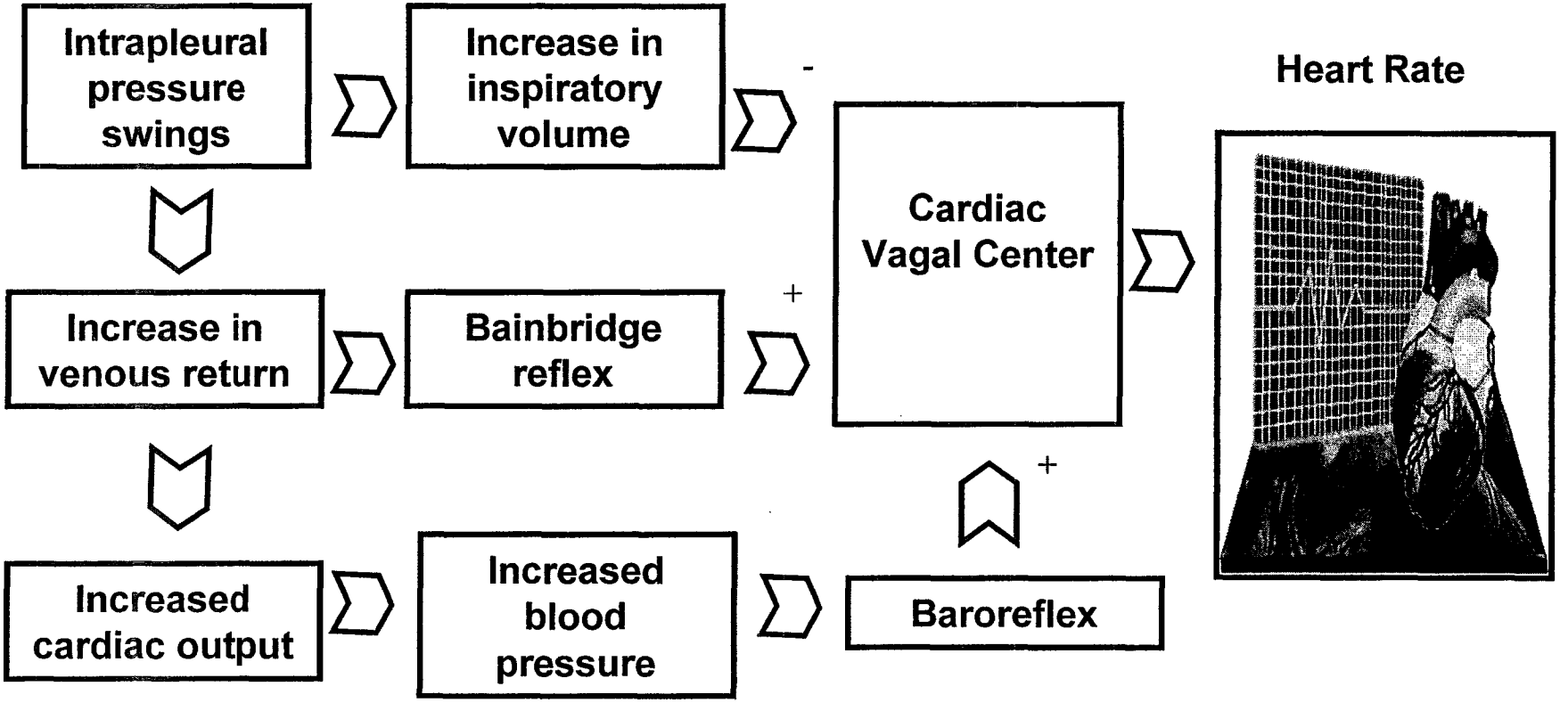


Figure 2. Adapted integrative model of respiratory sinus arrhythmia (A.S. Scott)

a diverse range of breathing patterns with varying breathing frequencies typically ranging from 6 to 31 breaths per minute (Quetelet, 1842, Hutchinson, 1850) with tidal volumes at rest ranging between
442 to 1549 ml per breath (Dejours et al 1961). Generally, as ventilation period lengthens the magnitude of RSA increases and as lung volume increases it is accompanied by increases in magnitude of RSA. These observations have been collected in several studies where subjects were asked to breathe spontaneously and then also to follow a breathing frequency given by an auditory or visual cue. Results showed the amplitude of RSA to be greater in lower breathing frequencies and with larger tidal volumes (Davies & Nielson, 1967, Hirsh and Bishop, 1981, Eckberg, 1983, Grossman & Kollai, 1993, Brown et al., 1993, Hayano et al., 1994, Haggenmiller et al., 1996, Zhang et al., 1997, Stark et al., 2000, Taylor et al., 2001, Calabrese et al., 2000). A study by Hirsh and Bishop (1981), which is commonly cited for its comprehensive collection of data depicting the influences of breathing frequency and tidal volume on respiratory related R-R interval changes, examined the selective influences of both breathing frequency and tidal volume on the extent of RSA. Subjects were instructed to keep tidal volume constant while breathing at several assigned breathing frequencies between 1 and 60 cycles/minute. Results confirmed previous findings of an inverse relationship between RSA amplitude and breathing frequency but also showed heart rate coupling not to be a major factor in determining the extent of RSA. In addition, these authors found the extent of RSA to have reached its greatest magnitude at breathing frequencies lower than 7 breaths per minute and to remain stable despite further lowering of the breathing frequency (Figure 3). The greater values of RSA reported at lower breathing frequency or longer inspiratory times may be explained by the fact that a sufficient lapse of time exists for expiratory cholinergic influences to be dissipated, resulting in a lower residual vagal tone and consequently a greater HR response (Calabrese et al., 2000).

2.1.2 Influence of tidal volume on RSA

A number of studies, which manipulated breathing frequency as previously discussed, also used tidal volume as an independent variable to study HRV and RSA (Hirsch and Bishop, 1981, Selman et al., 1982, Eckberg, 1983, Pagani et al., 1986, Bernardi et al, 1989, Brown et al, 1993, Haggenmiller 1996). Tidal volume can be voluntarily controlled by subjects through visual directives or involuntarily through passive ventilation. It is generally accepted that RSA is

dependent on the depth of breathing (Hirsh & Bishop, 1981, Eckberg, 1983, Brown et al., 1993, Kamath, 1993, Haggenmiller et al., 1996).R-R interval spectral analysis has revealed increases in tidal volume between 700 to 1,500 ml to result in 13 to 17% increases in spectral power components reflecting increased HRV (Haggenmiller et al., 1996, Brown et al., 1993).
Other investigations have examined the isolated influence of changing tidal volume on RSA *per se*. Hirsh & Bishop (1981) as well as Eckberg (1983) examined a total of 23 subjects ranging in age between 22 and 78 years during breathing at tidal volume of between 0.5 and 3 L . In addition, Hirsh & Bishop (1981) also obtained RSA amplitude during inspiratory breath-hold at different lung volumes. Results from the breath-hold volume indicated a linear positive relationship with r^2 values > 0.90 which was consistent with overall observations that RSA amplitude is characterized by independent relationships between lung volume and breathing frequency. It has been suggested that increased lung inflation or the increase atrial filling typical of inspiration was responsible for the cardiac acceleration of RSA. Selman et al (1982) also examined the interaction between heart rate and respiration with imposed changes in breathing frequency and tidal volume using a bode-plot method. The effect of tidal volume and breathing frequency on HRV resulted in a non-linear response. The significance of this finding remains to be clarified. On the other hand, their results from atropine administration also confirmed that respiratory frequency related spectral power was abolished indicating that vagal modulation of RSA ceases with muscarinic blockade. End-tidal CO_2 were corrected for decreases observed during increased tidal volumes but no change was observed in the extent of HRV.

Whether lung inflation and deflation *per se* is the main underlying mechanism for the initiation of RSA has been considered using a number of approaches both in animal and human models (Davies & Neilson, 1967, Hirsh & Bishop, 1981, Horner at al., 1995). For example, in a canine model (Horner et al., 1995) - (n=5, normal adult canines) using mechanical ventilation demonstrated that after cessation of ventilation at a maximal lung volume setting of 750 ml, RSA continued within the first phantom cycle (where respiratory cycle would have occurred) with a mean amplitude of (25.4±4.6(SE)b/m) both before and during the 1st phantom cycle was similar. During subsequent phantom cycles RSA became significantly different from mechanical cycles.

In humans, Hirsh and Bishop (1981) examined the extent of RSA at static and step-like changes in lung volumes between 0.5 and 3 L. Results indicated that RSA magnitude increased for higher static lung volumes, RSA persisted during apnea but its amplitude decreased over the first

10-20 s and then at about 30-50 s HR oscillations increase until the end of the breath hold. Therefore results showed RSA to disappear and reappear while lung inflation was maintained. These observations suggest that rhythmic afferent activity stimulated by dynamic respiratory movements is not completely responsible for the genesis of HR oscillations and does not solely dictate its amplitude (Hirsh and Bishop). Authors suggested this to be related to the importance of central control on the modulation of RSA. On the other hand, because of the observed RSA amplitude and volume relationship the stretch receptors in the lung also play a role making it difficult to separate the effect of lung volume receptor stimulation from that of phrenic nerve stimulation to induce the initial diaphragm contraction.

2.1.3. Influence of voluntary control of breathing versus spontaneous breathing

A number of investigations have been performed to determine whether the voluntary control of breathing as opposed to spontaneous breathing can influence the extent of HRV or RSA since it may interfere with inter-central communication as previously described (Figure 2). Imposing a breathing rate typically requires the preliminary assessment of the spontaneous breathing rate in a subject sitting quietly. A breathing rate selected to be either that of the spontaneous rate or another higher or lower than the spontaneous can then be provided with an auditory or a visual signal. Results of studies in which the breathing rate was imposed at the spontaneous breathing rate generally indicate voluntary pacing of breathing not to influence the outcome of RSA or HRV in a significant way (Hirsh & Bishop, 1981, Hayano et al., 1994 & Eckberg 1983, Dinh et al., 1999). Results obtained by Hirsh & Bishop (1981), Hayano et al (1994), Eckberg (1983) and Dinh et al (1999) using time-domain, power spectral density and breath-by-breath analysis respectively all confirm that mean R-R intervals(±), spectral components and RSA indices are not significantly different between spontaneous breathing and voluntary imposed breathing conditions. However, in one study by Stark et al (2000) a significant difference was observed in R-R interval between spontaneous and imposed (814±103 and 780±108ms) breathing at 12 breaths per minute, the authors suggested that inconsistencies between studies may be due in part to variation in methodology such as signalling (visual *versus* auditory) and selected breathing frequencies may confound spectral data because LF may reflect some HF power when breathing frequencies are selected below .15 Hz.

