Time course of adaptation in heart rate variability and Respiratory Sinus Arrhythmia to intensive endurance training.

A thesis

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ABSTRACT

The weekly time course of adaptation of Heart rate variability (HRV) and respiratory sinus arrhythmia (RSA) characteristics were examined over 4-weeks of intensive endurance training in 17 sedentary subjects (35.8 ± 10.0 yrs). Five-minute ECG and breathing recordings were obtained at rest at spontaneous breathing (SP) and at paced breathing rates of SP+4 (M4) and SP–4 (P4) breaths/minute. Spectral power components of HRV were computed from the R-R interval sequences; amplitude and phase of RSA were computed from the sinusoid fitted to the instantaneous heart rate within each breath. RSA sensitivity was obtained from the slope of the RSA amplitude versus breathing frequency relationship. Four weeks of training caused significantly increased in VO_2max (37.3 ± 4.4 versus 40.0 ± 5.4 ml/kg/min; ($p<0.01$); a significant decrease in DBP ($p<0.001$); a slight decrease in SBP ($p<0.08$), and max HR slightly decreased ($P<0.06$). None of these variables were changed significantly in control subjects. No change in resting HR was found after training (74 ± 14 versus 74 ± 7 bpm). Pre versus post-training results in total and high frequency spectral powers (ms^2) of HRV taken at SP were as was RSA amplitude. A significant reverse correlation was found between RSA-amplitude and breathing frequency ($r=0.40$). Results suggest a trend towards an increased gain in the amplitude of RSA for varying breathing frequencies after training. In conclusion, four weeks of intensive endurance training may significantly increase maximal aerobic power and provide some health related benefit such as a decrease in diastolic pressure, without concurrent changes in resting heart rate or its modulation by cardiac vagal activity.

Résumé

Cette étude porte sur la séquence hebdomadaire d'adaptation de la fréquence cardiaque et de sa variabilité (HRV) et de l'arythmie sinusale respiratoire (ARS) en réponse à un entraînement physique intense de quatre semaines. Un enregistrement de l'ECG et du débit ventilatoire a été obtenu chez dix-sept sujets sédentaires sains (35.8 ± 10.0 ans) au repos en respiration spontanée (SP) ainsi qu'au cours d'une respiration rythmée par un signal sonore à une fréquence respiratoire de SP-4 (M4) ainsi que SP + 4 (P4). Le spectre en fréquence de la puissance de la variabilité cardiaque a été calculé à partir de la séquence des intervalles R-R tandis que l'amplitude et la phase de l'ARS ont été calculés à partir d'un fonction sinusoïdale surperposée à la variation de la fréquence cardiaque instantanée au cours d'un cycle respiratoire. Le gain de la réponse de l'ARS en fonction de la fréquence respiratoire a été calculé à partir de la pente de la relation obtenue pour chaque sujet pour les 3 fréquences respiratoires. Une augmentation du VO_2max (37.3 ± 4.4 versus 40.0 ± 5.4 ml/kg/min; ($p<0.01$)) ainsi qu'une diminution de la pression artérielle diastolique ($p<0.001$) et une faible diminution de la pression systolique ($p\leq0.08$), ont été observées dans le groupe entraîné à la suite de la période d'entraînement mais non dans le groupe témoin. Aucune modification de la fréquence cardiaque au repos (74 ± 14 versus 74 ± 7 bpm) ou des paramètres spectraux de variabilité cardiaque n'a été observée après l'entraînement physique. De la même façon ni l'amplitude ni la phase de l'ARS n'ont été modifiées après l'entraînement. Les résultats confirment toutefois une relation significative inverse entre l'amplitude de l'ARS et la fréquence respiratoire ($r=0.40$). Les résultats suggèrent une tendance vers une

augmentation du gain de cette relation à la suite des 4 semaines d'entraînement. Il semble donc chez le sujet sain en bonne santé que quatre semaines d'entraînement physique intense permettent d'obtenir des gains de la performance physique ou des indices de la santé cardiorespiratoire sans pour autant conduire à une modification de la fréquence cardiaque ou de sa variabilité.

PART I: REVIEW OF LITERATURE

1. Effects of endurance training on heart rate and its control

Different research methods have been used to examine the effects of training on heart rate (Loimaala et al., 2000; Stein et al., 1999; Schuit et al., 1999; Levy et al., 1998; Tulppo et al., 1998; Ishida & Okada, 1997; Jensen-Urstad et al., 1997; Goldsmith et al., 1997; Gregoire et al., 1996; Davy et al., 1996; Lazoglu et al., 1996; Sacknoff et al., 1994). Research designs addressing this question include both cross-sectional and longitudinal approaches. Cross-sectional studies generally compare an experimental to a control group with the same general characteristics except for the experimental manipulation of interest. In this type of study, populations studied have varied from sedentary to athletic. In addition, the athletic populations studied varied to include endurance as well as non-endurance athletes such as weight lifters or to include athletes of differing calibers such as professional versus varsity sports. Cross-sectional studies do not permit researchers to draw conclusions on the effectiveness of a particular training program to bring about change. Rather they "suggest" effects due to training but these "effects" can also be explained by genetics or chance differences in the characteristics of the subjects in study not due to training.

Longitudinal studies on the other hand, if carefully designed and controlled permit researchers to evaluate the influence of training on dependent variables monitored over time in the same group. Many longitudinal studies of resting heart rate (HR) with exercise and training involving human and animal subjects have been conducted (Loimaala et al.,

2000; Torii et al., 1992; Dart et al., 1992; Krzeminski et al., 1991; Steinhaus et al., 1990; Seals & Chase, 1989). Longitudinal studies have been conducted on both healthy human subjects and diseased populations, especially those suffering from heart disease.

1.1 Cross-sectional comparisons of exercise training and heart rate

The scope of the review is limited to studies dating back to the 1960's, although most are less than fifteen years old. These studies compare healthy active and inactive subjects. Most studies compare two categories of subjects; endurance trained versus sedentary or untrained subjects (Ishida & Okada, 1997; Jensen-Urstad et al., 1997; Davy et al., 1996). In some studies additional comparative groups formed part of the design of the study including active individual with varying fitness levels. Some studies grouped subjects on the basis of a fitness test without consideration for leisure time activity (Tulppo et al., 1998).

Results from 10 studies comparing athletes to sedentary individuals and/or different levels of fitness are summarized in Table 1. A total of 133 athletes were compared to 136 control subjects. Respective values for resting heart rate (HR), VO_2max and the differences between groups are summarized in the table. Nine of the ten studies showed a significant difference in resting heart rate between athletes and their sedentary counterparts (Tulppo et al., 1998; Ishida & Okada, 1997; Jensen-Urstad et al., 1997;

Davy et al., 1996; Gregoire et al., 1996, Sacknoff et al., 1994; Jost et al., 1989; Katona et al., 1982). Resting HR ranged from between 43 and 65 bpm in the athletes subjects. Mean resting heart rate was 58 bpm in the athletes and 65 bpm in the control subjects. This represents an 11% lower resting HR in athletes compared to the sedentary controls.

Lower resting HR seems to be tied to endurance training since athletes involved in sports that were not endurance related often did not have resting HR that were different from untrained control subjects. For example, when weightlifters, controls and endurance-trained subjects were compared, the resting HR of weightlifters was not different from controls and significantly higher than endurance-trained subjects (Lazoglu et al., 1996; Jost et al., 1989).

Endurance athletes have higher VO_2max values, ranging from 50.0 $ml \cdot kg^{-1} \cdot min^{-1}$ to 73.0 $ml \cdot kg^{-1} \cdot min^{-1}$ and a mean value of 60.7 $ml \cdot kg^{-1} \cdot min^{-1}$. In comparison control subjects exhibit a VO_2max ranging between 27 and 50 $ml \cdot kg^{-1} \cdot min^{-1}$, such that endurance athletes on average had a VO_2max approximately 60% greater than that of control subjects (Lazoglu et al., 1996; Jost et al., 1989; Ishida & Okada, 1997; Jensen-Urstad et al., 1997; Goldsmith et al., 1997). The relationship between the difference in resting HR in athletes and sedentary and the difference in VO2 max in athletes and sedentary seems to be a negative linear one ($r = 0.60$). Investigators seem to have usually chosen younger athletes, between the ages of 20 to 30 years, to participate in these research studies (Lazoglu et al., 1996; Ishida et al, 1997;