In a study by Calabrese et al (2000) the use of resistive load was applied to the cardiorespiratory interaction to examine changes in breathing pattern, negative intrathoracic

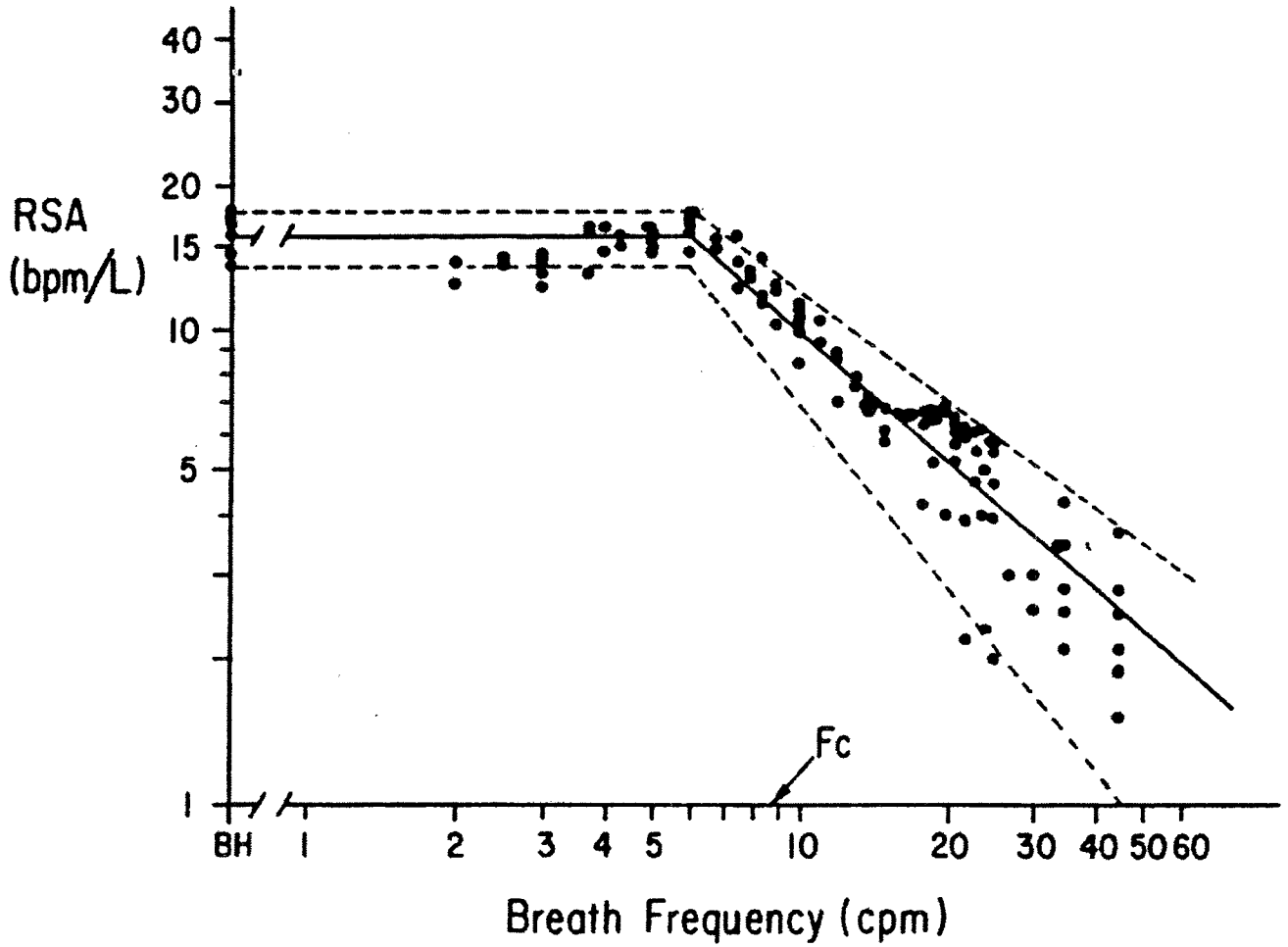


Figure 3. Respiratory sinus arrhythmia amplitude versus breathing frequency at 1L tidal volume. (Hirsh & Bishop, 1981)

pressures and concomitant effects on HRV and RSA response. Increases in resistive load indirectly caused increases in respiratory period, reducing breathing frequency. Results indicated unchanged HR with increasing HRV and RSA parameters. These observations may be taken to reflect that involuntary changes in breathing rate similarly affects RSA suggesting that the rate of breathing *perse* may be the primary determinant of RSA.

2.1.4 Influence of entrainment on respiratory rhythm

It has been demonstrated in various mammals that locomotion or exercise such as walking or pedaling can result in entrainment of the respiratory rhythm to the rhythm of the exercise (Bramble & Carrier, 1983, Persegol et al., 1991, van Alphen & Duffin, 1994, Funk et al., 1992, Mateika & Duffin, 1995). Bramble and Carrier collected data from simultaneous recordings of gait and ventilation in rabbits, dogs, horses and humans. Results indicated that breathing and gait were tightly coupled in quadrapedic mammals as well as in striding bipedal gaited humans with specific respiration-locomotion coupling ratios varying in pattern between species. For example horses employ a constant ratio of 1:1 (strides per breath) during trot and gallop whereas humans have a repertoire of coupling patterns that can appear while running (4:1, 3:1, 2:1, 1:1, 5:2, and 3:2) although a two stride per breath ratio (2:1) appears to be dominant. Similarly in the Canada goose studies by Funk et al (1992 II) resulted in a repertoire of observed ratios of wing flapping coupled to respiration of (1:1, 2:1, 3:1, and 4:1) and after decerebration a 1:1 pattern predominated. Removal of all afferent activity did not hinder the ability of continued passive wing flapping to entrain respiration (Funk et al, 1992 I).

Locomotion is not the only activity observed to influence the entrainment of breathing pattern. Mechanical ventilation has been used in animal and human models to examine machine generated ventilatory variations in volume and frequency conditions and entrainment (Simon et al., 1999, Baconnier et al., 1992, Muzzin et al., 1992, Graves et al., 1986). Entrainment in this case implies the resetting of respiratory rhythm to a respiratory rate such that a fixed, repetitive, temporal relationship exists between the onset of neural inspiratory activity and a mechanical breath. Entrainment with mechanical ventilation has been of clinical importance in the area of intensive care where patients are dependent on ventilator machines and the need for effective ventilatory generated breathing patterns. Respiratory entrainment using other external modes such as visual signalling to modulate flow volume and frequency have been used therapeutically in

some pathologies such as chronic obstructive pulmonary disease to adopt more efficient patterns of respiration.

A few studies have examined the locomotor-respiratory coupling specific to a sport and investigated the hypothesis that entrainment occurs due to training (Mahler et al., 1991, Steinacker et al., 1993, Bonsignore et al., 1998). In general the observations from these studies indicate that entrainment does occur. For example, in a study of novice female rowers by Mahler et al (1991) it was observed that after 4 months of training a new pattern of breathing during exercise was observed which was more tightly coupled to the movement pattern. To our knowledge the effect of the change in breathing pattern through entrainment on the extent of RSA has not been investigated.

3.0 The influence of training state on HRV and RSA

Many investigations have been conducted to examine the influence of physical training on the heart and its modulation using heart rate variability analysis in the time and frequency domains. These studies have used experimental approaches including both cross-sectional comparison of athletic and non-athletic subjects as well as pre- and post-training comparisons of healthy subjects. Table 3 and 4, summarize findings from these studies. Due to the large range of methods used (time or frequency domain) and differences in the units of measurements (absolute in s^2/Hz; in beats^2/Hz; in normalized units) differences or changes are reported using percentage difference in athletes from non-athletes or following training as compared to before training.

3.1 Cross-sectional comparisons of athletes versus non-athletes

Cross sectional comparisons of athletes versus non-athletes, fit versus inactive sedentary healthy individuals have been used as indirect examination of the influence of endurance training on heart rate variability. The standard deviation of the R-R interval as well as frequency spectral density have been used as indices of parasympathetic and sympathetic modulatory influences on the sinoatrial node. Table 3. summarizes nine studies published over the last 8 years on a total of 339 athletic (Jensen-Urstad et al; 1997, Ishida et al; 1997, and Lazoglu et al; 1996)or fit subjects (Tulppo et al; 1998 and Gregoire et al; 1996). Both these groups were compared to a sedentary one or to subjects that were significantly less fit than the experimental group. As expected, results indicate in most cases, a significantly higher VO_2max (20% to 88%) in the trained groups, while the mean R-R intervals were considered 6% to 47% higher in highly trained individuals compared to controls, with a mean difference of 25.7% (Jensen-Urstad et al; 1997, Ishida et al; 1997, Goldsmith