Jensen-Urstad et al., 1997; Goldsmith et al., 1997) although some studies have also examined these phenomena in older subjects, between 40 and 60 years of age (Tulppo et al., 1998; Davy et al., 1996). VO_2max values were lower in older athletes (50-51 $ml \cdot kg^{-1} \cdot min^{-1}$) (Tulppo et al., 1998; Davy et al., 1996) compared to younger athletes (51-73 $ml \cdot kg^{-1} \cdot min^{-1}$) (Ishida & Okada, 1997; Jost et al., 1989), but a significant difference was still apparent between older athletes and older control subjects (50-88%) (Ishida & Okada, 1997; Jost et al., 1989). Similarly, most studies have been conducted on mostly male subjects. Only two studies have been conducted on women athletes and non-athletes (Davy et al., 1996 & Gregoire et al., 1996) and again indicate changes in resting heart rates to be in opposite direction to the changes in maximal oxygen uptake. There is no evidence in the literature for a different training response in women.

Overall, the data indicate that endurance athletes and other aerobically fit subjects have significantly lower resting HR and higher VO_2max values compared to control subjects. Although differences in resting HR (HRr) and VO_2max between endurance athletes and control subjects are significant cross-sectional studies do not clearly demonstrate the influence of training since genotype and lifestyle differences such as dietary habits may be influential and were not controlled in these studies. Longitudinal studies are necessary to determine the influence of training on the cardiorespiratory function.

1.2 Longitudinal studies of exercise training and heart rate

Animal studies

In longitudinal studies a common approach consists of examining pre and post-test measurements of maximal and sub maximal exercise cardio-respiratory responses to endurance training. Studies have included the use of both humans and other animal species (Negaro et al., 1992; Hughson et al., 1977; Yu-Chong & Horvath, 1972). The advantages of using animals for research studies include a better control on potential confounding factors such as animal behaviour, food consumption or quantification of exercise variables. In contrast, animal studies then lead to the often-erroneous generalization of results to humans. Different species respond differently to exercise and many species also have different physiological characteristics prior to the commencement of an exercise program. For example, many animals exercise in a body position which is not linked to orthostatic stress resulting in very different circulatory responses from that seen in the upright exercising condition. Some smaller species of animals such as rats have heart rates that may be five to six time faster than the heart rates of humans.

The exercise training programs used in animal studies have generally included walking and/or running on a motor driven treadmill using a speed ranging between 19.0 and 26.8 m/min. Swimming with or with out weights equivalent to approximately 2% of body weight attached to the tail have also been used. Negaro et al. (1992) and Hughson et al. (1977) used a running training program for 8 to 13 weeks, while others (Yu-Chong & Horvath, 1972)

used a swimming protocol. Training programs consisted of between 5 and 7 sessions per week although 5 sessions per week was the most common frequency of training (Yu-Chong & Horvath, 1972; Barnard et al., 1976; Hughson et al., 1976; Negaro et al., 1992; Hassan, 1991). In these training studies the resting heart rate was significantly lower in animals that participated in endurance training programs compared to sedentary control animals. Overall results show a significant decrease in resting heart rate ranging from 9 to 71 bpm or 3% to 17% compared to pre-training values (Yu-Chong & Horvath, 1972; Barnard et al., 1976; Hughson et al., 1976; Negaro et al., 1992; Hassan, 1991). The different protocols and intensities performed in the training programmes could contribute to the variation in the changes in resting heart rate after the training.

Humans with Heart Disease

Many studies have examined cardio respiratory responses to exercise training in cardiac patients. These patients often undergo an aerobic exercise program as part of the rehabilitative process following myocardial infarction or coronary artery bypass procedure. Periodic evaluations of fitness levels pre and post training are often conducted and therefore yield information on peak and/or sub maximal cardio respiratory responses to the training program. For example, peak VO_2, stress tests and maximal endurance cycle or treadmill ergo meter tests have been used to evaluate the effects of training (Malfatto et al., 1996). Most training programs consist of training intensities ranging