et al; 1997, Gregoire et al; 1996, Davy et al; 1996, and Sacknoff et al; 1994). As seen in table 3, the SDRR interval was generally higher in endurance athletes and highly fit individuals. Six of 8 studies showed statistically higher SDRR values of 5% to 67% in the experiment groups (Tulppo et al; 1998, Yataco et al; 1997, Jensen-Urstad et al; 1997, Goldsmith et al; 1997, Davy et al; 1996, and Sacknoff et al; 1994) while Gregoire et al (1996) found non-significant differences between 4 groups of trained subjects and 4 groups of untrained subjects. The experimental group trained at least 5 times a week for 45 min, however this training regimen was associated with differences in SDRR. More specific indices of HRV from the frequency domain analysis are shown in Table 4. The table summarizes results from 8 cross-sectional studies published over the last decade comparing frequency domain heart rate variability in trained and untrained individuals. In these studies 165 males and 88 females between 21 and 69 years old were evaluated under standard resting conditions. Maximal oxygen consumption was measured in 5 of 8 studies and attests to an average 61% higher value in athletes than non-athletes with differences ranging from 20 to 88% (Yataco et al; 1997, Goldsmith et al; 1997, Gregoire et al; 1996, Davy et al; 1996, Lazoglu et al; 1996, Sacknoff et al; 1994). Resting heart rate was measured in 7 studies and was found to be 4% to 33% significantly lower. (Yataco et al., 1997, Jensen-Urstad et al., 1997, Ishida et al., 1997, Goldsmith et al., 1997, Gregoire et al., 1996, Davy et al., 1996, Lazoglu et al., 1996). Frequency domain variables generally appeared higher in highly trained individuals compared to the sedentary sample population. Total spectral power of R-R was generally found to be statistically higher in endurance trained subjects compared to controls which may be associated with statistically higher HF power components in endurance trained individuals compared to sedentary individuals the difference ranging from +25% to +353% (Davy et al., 1996, Jensen-Urstad et al., 1997, Ishida et al., 1997, Goldsmith et al., 1997, and Yataco et al 1997). Similarly, significantly lower values ranging in magnitude from 8% to 177% were found in 50% of these studies for the LF component of endurance athletes compared to sedentary controls (Yataco et al; 1997, Jensen-Urstad et al; 1997, Goldsmith et al; 1997, and Davy et al; 1996). On the other hand, Sacknoff et al (1994) and Ishida et al (1997) found significant lower values, 33% to 47 %, in athletes compared to sedentary controls. A clear explanation for this discrepancy remains to be provided. In addition, a close look at these results indicates some controversy. Total power and low/high frequency values may be coerced by large within subject variability, and often the results of some studies did not reach statistical significance. The only somewhat consistent finding appears to be of a higher HF

Table 3. Time domain heart rate variability in trained subjects compared to untrained subjects.

Authors	Subjects N F/M	Age (Years)	Training	VO_2max Δ%	HR Δ%	R-R Δ%	SDRR Δ%
Tulppo et al;1998	25 M	43 ± 10	Poor fitness				
	36 M	40 ± 9	Average fitness	+20.6†			+66.7†
	25 M	40 ± 9	Good fitness	+24.4†			+30.0†
Yataco et al; 1997	14 M	69 ± 4	Sedentary				
	15 M	69 ± 7	Athletes	+70†			+4.8†
Jensen-Urstad et al; 1997	13 M	25 ± 1	Sedentary				
	16 M	25 ± 3	Elite runners		-33.6†	+29.4†	+48.8†
Ishida et al; 1997	16	27 ± 12	Sedentary				
	8	27 ± 12	Athletes		-12.7†	+14.6†	
Goldsmith et al; 1997	8 M	29 ± 3	Sedentary				
	8 M	28 ± 4	Endurance	+87.6	-25.4†	+47.4†	+7.6†
Gregorie et al; 1996	10 M	21 ± 2	Untrained				
	10 M	27 ± 4	trained	+20.7	-12.9†	+15.6†	+3.0
	9 F	22 ± 2	Untrained				
	10 F	22 ± 3	trained	+23.0	-5.1†	+5.6†	+3.9
	8 M	44 ± 8	Untrained				
	9 M	43 ± 5	trained		-23.3†	+30.5†	+24.0
	7 F	44 ± 8	Untrained				
	10 F	44 ± 7	Trained		-16.9†	+20.3†	+37.6
Davy et al; 1996	11 F	56 ± 2	Sedentary				
	9 F	53 ± 1	Runners	+88.4†	-19.1†	+24.2†	+53.1†
Lazoglu et al; 1996	10	28 ± 3.	Sedentary				
	10	27 ± 2	Weight lifters	+30.7†	-7.4	+4.5	+8.4
	12	29 ± 3	Cyclists	+46.5†	-3.9	+6.4	+19.9
Sacknoff et al; 1994	3F/9M	26 ± 2	Sedentary				
	1F/17M	30 ± 0	Endurance		-22.7†	+37.9†	+26.0†
Mean	**251M 88F**	**37**		**+45.8**	**-16.6**	**+21.5**	**+25.7**

M/F=male/female; HR=heart rate; Δ%=difference between trained and less trained; R-R=mean R-R intervals; SDRR= standard deviation between R-R interval;.†= significant difference compare to the group above ($p<0.05$).

component of HRV in highly endurance trained individuals, which may be suggestive of a higher vagal influence. Few studies (Kenney et al; 1985, Benedito et al; 1985) have however examined the more specific vagally mediated respiratory component of HRV, RSA. Kenney et al (1985) found a relationship of r=0.92 between variation in heart period during cardiopulmonary synchronization of respiration and VO_2max (ml/kg/min) in 21 healthy subjects. On the other hand, Benedito et al (1985) found no differences in the amplitude of RSA between 7 endurance athletes and 13 sedentary control subjects. Similarly, using a longitudinal approach these investigators observed no change in the index of RSA after 10 weeks of endurance training (5 times per week) in 7 previously sedentary subjects. Due to the limited number of studies it is difficult to fully interpret findings from HRV and RSA studies of athletes although the HF component appears to be predominant in endurance-trained athletes compared to non-athletes.

Table 4. Spectral power results of heart rate variability in trained subjects compared to untrained subjects.

Authors	Subjects N F/M	Age (Years)	Training	VO_2max Δ%	HR Δ%	TP Δ%	LF Δ%	HF Δ%
Yataco et al; 1997	14 M 15 M	69 ± 4 69 ± 7	Sedentary Athletes	+70†		+8.9†	+8.1†	+24.9†
Jensen-Urstad et al; 1997	13 M 16 M	25 ± 1 25 ± 3	Sedentary Elite runners		-33.6†	+117.4†	+67.1†	+90.0†
Ishida et al; 1997	16 8	27 ± 12 27 ± 12	Sedentary Athletes		-12.7†		- 33.3†	+66.7†
Goldsmith et al; 1997	8 M 8 M	29 ± 3 28 ± 4	Sedentary Endurance	+87.6	-25.4†	+118.9†	+177.2†	+339.9†
Gregorie et al; 1996	10 M 10 M 9 F 10 F 8 M 9 M 7 F 10 F	21 ± 2 27 ± 4 22 ± 2 22 ± 3 44 ± 8 43 ± 5 44 ± 8 44 ± 7	Untrained trained Untrained trained Untrained trained Untrained Trained	 +20.7 +23.0 	 -12.9† -5.1† -23.3† -16.9†	 +17.4 +7.7 +48.9 +119.1†		
Davy et al; 1996	11 F 9 F	56 ± 2 53 ± 1	Sedentary Runners	+88.4†	-19.1†	+156±	+112.9†	+353.5†
Lazoglu et al; 1996	10 10 12	28 ± 3. 27 ± 2 29 ± 3	Sedentary Weight lifters Cyclists	 +30.7† +46.5†	 -7.4 -3.9	 +27.7 +28.5	 +52.1 -18.0	 +119.8 -13.5
Sacknoff et al; 1994	3F/9M 1F/17M	26 ± 2 30 ± 0	Sedentary Endurance		-22.7†	-52.3†	-43.6†	-188.0†
Mean	**165 M 88 F**	**36**		**+61.2**	**-17.8**	**+54.4**	**+40.3**	**+99.1**

M/F=male/female; HR=heart rate; Δ%=the difference between trained and untrained groups; TP=total power; LF=low frequency; HF=high frequency; †= significant difference compared to the group above ($p<0.05$).

3.2 Comparison of pre- and post-training HRV

Investigations using a longitudinal approach to examine the influence of endurance training on heart rate variability have been conducted in both healthy sedentary individuals as well as on heart disease patients undergoing exercise rehabilitation.

Tables 5 and 6 summarize the effects of endurance training on time domain and frequency domain HRV variables in healthy endurance trained populations. These tables report on a total of 182 experimental subjects and controls ranging in age between 20 and 67 years old participating in a total of 9 studies over the last 13 years (Loimaala et al., 2000, Schuit et al., 1999, Levy et al., 1998, Stein et al., 1999, Sheldahl et al., 1994, and Seals et al., 1989, Ishida & Okada, 1998, Al-Ani et al., 1996, Boutcher et al., 1995). Endurance training consisted of jogging, walking and/or cycling between 2 and 7 times per week for durations lasting as little as 6 or 8 weeks up to 52 weeks. No significant differences were found between subjects and controls in VO_2max, resting heart rate, and time domain heart variability (Loimaala et al., 2000, Schuit et al., 1999, Levy et al., 1998, Stein et al., 1999, Sheldahl et al., 1994, and Seals et al., 1989). An increase in VO_2max of between 8.6 to 30.5% was generally reported. A concurrent statistically significant decrease (6.7%) in resting heart rate was observed in 8 of these 9 studies measured a lower resting heart rate. After training an significant increase in SDRR was found in 3 studies (Levy et al; 1998, Stein et al; 1999, and Seals et al., 1989). As seen in Table 6, a statistically significant increase in the power of the HF or vagal component of HRV was only seen in one study of young subjects undergoing 25 min cycling sessions every day for a period of 6 weeks (Al-Ani et al 1996). Three studies showed non-significant decreases ranging from 0.9% and 25% (Schuit et al., 1999, Ishida & Okada, 1998, Boutcher et al., 1995), while another two studies showed non significant increase ranging between 2.7% and 5.1% (Loimaala et al., 2000 and Stein et al., 1999) despite longer training durations. Interestingly, while indicating a significant increase in the HF component of HRV, results from Al-Ani et al also indicate a significant increase from pre-training in the LF band of HRV. Other studies have reported either small (1% to 20 %) non-significant increases or a decrease in the LF component (Boutcher et al; 1995). Unlike the cross-sectional observations which may be suggestive of a higher vagal influence on HRV in endurance trained athletes, results from longitudinal studies are not consistent in showing an increase in the vagal component following training. These inconsistencies could be related to differences in subject characteristics, exercise

Table 5. The Effect of endurance training on time domain HRV during rest in sedentary healthy population.

Authors	Subjects N F/M	Age (Years)	Training (weeks)	VO_2max Δ%	HR Δ%	R-R Δ%	SDRR Δ%
Loimaala et al; 2000	26 M	46 ± 6	22	+10.9	-1.5	+1.5	+6.4
	28 M	47 ± 6	22	+15.0†	-5.9†	+6.2†	+8.6
Schuit et al; 1999	27 F/M	67 ± 5	27	+8.6†	-1.6	+2.4	+5.3
Levy et al; 1998	13 M	68	26	+21†	-9.0†	†	+67.7†
	11 M	28	26	+17†	-5.0†	†	+17.2†
Stein et al; 1999	7M/ 9F	66 ± 4	52	+30.5†	+6.5†	+4.6†	+12.7†
Sheldahl et al; 1994	10 M	54 ± 8	12	+17.0†	-7.8†	+8.5†	+7.3
Seals et al: 1989	11 M	53 ± 2	30	+29.7†	-7.9†	+8.8†	+15†
Mean	**120M 22 F**	**54**	**12-52**	**+13.4**	**-4.8**	**+4.5**	**+14.5**

M/F=male/female; HR=heart rate; R-R=mean R-R intervals; SDRR= standard deviation in R-R intervals; Δ%=difference between pre and post endurance training, †=significant different between pre and post endurance exercise ($p<0.05$).

Table 6. The effects of endurance training on resting spectral power values of HRV in healthy individuals.

Authors	Subjects N F/M	Age (Years)	Training (weeks)	VO_2max Δ%	HR Δ%	TP Δ%	LF Δ%	HF Δ%
Loimaala et al; 2000	26 M	46 ± 6	22	+10.9	-1.5		+1.0	+2.7
	28 M	47 ± 6	22	+15.0†	-5.9†		+2.5	+5.1
Schuit et al; 1999	27 F/M	67 ± 5	27	+8.6†	-1.6		+8.2	-3.6
Ishida et al; 1998	4 M/ 8F	38 ± 9	8		-10.6†		+20	-25.0
Stein et al; 1999	7M/ 9F	66 ± 4	52	+30.5†	-6.5	+3.1†	+3.3	+4.1
Al-Ani et al; 1996	9 F/M	20 ± 1	6	+13.3†	-17.4†		+190.1 †	+150.0 †
Boutcher et al; 1995	19 M	46 ± 1	8	+12.0†	-4.8 †		-2.1	-0.9
Mean	**102 M 35 F**	**50**	**6-52**	**14.2**	**-5.3**	**+3.1**	**+16.6**	**+8.9**

M/F=male/female; HR=heart rate; TP=total power; LF=low frequency; HF=high frequency; Δ%=difference between pre and post endurance training, †=significant different between pre and post endurance exercise ($p<0.05$).

training protocols or HRV analysis method. On the other hand, it is possible that observed differences between athletes and non-athletes are not due to a training effect but may be related to individual physiological characteristics.

4.0 Position of the Problem

It has been well documented that respiration is the prime modulator of heart rate variability. Several studies have shown HRV and RSA to be related to breathing pattern. More specifically, it has been shown that RSA is related to breathing frequency and tidal volume such that for a given heart rate, increasing tidal volume or decreasing breathing frequency results in an augmentation in the extent of RSA. Other factors have also been found to modulate the extent of RSA such as age and/or physical training state. For example, cross-sectional studies of endurance athletes indicate a higher HF or vagal component of HRV in athletes compared to non-athletes. Not too many studies have examined RSA parameters in athletes and overall findings are inconclusive.
A clear mechanism for the integration of heart and lung characteristic to determine amplitude or phase of RSA is still lacking. Evidence obtained during breath-hold indicates RSA to persist during the first phantom cycle (where the respiratory movement would have appeared) of apnea and to be of greater magnitude for increased static lung inflation. Moreover, this RSA amplitude has been seen to disappear but to reappear during breath-hold while lung volume is static, suggesting the important involvement of central neural factors.

On the other hand, it has been shown that breathing patterns can be modified involuntarily to maximize breathing efficiency. For example, during exercise, a coupling ratio of muscle movement with respiration exists in both animals and humans. If these changes in breathing pattern persist for a period of time entrainment is also possible.
Athletes have also been shown to have a higher vagally mediated HRV although not too many studies of RSA to our knowledge have examined the influence of their athletic status on the gain of the RSA amplitude to changes in breathing pattern.

Physical training has also been suggested to lead to entrainment of the breathing pattern. Swimmers are required to adapt their breathing pattern to their activity such that inspiration does not occur during the immersion phase of the activity. It is thus possible that such a repeated perturbation of the breathing pattern may result in some respiratory entrainment in these subjects.

The purpose of the present study was thus twofold; first to examine whether the gain in RSA to changes in breathing rate is different in endurance athletes and healthy non-athletes aged matched and secondly, to examine whether the repeated swimming-induced perturbation affected the gain of the RSA response to variations in breathing frequency as compared to that of running-induced breathing entrainment RSA response.

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Part II

Experimental Article

Introduction

Respiratory sinus arrhythmia (RSA) is the most important determinant of resting heart rate variability. As vagal inhibition leads to increasing heart rate during inspiration and lifting of this inhibition at the end of inspiration results in a decrease in heart rate during expiration (Katona & Jih, 1975, Hirsch & Bishop, 1981, Selman et al., 1982, Brown et al., 1983). The extent of RSA is generally reported as the amplitude of the maximum change in spontaneous heart rate occurring throughout a breathing cycle. It is commonly accepted that RSA amplitude increases when heart rate is reduced and inversely, decreases when heart rate increases (Katona & Jih, 1975, Eckberg, 2000). A common method for the assessment of RSA is to ask subjects to follow auditory or visual cues in order to manipulate breathing frequency and/or tidal volume. Results from these studies indicate that for a given steady-state heart rate, RSA amplitude increases with longer respiratory intervals. Thus, reducing breathing frequency or increasing tidal volume increases RSA amplitude (Davies & Nielson, 1967, Hirsch & Bishop, 1981, Eckberg, 1983, Grossman & Kollai, 1993, Brown et al., 1993, Hayano et al., 1994, Zhang et al., 1997, Stark et al., 2000, Haggenmiller et al., 1996, Cooke et al., 1998, Calabrese et al., 2000, Taylor et al., 2001).

Observations in several mammalian species indicate the existence of locomotor-respiratory coupling (Bramble & Carrier, 1983, Persegol et al., 1991, Funk et al., 1992, Van Alphen & Duffin, 1994, Mateika & Duffin, 1995). Bramble and Carrier collected data from simultaneous recordings of gait and ventilation in rabbits, dogs, horses and humans. Results indicated that breathing and gait were tightly coupled in quadrapedic mammals as well as in striding bipedal gaited humans with specific respiration-locomotion coupling ratios varying in pattern between species. For example horses employ a constant ratio of 1:1 (strides per breath) during trot and gallop whereas humans have a repertoire of coupling patterns that can appear while running (4:1, 3:1, 2:1, 1:1, 5:2, and 3:2), although a two stride per breath ratio (2:1) appears to be dominant (Bramble & Carrier, 1983). Moreover, in decerebrated Canada geese (Funk et al 1992 l), passive wing flapping resulted in a similar coupling of wing beat frequency and respiratory frequency as that observed during free flight suggesting that central feedback from the chest wall and or lung may be important in producing entrainment. A few studies have examined the locomotor-respiratory coupling specific to a sport and examined the hypothesis that entrainment occurs due to repeated bouts of acute dynamic exercise (Mahler et al., 1991, Steinacker et al., 1993, Bonsignore et al., 1998). In general the observations from these studies indicate that entrainment can be

induced by physical training such that there exists a closer relationship between frequency of movement and frequency of breathing after training (Mahler et al., 1991, Steinacker et al., 1993). For example, in a study of novice female rowers by Mahler et al (1991) results indicate a new pattern of breathing during rowing practice after 4 months of training, which was reflective of a tighter coupling of movement to breathing pattern. It remains to be seen however whether respiratory entrainment resulting from repeated exercise training would have an impact on the extent of modulation of heart rate by respiration or RSA.

Several studies using spectral analysis of R-R intervals have been conducted on endurance-trained athletes and non-athletes to examine the effect of training state on resting cardiac vagal influence (Davy et al., 1996, Goldsmith et al., 1997, Tulppo et al., 1998, Yataco et al., 1997, Jensen-Urstad et al., 1997 & Ishida & Okada, 1997). Results from these studies generally reveal a larger HF or vagal component of the HRV in endurance athletes (Jensen-Urstad et al; 1997 and Shin et al; 1996) compared to non-athletes, which, has been taken to suggest a greater resting parasympathetic sinoatrial influence. On the other hand, reports of power spectral density as well as of standard deviation of R-R intervals at rest have also shown lesser or no differences in cardiac vagal influence in endurance athletes compared to non-athletes (Sacknoff et al., 1994, Lazoglu et al., 1996) The inconsistency in results may be ascribed in part to the fact that the breathing pattern was not taken into account in these studies resulting in an undefined respiratory contribution to the R-R spectral power distribution. Individuals at rest exhibiting a breathing frequency below the minimum value of the standard High Frequency band of HRV i.e. 0.15 Hz or 9 breaths/minutes will exhibit a respiratory contribution to HRV appearing in the frequency band of the R-R power spectral density distribution designated as the standard Low Frequency or non-vagal band. As a result, the power spectral density of the standard HF component will be erroneously underestimated while that of the technically non-respiratory standard LF band will be overestimated. An alternate way to specifically assess the respiratory-related influence of HRV is to measure respiratory sinus arrhythmia (RSA).