between 60 and 85% of peak heart rates, 3-4 times per week with some studies using up to 12 sessions per week (Lellamo et al., 2000). In the past 10 years results from studies in which subjects trained for 2-8 weeks showed significant increases in peak VO_2 with changes ranging from 5% to 29% compared to pre-training levels (Lellamo et al., 2000; Duru et al., 2000). Time to exhaustion/maximal effort (exercise duration) increased significantly to 15± 3 min post training versus 11 ± 2 min before training ($p < 0.01$)(La Rovere et al.,1992). In addition, these authors reported a 3.3 min increase in exercise duration after 4 weeks of training in 11 patients suffering from their first recent myocardial infarction. Resting heart rate was also found to decrease in patients with ischemic heart disease (Malfatto et al., 1996; Pardo et al., 2000; Lellamo et al., 2000; Duru et al., 2000). Malfatto et al. (1996) found a significantly lower resting heart rate after 8 weeks of training (60 ± 8.1 bpm versus 65.3 ± 7.2 bpm) in 52 ± 7 year old patients that had their first, uncomplicated, myocardial infarction. In summary, cardiac patients undergoing a training program seem to respond to the training regimen with increases in peak VO_2 and endurance time and decreases in resting heart rate. However, most patients involved in these studies were taking medication and the adaptation to training may therefore be confounded by the fact that medication may have affected heart rate and other cardio respiratory response to exercise independent of the training program. Although, due the fact that no medication were changed in most of the subjects it is probably a true training effect which show that effects can occur even when on

medication. The healing process, which could have caused the changes in resting heart rate calculate by comparing to the controls.

Healthy subjects

Table 2 summarizes results from 19 studies conducted over the past 30 years on the effects of exercise training on HR and VO_2max in healthy subjects (Loimaala et al., 2000; Mier et al., 1997; Torii et al, 1992; Steinhaus et al, 1990).

A total of 358 subjects (256 males and 102 females) approximately equally divided between experimental and control subjects participated in these studies. Control subjects were considered sedentary or untrained.

The criterion for defining "sedentary state" differed from study to study. In general, a subject was considered sedentary if he/she was not involved in any type of physical activity during his/her leisure time. Subjects' ages ranged from 20-68 years with a mean of 46.7 years of age. Training programs in these studies consisted of 2-7 endurance exercise sessions (aerobics classes, biking, walking, running, stair climbing or any other aerobic activity). Each session lasted between 30 and 60 minutes and the intensity of training varied from 50-90% of VO_2max or maximal heart rate reserve. Most training programs were between 3 to 20 weeks in duration. The shortest durations are found in studies by Mier et al. (1997), Torii et al. (1992) and Steinhaus et al. (1990) using training programs of 3 to 4 weeks, while the longest duration is presented by Stein et al. (1999) reporting 41 weeks of endurance training. Most studies reported the pre and post changes in VO_2max, resting heart rate

Table 2. The effect of endurance training program on HR and VO_2 max.