There exists only a few studies having examined the influence of the endurance training status on RSA (Kenney, 1985, Benedito et al; 1985, De Meersman, 1991). The magnitude of RSA has generally been quantified as the difference between the maximum and minimum instantaneous heart rates recorded throughout each breath cycle over a series of successive respiratory cycles. Benedito et al (1985) found no differences in the amplitude of RSA between 7 endurance athletes

and 13 sedentary control subjects while Kenney (1985) reported a significant increase in RSA for trained and moderately active subjects compared to sedentary subjects (n=21). Benedito et al (1985) also observed no change in the index of RSA after 10 weeks of endurance training (5 times per week) in 7 previously sedentary subjects. De Meersman (1991) found an increase in the amplitude of RSA in 8 track athletes following an 8-week high-intensity running program (7 days a week). Overall, results from these studies as well as those examining HRV do not conclusively attest to an influence of regular endurance training on tonic vagal activity and/or on the extent of vagally-mediated modulations of heart rate by respiration. It is well know that many factors related to inspiration are involved in determining the extent of the vagal effector response of the sinus node throughout the breathing cycle (Eckberg, 1995, 2000). To our knowledge, there has been no study examining the influence of the training status or of the potential respiratory entrainment resulting from the regular exercising pattern on the RSA response

In the present study we compared the extent of RSA in varsity athletes and age-matched non-athletes under baseline spontaneous breathing conditions as well as in response to various imposed breathing frequencies. We hypothesized that if a parallel upward shift of the RSA amplitude *versus* breathing period relationship was seen for the endurance trained group compared to that of the untrained group, we could infer an effect of training status on cardiac vagal influence. The slope of the RSA amplitude *versus* breathing period relationship defines the sensitivity or gain of the RSA response. It is also conceivable that instead of causing an upward shift in the RSA amplitude *versus* breathing period relationship, regular endurance exercise could affect its sensitivity or gain such that a greater modulatory influence would be observed for the same decrease from spontaneous breathing rate.

The main purpose of this study was thus to characterize the RSA amplitude *versus* breathing period relationship in order to bring further insight into the mechanism of training-induced bradycardia and the influence of training on the sensitivity of the respiratory frequency related modulation of RSA.

A secondary purpose was to examine whether regular perturbations of the breathing pattern associated more specifically with the activity of swimming might be associated with locomotor-respiratory entrainment and modify the RSA integration mechanisms. We reasoned that the existence of a locomotor-respiratory entrainment resulting from the water-immersion breathing pattern of swimmers would be reflected in differences in the breathing frequency-RSA response

curves between endurance swimmers and non-aquatic endurance athletes. We therefore compared the RSA amplitude *versus* breathing period relationships of varsity swimmers to that of varsity runners.

Methodology

Subjects

Thirty-two healthy subjects, non-smokers, agreed to participate in this study on a voluntary basis. After a description of the experimental design and protocol each subject signed a consent form approved by the institutional Ethics Review Board. Volunteers were assigned to an athletic or sedentary group on the basis of their regular swimming or running endurance exercise regimen. Swimmers were considered to be in the experimental group because of their regular exposure to water-dependent breathing patterns during training, which might impact on the integration of respiratory sinus arrhythmia in addition to an endurance-training effect. Runners were used as the experimental control group on account of their endurance training effect comparable to that in swimmers but different from that of sedentary group.

***Experimental Group*:** The experimental group consisted of eleven varsity swimmers, six females and five males ranging in age from 18 to 22 years with a mean of 20 ± 1.4 years performing more than 15 hours per week of training in the pool. The average height and weight were 170.7± 18.8 cm and 69 ± 8 kg. The mean maximal heart rate was 188.6 ± 14.5 beats per minute while maximal oxygen consumption in swimmers ranged from 59 to 75 $ml \cdot kg^{-1} \cdot min^{-1}$ with a mean value of 66.5 ± 7.8.

***Experimental Control Group*:** The experimental controls consisted of 9 varsity endurance runners, four female, six male with a mean of 22 ± 2.4 years performing more than 10 hours of endurance running a week. The average height and weight were 169.7 ± 8.9 cm and 64.6 ± 7.9 kg. The mean maximal heart rate was 196 ± 10 beats per minute while maximal oxygen consumption in runners ranged between 60 to 76 $ml \cdot kg^{-1} \cdot min^{-1}$; with a mean of 67.4 ± 5.9.

***Sedentary Control Group*:** The sedentary group consisted of 12 subjects, 7 female and 5 male performing less than 3 hours of physical activity a week. The age range of sedentary subjects

were 18 to 36 years, with mean 24 ± 3.4 yrs, height and weight 173.7 ± 8.4 and 66.1 ± 12.5 kg. The mean maximal heart rate was 201 ± 13 beats per minute while maximal oxygen consumption ranged between 31 to 52 ml• kg^{-1}• min^{-1}; with a mean of 40 ± 7.3.

Experimental Protocol

Subjects were asked to avoid consumption of caffeine 2 to 3 hours prior to testing and were asked to report to the laboratory on two separate occasions. During the first visit subjects were submitted to maximal oxygen consumption determination and on the second visit heart rate variability was assessed. During the heart rate variability procedures subjects were asked to refrain from talking and to remain as motionless as possible while the laboratory was dimly lit, temperatures were ambient (22° to 24°) and all visual and auditory distractions were minimized.

VO_2 max determination: Maximal oxygen consumption was measured using an incremental running protocol on a treadmill to volitional exhaustion. Ventilation, respiratory rate, tidal volume, VO_2, CO_2 production, and respiratory exchange ratio were continuously monitored using an automated metabolic cart (Model 2900, Sensormedics, Anaheim, CA). True maximal effort was assumed if two of the following criteria were met: 1) a lack of O_2 increase with an increase in work rate 2) attainment of maximal heart rate predicted for each subject's age 3) a respiratory exchange ratio > 1.10.

Assessment of Heart Rate Variability and respiratory sinus arrhythmia:
Electrocardiographic and ventilatory flow parameters were obtained as previously described (Pham Dinh et al., 1999, Calabrese et al., 2000). Briefly, subjects were seated and wore a facemask mounted a flowmeter (Hans-Rudolph pneumotach) and a differential pressure transducer. A series of three recordings of at least 5 minutes each were obtained under conditions of spontaneous breathing or during paced breathing as to explore respiratory sinus arrhythmia over a specified range of breathing rates. Following the first recording obtained at the spontaneous breathing rate **(SP)**, subjects were asked to follow a breathing rate given by an auditory cue at 1) four breathing cycles lower **(M4)** and 2) four breathing cycles higher **(P4)** than their spontaneous rate. Subject's blood pressure was determined during the last minute of each recording using an electronic arm sphygmomanometer (Sunbeam model 7650) placed on the arm.

Heart rate variability data acquisition and analysis

The acquisition of data was performed on an IBM microcomputer equipped with an analog-digital interface card (National Instruments, BNC-2110). Sampling rate was 250 Hz. For each given recording, a breath-by-breath analysis was performed to calculate respiratory period (T_{TOT}), inspiratory time (T_I) and tidal volume (V_T) and an average value and standard deviation was computed over all cycles. The ECG signal was processed and the R-R interval series were extracted and displayed on the computer screen in order to verify that the signal exhibited no noticeable trend and to show possible errors. Means ± SD of the R-R intervals were calculated for each recording. R-R intervals were linearly interpolated at 0.25-sec intervals to obtain equidistant time samples, and power spectral analysis was performed using a recording length of at least 1000 sample data points. A Fast Fourier Transform procedure was applied to obtain the low (LF:0.04-0.15 Hz) and high-frequency (HF:0.15-0.40 Hz) spectral power components. For each recording, a restricted respiratory frequency power component identified as the Respiratory Centred Frequency (RCF) component was also calculated, using the frequency range corresponding to ±10% of the respiratory rate averaged over the entire recording (Pham Dinh, 1999) . Values for spectral components are reported in (s^2, ms^2, and nu, normalized units). Normalized units are calculated by dividing total power by either the LF or HF and subtracting the VLF component of total power.

A more specific analysis of RSA was performed using a breath-by-breath analysis of heart rate variability (Pham Dinh, 1999). To quantify the extent of within-respiratory cycle RSA, a sinusoid is calculated, fitting to the changes in instantaneous heart rate within the respiratory cycle. Its amplitude, which may be considered the maximum heart rate within each breath is used as a measure of the magnitude of RSA. The instant of occurrence of this maximum is expressed either as a fraction of breath duration (phase) or in seconds (delay). Average amplitude, phase and delay values over several breaths are then calculated for each recording.

For each subject, the RSA amplitude measured for each of the three breathing rates was plotted and the slope of the RSA amplitude *versus* breathing rate response curve was considered an index of RSA sensitivity or gain of the RSA response to breathing rate.

Statistical Analyses

The results for descriptive variables such as age, height, weight, and maximal oxygen consumption were expressed as group means and standard deviations. Group mean comparisons were made using a one-way analysis-of-variance (ANOVA) on selected response variables.

For each recording group, mean comparisons of ventilatory parameters: tidal volume, total respiratory time, inspiratory time, delay, and the inspiratory time:total respiratory time ratio and tidal volume:inspiratory time ratio, blood pressure, heart rate and RSA amplitude, phase were performed using multivariate ANOVA (MANOVA-RM) to assess the impact of breathing condition on two or more dependent variables simultaneously using Statistica (StatSoft®, Statistica for Windows 5.0). The same method was used to compare power spectral components. Post-hoc comparisons were achieved using a Tukey Honest Significance test when a main effect was found to be statistically significant ($p \leq 0.05$). Spearman (non-parametric) and Pearson correlations were performed for heart rate, RSA amplitude, and phase for group and condition effects (Statistical Analysis System, SAS® Institute Inc., Cary, NC).

Results

Subject characteristics:

No significant differences were found for either height or weight between subject groups. Maximal oxygen consumption was significantly ($p \leq 0.05$) higher in both groups of athletes compared to non-athletes attesting to their highly trained state. However no significant difference in VO_2 max was found between runners and swimmers. Runners and swimmers had similar training schedules comprised of twelve hours or more of endurance training per week.

Circulatory and ventilatory measurements during spontaneous and imposed breathing conditions.

Mean values and standard deviations for circulatory and ventilatory parameters are shown in Tables 1 and 2. As can be seen, results indicate resting heart rate and blood pressure values within expected ranges for a normal healthy population. R-R interval and heart rate were both found to be significantly different in athletes compared to non-athletes, which confirms the bradycardia typically seen in athletes. There was no significant difference in resting heart rate between runners and swimmers. Overall there was no significant change in HR when breathing

was decreased or increased from spontaneous frequencies. Results from the ANOVA indicate no significant main group or condition effect for either systolic or diastolic blood pressure. The standard deviation of the R-R interval measured under spontaneous condition was not significantly different between groups. Changing breathing frequency did not affect the R-R interval however the standard deviation of the R-R interval was significantly different between each breathing condition. The mean and standard deviation for breathing frequency were comparable in all groups regardless of the breathing condition. Increasing or decreasing breathing frequency was not seen to have a significant effect on standard deviation of the breathing frequency irrespective of subject group.

The findings indicate tidal volume during spontaneous breathing to be comparable for all groups. However, under M4 and P4 breathing conditions tidal volume for both runners and swimmers are significantly greater than that of control subjects. As expected increasing breathing frequency decreased tidal volume while the inverse is observed with decreased breathing frequency. Total breathing time was significantly affected by breathing condition. Inspiratory time varied significantly with breathing conditions such that it was longer when breathing rate was slower than spontaneous and shorter when breathing rate was faster than spontaneous. Group differences for inspiratory time were not seen under any breathing condition. No significant findings were observed for tidal volume: inspiratory time and inspiratory time: total breathing time ratios.

Heart rate variability and respiratory sinus arrhythmia under spontaneous and imposed breathing conditions

Figures 1 (a), (b), (c) and (d) show the mean values of the power spectral density components (PSD) of the R-R interval variability, under spontaneous breathing condition as well as lower (M4) and higher (P4) breathing rates. Results from the ANOVA for total power in ms^2 indicate no main effect for either group or condition (Figure 1 (a)). Under the spontaneous breathing condition results indicate a slightly lower HF PSD component in controls compared to both swimmers and runners, although differences did not reach statistical significance. Similarly, no significant differences in HF PSD were observed between groups under the P4 and the M4 breathing conditions (Figure 1 (b)). Decreasing or increasing breathing frequency did not significantly affect the HF PSD. An overall main group effect for LF PSD was found showing that swimmers are significantly different from controls and runners although post-hoc analyses did not

reveal any significant group difference under any specific condition (Figure 1 (c)). Again, there was no effect of breathing condition on LF PSD. Finally, the RCF PSD component is not significantly different between groups. However, statistical analyses revealed a main condition effect for RCF PSD resulting in the significant difference of the M4 breathing condition compared to P4 and spontaneous breathing conditions while spontaneous and P4 are comparable (Figure 1 (d)).

RSA amplitude and RSA phase and RSA delay are shown in Figures 2 (a), (b), and (c). Results indicate a significant increase in RSA amplitude at M4 compared to SP and inversely a significant decrease in RSA amplitude at P4 compared to SP in all subject groups (Figure 2 (a)). No statistically significant difference in RSA amplitude was however observed between groups under any breathing condition. The RSA phase expressed as the fraction of the breath cycle is shown in Figure 2 (b). Results indicate no significant difference between groups or over breathing conditions. Statistical analyses on the RSA delay (Figure 2 (c)) expressed in seconds indicate no overall main effect of condition or group.

SENSITIVY OF THE RSA RESPONSE TO CHANGES IN BREATHING FREQUENCY.

Figures 3 (a) and (b) illustrate the relationship between RSA amplitude and breathing frequency expressed either relative to the spontaneous breathing rate (M4, P4) or as breathing frequency (breaths/min) per se (Figure 3 b). As can be seen in Figure 3 (a), a negative relationship is seen between RSA amplitude and breathing frequency with similar slopes of the relationship being found in all groups. However, results indicate a greater upward shift of the response curve for the runners compared to that of the swimmers and controls resulting in a higher parallel line for this group (not significant). When expressed relative to absolute breathing rates, results confirm the negative relationship between RSA amplitude and breathing rate. Figure 3 (c) depicts the sensitivity of the RSA phase response to absolute breathing rates under spontaneous and imposed breathing conditions. No differences are observed between groups or condition.

Discussion

The main findings from this study indicate no significant difference in the extent of RSA or heart rate variability in highly trained competitive endurance athletes exhibiting a significant resting bradycardia compared to age-matched untrained healthy controls at rest and in response to changes in breathing frequency. This observation thus suggests that HRV and RSA determination

may be not reflective of resting vagal tone as a lower vagal tone would be suspected in the athletes exhibiting a significantly lower heart rate. The present findings confirm a negative relationship between RSA amplitude and breathing frequency such that decreasing and increasing breathing frequency without affecting heart rate resulted in a significant increase and decrease in RSA amplitude independent of the specificity of training or training state. The slope of the RSA amplitude *versus* breathing period relationship curve was comparable for all groups. As reflected by slope characteristics, the gain of the RSA amplitude-breathing frequency response was not significantly different between groups suggesting that there are no influences of endurance training state on the extent of vagally-mediated respiratory induced sinoatrial responses.

Heart rate variability and RSA in endurance trained athletes

Over the last 8 years, spectral analysis of heart rate variability has been applied in some 14 studies to examine the relationship between HRV and exercise training (Sacknoff et al., 1994, Boutcher et al., 1995, Davy et al., 1996, Gregoire et al., 1996, Yataco et al., 1997, Jensen-Urstad et al., 1997, Goldsmith et al., 1997, Al-Ani et al., 1996, Lazoglu et al, 1996, Ishida & Okada., 1997, 1998, Schuit et al., 1999, Stein et al., 1999, loimaala et al., 2000). These studies have been mostly completed using a cross-sectional approach comparing active and inactive subjects either in the general population or more specifically comparing athletes to non-athletes. A number of studies have also focused on the changes in HRV resulting from 12 to 52 weeks of endurance training in healthy sedentary individuals (Boutcher et al., 1995, Al-Ani et al., 1996, Bonaduce et al., 1998, Ishida et al., 1998, Schuit et al., 1999, Stein et al., 1999, loimaala et al., 2000, Yamamoto et al., 2001). Results from these studies indicate higher HF component of HRV after training in five of eight studies (Al-Ani et al., 1996, , Schuit et al., 1999, Stein et al., 1999, loimaala et al., 2000, Yamamoto et al., 2001) while small decreases in HF were reported in two studies (Boutcher et al., 1995, Ishida & Okada., 1998). and no change in HF was reported in one study (Bonaduce et al., 1998).

A clear explanation for the discrepancy has not been provided. In healthy "active" *versus* " inactive" populations, a significant difference in total HRV or in the spectral marker of the vagal component of HRV do not appear to be observed (Gregoire et al., 1996). Results in highly trained endurance athletes *versus* non-athletes however have generally shown athletes as having higher HF or vagal component of HRV (Davy et al., 1996, Jensen-Urstad et al., 1997, Ishida et al., 1997,

Goldsmith et al., 1997, and Yataco et al 1997, Shin et al, 1997). In many of these studies however, the description of the HRV was based on the analysis of a 24-hour recording (Goldsmith et al., 1997, and Yataco et al., 1997, Jensen-Urstad et al., 1997) and the data was presented either as an analysis of the entire recording or a segment. The present data obtained through consecutive 5-minute recordings under standardized conditions of rest, posture and breathing frequency do not support that the power spectral density HF component of swimmers and runners is significantly different from that of untrained healthy control subjects.

A limitation in the use of the standard spectral analysis of HRV and the HF-band to define the respiratory-related or vagal component of HRV is to ensure that the average breathing rate of individuals throughout the recording indeed falls within the pre-defined frequency band. A review of the investigations of heart rate variability in athletes indicate three of the 14 studies to have used standardized short-term ECG recordings, of which two also controlled breathing frequency to ensure its occurrence within the HF band (Davy et al., 1996, Yamamoto et al., 2001) . This lack of conformity in method has lead to inconsistencies in reporting and interpreting of data for the HF component because of the possible loss of HF power to the LF band. Therefore this limitation makes it difficult to interpret the respiratory-related component being reported as uniformly representing the vagal component of HRV.

The present study incorporated a specific Respiratory Centred Frequency band in addition to the standard HF band, to delineate the respiratory or vagal component of HRV. Results again indicate no significant difference between groups for the PSD of the RCF of heart rate variability under all experimental conditions. The RCF PSD component of heart rate variability has been previously shown to be a better marker of RSA than the standard HF PSD of heart rate variability (Pham Dihn et al., 1999). There exists few studies on the interaction of physical training status and RSA characteristics (Kenney et al; 1985, Benedito et al; 1985, De Meersman, 1991). Comparison of the extent of RSA in endurance-trained *versus* untrained individuals indicate no differences in the amplitude of RSA between 7 endurance athletes and 13 sedentary control subjects (Benedito et al; 1985). Similarly, using a longitudinal approach these investigators observed no change in the index of RSA after 10 weeks of endurance training (5 times per week) in 7 previously sedentary subjects (Benedito et al., 1985) while De Meersman (1991) reported an increase in the maximum heart rate change throughout the breathing cycle after 8 weeks of daily two-hour running sessions in competitive athletes. Finally, results obtained during a breathing cycle extending over 7 heart

beats in sedentary, active and competitive endurance athletes indicate a significantly higher change in mean heart rate in competitive and moderately active individuals compared to sedentary subjects (Kenney et al, 1985).

The present findings indicate the extent of RSA amplitude to be similar in all groups of subjects independent of training state and differences in baseline heart rate. The discrepancy in findings between the present results and those obtained by Kenney et al (1985) may be related to the total breath duration, which has been known to affect RSA amplitude. Indeed, in the present study, RSA amplitude was measured in all groups at their spontaneous breathing rate and no significant difference between groups were found for breathing frequency under any testing condition.

In the study by Kenney et al (1985) the breathing rate was dictated by heart rate such that inspiration extended over three and expiration over four heartbeats. Because, resting heart rate was lower in athletes, the observed difference in mean heart rate change could also result from their lower breathing frequency and total breath duration.

RSA amplitude has also been negatively related to both heart rate and breathing frequency and positively related to tidal volume (Hirsch and Bishop, 1981, Selman et al., 1982, Eckberg, 1983, 1989, Brown et al, 1993, Kamath, 1993, Haggenmiller 1996). In the present study tidal volume was similar in all groups under spontaneous breathing but was smaller in the control subjects than in athletes under the M4 and P4 breathing conditions. Despite these latter differences in tidal volume, no significant difference in RSA amplitude was observed between groups under spontaneous breathing as well as conditions of enhanced or depressed breathing rates. Our results thus indicate no significant correlation between tidal volume and RSA amplitude whether calculated for all subjects pooled or within each subject group. This is in agreement with previous observations showing the extent of RSA to be predominantly determined by breathing frequency regardless of the observed increases in tidal volume from the resistive breathing (Calabrese et al., 2000). Our results further suggest no influence of training state on the timing of respiratory sinus arrhythmia as the present results indicate no significant difference in RSA phase or delay is found in response to changing breathing condition and a similar pattern is found in all groups.

Measurements of HRV have often been taken to make inferences regarding the parasympathetic cardiac tonic influence in a given individual. In a healthy subject the recorded resting heart rate is a measure of both the tonic vagal cardiac discharge and its modulation associated with the

respiratory cycle activity. Whether the assessed HRV reflects a tonic vagal influence or that, resulting from modulatory influences has not been clearly established. In the present study, RSA amplitude was not found to be correlated to resting heart rate in any of the experimental groups either under spontaneous breathing or when the amplitude of RSA was increased through a decrease in breathing frequency. The present results suggest RSA amplitude to reflect vagally-mediated respiratory-induced modulatory influences on the sinus node rather than a tonic vagal activity since similar RSA amplitude is found in all groups despite lower heart rates and thus presumably a higher vagal tonic influence in athletes.

The relationship between RSA amplitude and breathing rate.

In accordance with previous observations in healthy subjects the present observations indicate a significant relationship between frequency of breathing and the RSA amplitude (Davies & Nielson, 1967, Hirsh and Bishop, 1981, Eckberg, 1983, Grossman & Kollai, 1993, Brown et al., 1993, Hayano et al., 1994, Haggenmiller et al., 1996, Zhang et al., 1997, Stark et al., 2000, Taylor et al., 2001, Calabrese et al., 2000). The relationship is shown with respect to the relative rate of breathing in figure 3 (a) and to the absolute breathing rate in figure 3 (b). In both representations, the line plot obtained for runners appears higher than that of swimmers and age-matched controls although it did not reach statistical significance. This finding is contrary to our experimental hypothesis of a potential difference in the breathing – RSA integration of swimmers on account of a particular pattern of entrainment related to the swimming activity. A few studies have examined the locomotor-respiratory coupling specific to a sport and examined the hypothesis that entrainment occurs due to repeated bouts of acute dynamic exercise (Mahler et al., 1991, Steinacker et al., 1993, Bonsignore et al., 1998). In general the observations from these studies indicate that entrainment does occur with physical training such that there is improved ventilatory efficiency and entrainment of mechanical stroke frequency to that of breathing. In light of the similarities in the breathing frequency- RSA response curve of all groups, it would appear from our observation that any respiratory entrainment resulting from repeated swimming does not influence the respiratory feedback loop for the determination of RSA amplitude. Results of VO2 max clearly confirm the endurance-trained state of both athletic groups compared to the untrained healthy controls. Furthermore, examination of the training logs provided both by swimmers and the runners do not show differences in the intensity or volume of training and both groups show a similar bradycardia

compared to untrained control subjects. While running does not involve interruption of the natural breathing pattern because of water immersion, evidence of locomotor-respiratory coupling has been provided during running both in animals (Bramble & Carrier, 1983, Funk et al., 1992) and in humans (Persegol et al., 1991, van Alphen & Duffin, 1994, Mateika & Duffin, 1995). The present observations could thus be taken to suggest an influence of a running-induced respiratory entrainment on the vagal-induced respiratory modulation of heart rate. A clear mechanism for this phenomenon can however not be suggested from the present descriptive observations.

In conclusion, results from the present study contribute to the advancement of knowledge on the interaction of exercise-training and the autonomic control of heart rate and its modulation by bringing forth specific quantitative evidence of the vagally-mediated respiratory influence on heart rate on similarly trained and untrained subjects of comparable age. The present observations may be the first to provide grounds for a distinction between the influence of endurance training on the tonic vagal cardiac motor activity and on its modulation by changes in breathing characteristics. Further investigations are needed to examine the role of locomotor-respiratory entrainment on the vagally-mediated respiratory induced sinoatrial response in endurance trained atheletes.

Table 1. Circulatory subject characteristics at rest and in response to changes in breathing frequency (N=32)

Variables	Group	Spontaneous Breathing	Spontaneous minus 4 breathing cycles	Spontaneous plus 4 breathing cycles
HR (beats/min)	Runners	60.3 (5.7)*	60.3(9)	60.3(7.7)
	Swimmers	66(5.2)*	64.8(5.2)	65(5.9)
	Controls	75.2(9.0)	74.4(8.4)	75.9(8)
SD of R-R interval	Runners	0.09(0.05)	0.12(0.06)	0.09(0.04)
	Swimmers	0.09(0.04)	0.12(0.04)	0.08(0.04)
	Controls	0.07(0.02)	0.10(0.03)	0.06(0.02)
Systolic BP (mmHg)	Runners	122(7.6)	114(8.4)	118(9.4)
	Swimmers	122(9.7)	118(6.4)	122(10.6)
	Controls	124(10.3)	123(19.6)	121(19.2)
Diastolic BP (mmHg)	Runners	75(8.3)	73(5.7)	74(8.3)
	Swimmers	75(4.6)	72(8.4)	73(8.2)
	Controls	73(5.6)	73(4.4)	75(5.7)

Values are means (SD), HR=heart rate, BP=blood pressure, * indicates $p < 0.05$ from controls.

Table 2. Respiratory subject characteristics at rest and in response to changes in breathing frequency (N=32)

Variables	Group	Spontaneous Breathing	Spontaneous minus 4 breathing cycles	Spontaneous plus 4 breathing cycles
Breathing frequency (breath/min)	Runners	13.6(2.8)	9.7(3.3)	17.1(2.9)
	Swimmers	13.3(3.4)	9(3.5)	17.2(3.5)
	Controls	13.5(3.6)	9.8(3.2)	17(4.1)
Tidal volume (L/min)	Runners	0.74(0.1)	1.4(0.6)* †	0.77(0.2)* †
	Swimmers	0.84(0.3)	1.3(0.6)* †	0.73(0.3)* †
	Controls	0.70(0.2)	0.80(0.5)	0.60(0.3)
Inspiratory time (s)	Runners	2.10(0.5)	2.90(1.1)	1.80(0.4)
	Swimmers	2.4(1.1)	3.8(2.1)	2.2(0.8)
	Controls	2.1(0.7)	3.2(1.2)	1.8(0.6)
Tidal volume: Inspiratory time (ratio)	Runners	0.38(0.9)	0.52(0.34)	0.53(0.17)
	Swimmers	0.38(0.14)	0.37(0.15)	0.57(0.21)
	Controls	0.32(0.11)	0.32(0.10)	0.39(0.16)
Inspiratory time: total breathing time (ratio)	Runners	0.45(0.09)	0.43(0.08)	0.45(0.07)
	Swimmers	0.43(0.12)	0.43(0.12)	0.45(0.11)
	Controls	0.44(0.03)	0.47(0.07)	0.47(0.04)

Values are means (SD), * indicates $p < 0.05$ from controls; † indicates $p < 0.05$ from spontaneous breathing condition.

Figure 1 (a)

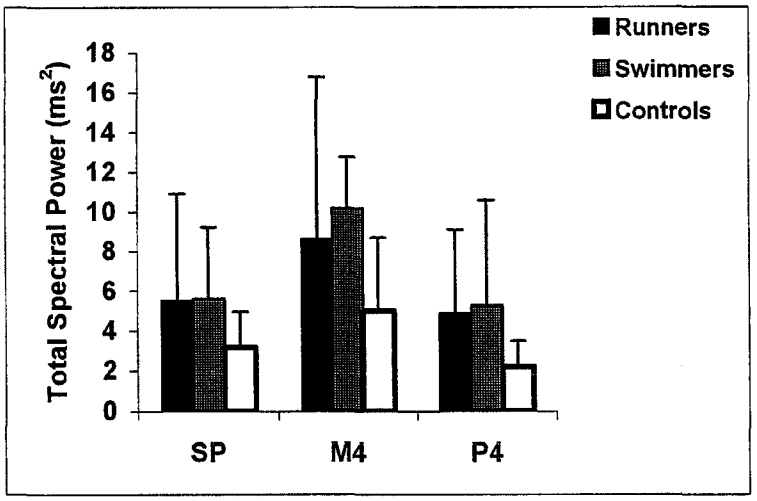

Figure 1 (b)

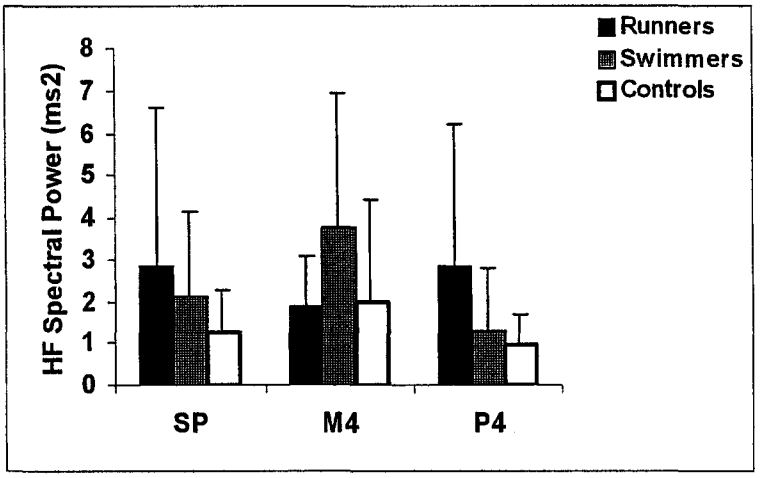

Figure 1. (a) Total and HF power spectral density (b) for each subject group at different breathing rates, SP=spontaneous breathing, M4=spontaneous breathing rate minus 4 breath cycles, P4= spontaneous breathing rate plus 4 breath cycles.

Figure 1 (c)

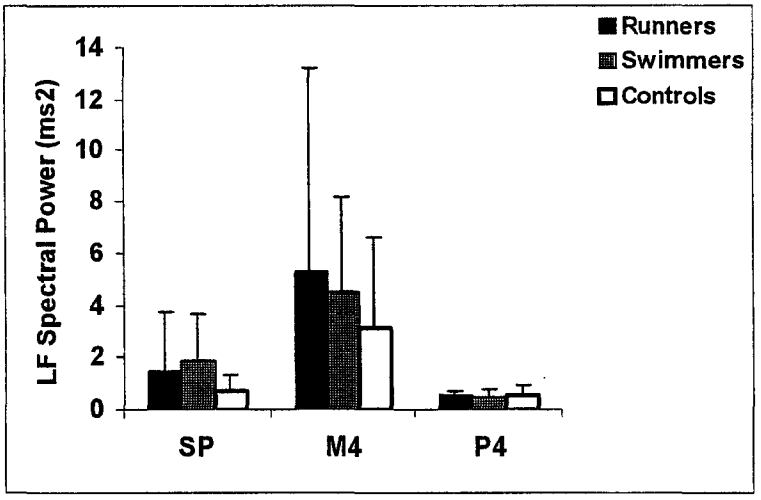

Figure 1 (d)

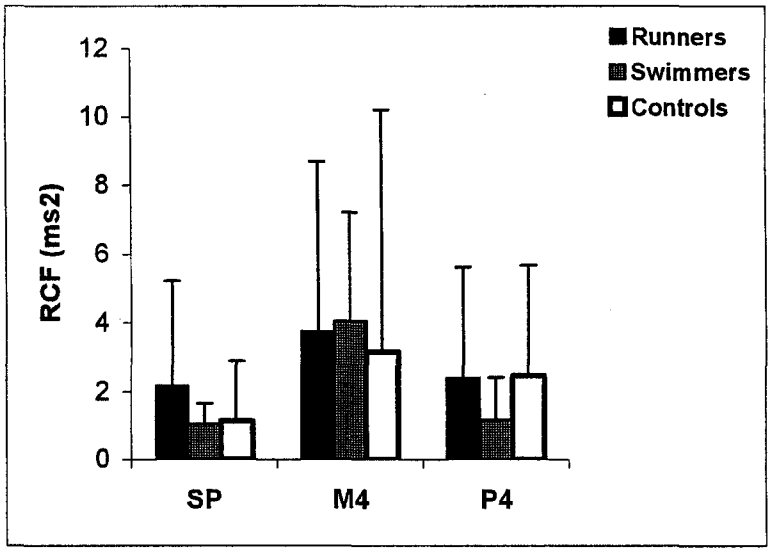

Figure 1 (c) LF and respiratory centered frequency power spectral density (d) for each subject group at different breathing rates, SP=spontaneous breathing, M4=spontaneous breathing rate minus 4 breath cycles, P4= spontaneous breathing rate plus 4 breath cycles.

Figure 2 (c)

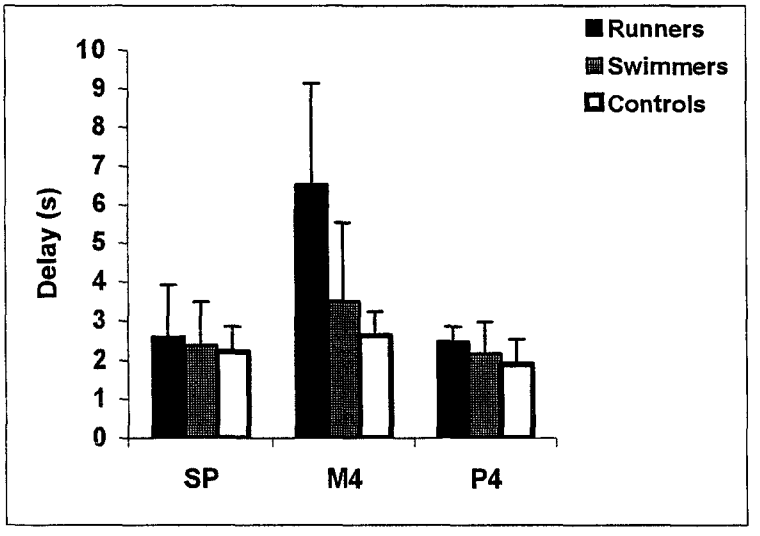

Figure 2 (c), RSA delay for each subject group at different breathing rates, SP=spontaneous breathing, M4=spontaneous breathing rate minus 4 breath cycles, P4= spontaneous breathing rate plus 4 breath cycles.

Figure 3 (a)

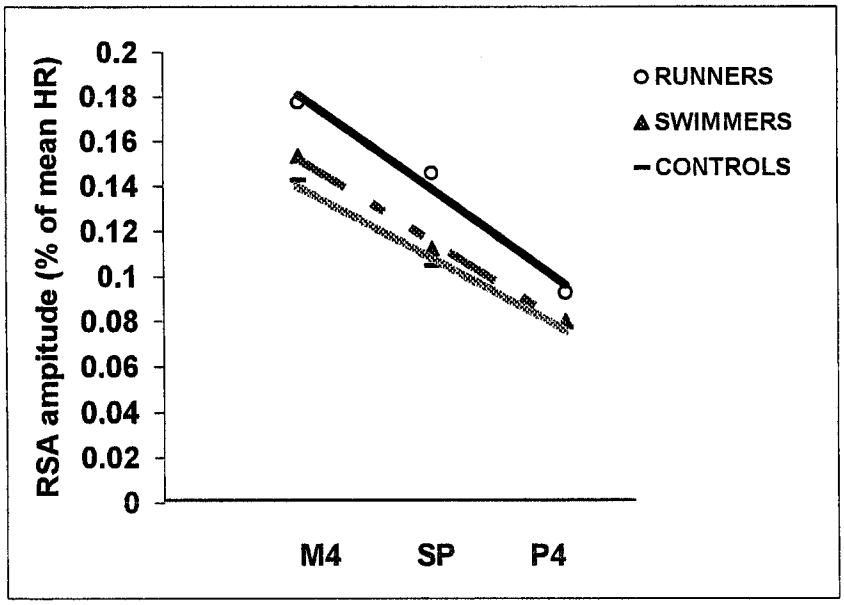

Figure 3 (b)

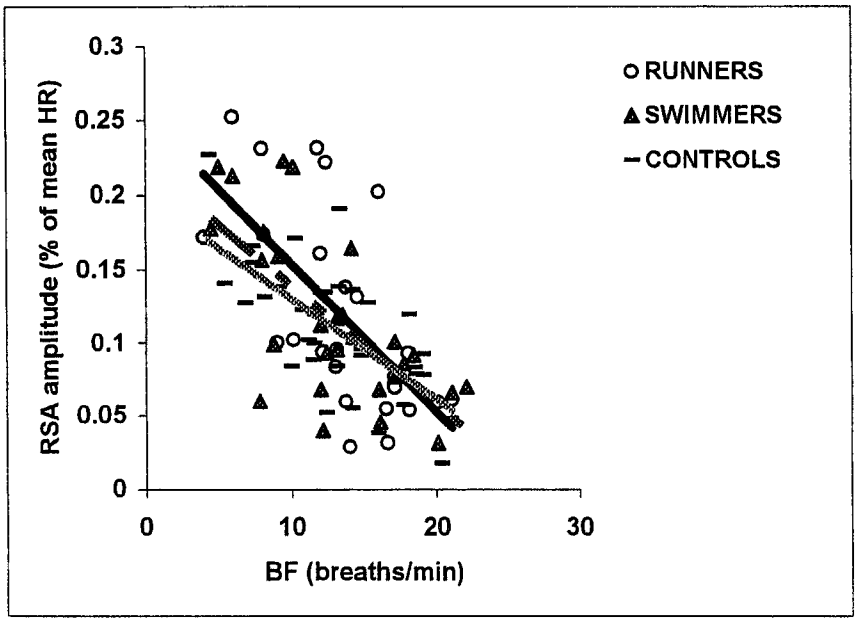

Figure 3 (a) Relative and absolute (b) amplitude for each subject group at different breathing rates, SP=spontaneous breathing, M4=spontaneous breathing rate minus 4 breath cycles, P4= spontaneous breathing rate plus 4 breath cycles.

Figure 3 (c)

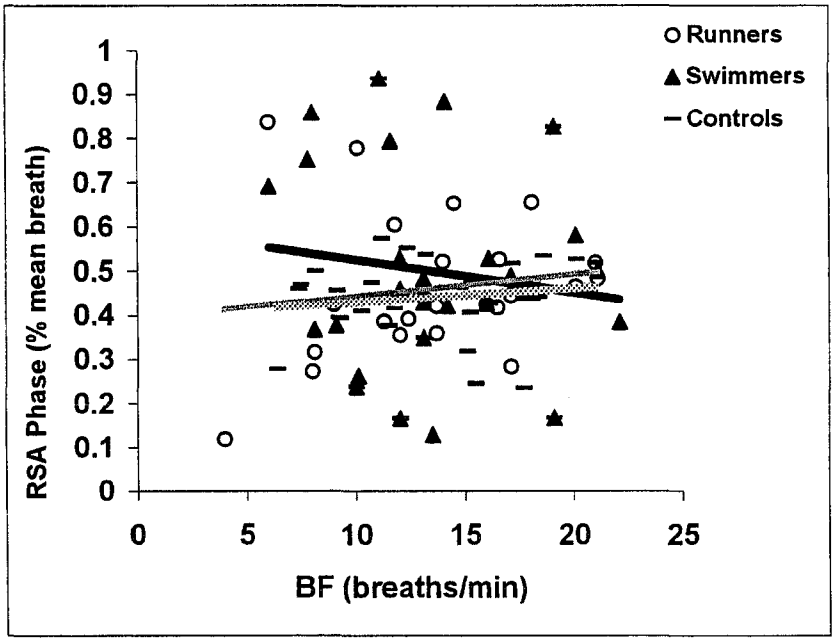

Figure 3 (c) RSA phase for each subject at absolute breathing rates during spontaneous and imposed breathing conditions.

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