Authors	N M/F	Mean Age (yrs)	Training (wks)	Resting HR (beats/min)			VO_2max (ml·kg^{-1}·min^{-1})			Hr max (beats/min)		
				Pre	Post	% Δ	Pre	Post	% Δ	Pre	Post	% Δ
Loimaala et al., 2000	26M	47	20	66±12	65±12	-1.5	38.4±5.5	42.6±6.3	10.9			
	28M	47	20	68±11	64±9	-5.9†	37.3±4.0	42.9±6.1	15.0†			
Stein et al., 1999	9F/7M	66	41	71±8	66±9	-7.0†	23.6±3.8	30.8±5.2	30.5†	163±8	163±9	0
Schuit et al., 1999	36(M/F)	65	27	73±7	72±8	-1.4	27.9±8.3	30.3±7.8	8.6†			
Levy et al., 1998	13M	68	27			-9.0†			21†			
	11M	28	27			-5.0†			17†			
Ahmaidi et al., 1998	11(F/M)	62	12				25.4±1.2	30.6±2.3	20.5†	162±5	162±3	0
Ishida & Okada, 1998	8F/4M	38	8	70±10	63±11	-10.0†						
Mier et al., 1997	5F/M	26	3	67±4	64±4	-4.5	35.4±2.5	38.9±2.9	9.9†	189±2	184±2	-2.6
Al-Ani et al., 1996	1F/10M	20	6	69±3	52±2	-24.6†	45.0±2.0	51.0±2.0	13.3†	196±3	194±2	-1.0†
Wilmore et al., 1996	21F/26M	36	20	60±8	58±8	-3.3†	30.9±10.0	35.8±11.0	15.9†			
Sheldahl et al., 1994	10M	54	12	68±2	63±3	-7.4†	30.1±1.5	35.1±2	16.6	179±	175±	-2.2
Boutcher et al., 1994	19M	46	8	71±2	68±3	-4.2†	34.3±2.2	38.6±2.6	12.5†			
Torili et al., 1992	4M	32	4	75±8	71±6	-5.3	43.3±7	43.5±8	0.5			
	4M	31	4	77±3	76±5	-1.3	36.8±6	41.2±6	12.0†			
	4M	30	4	86±12	74±4	-14.0	43.8±6	47.4±4	8.2			
Dart et al., 1992	5(F/M)	25	4	67±2	60±2	-10.4†	37.8	43.8	15.9†			
Krzeminski et al., 1991	7M	22	10	87±12	72±12	-17.2†	39.2±4.7	46.3±5.6	18.1†			
Steinhaus et al., 1990	4F/9M	60	4	82±16	78±12	-4.9	19.4±5.7	24.6±4.8	26.8†	148±13	148±13	0
Seals et al;1989	11M	53	30	63±2	58±1	-8.1†	31.6±1.2	41.0±1.8	15.5†	174±3	169±3	-2.9†
Redenhop et al., 1983	10F/4M	66	9				21.1±3.6	24.5±4.8	16.1†	156±12	159±13	1.9
	11F/3M	68	9				20.6±3.8	23.7±5.9	15.0†	160±9	158±8	-1.3
Pollock et al., 1977	9M	30-40	26	67±10	62±8	-7.5†	43.0±3.3	50.6±5.5	17.7†	195±9	187±5	-4.1†
	5M	30-40	20	72±3	60±2	-16.7†	37.7±4.6	46.2±8.3	22.5†	192±5	183±4	-4.7†
Oscai et al., 1968	14M	37	14	64	58	-9.4†	38.5	47.0	22.1†			
Mean	**358**	**46.7**		**69**	**65**	**-6.3**	**33.0**	**37.1**	**+12.4**	**170**	**169**	**-0.9**

HR=heart rate; %Δ = percentage change between pre and post training values. M/F: Male/ Female. † Significant difference P<0.05.

and maximal heart rate as may be seen in Table 2. All studies presented in the table showed significant increases in VO_2max (from 20.6 and 43.0 ml·kg^{-1}·min^{-1} to 23.7 and 51.0 ml·kg^{-1}·min^{-1}). The mean increase in VO_2max was 12.4% after training in these studies. Aging (60-69 years of age) is accompanied by a decrease in VO_2max. Compared to younger subjects (20-29 years of age) with a VO_2max of 40.5 ml·kg^{-1}·min^{-1} older subjects averaged 21.6 ml·kg^{-1}·min^{-1}. However, in both age groups exercise training resulted in increases up to 46.4 and 25.0 ml·kg^{-1}·min^{-1} respectively. Thus the magnitude of improvement is not linked to aging with the older group increasing VO_2max by 15.5% with a corresponding increase of 14.6% in younger subjects.

In 13 of 19 experiments a significant decrease in resting heart rate was noted after endurance training (Loimaala et al., 2000; Stein et al., 1999; Levy et al., 1998; Ishida & Okada, 1998; Al-Ani et al., 1996; Wilmore et al., 1996; Sheldahl et al., 1994; Bouthcher et al., 1994; Dart et al, 1992; Krzeminski et al., 1991; Seals & Chase, 1989; Pollock & Ayres, 1977; Oscai et al., 1968). The decrease in heart rate ranged from 4 to 17 bpm corresponding to a 3% to 25% decrease compared to pre-training levels. In 5 studies a non-significant decrease was reported after training (Loimaala et al., 2000; Schuit et al., 1999; Mier et al., 1997, Torii et al., 1992; Steinhaus et al., 1990). In 3 of these 5 studies the experimental group was older than 45 years of age (Loimaala et al., 2000; Schuit et al., 1999; Steinhaus et al., 1990). It is possible that the inconsistency of the training effect was due to age and not training. It has been previously suggested that endurance training results in a more modest

bradycardia in middle-age and older individuals compared to younger subjects (Levy et al., 1998). Alternately, other factors may have contributed to these results. For example, the intensity of training may be important as Loimaala et al. (2000) noted a significant decrease in HRr with high intensity training but not with lower intensity training in two training groups of the same age.

Some investigators have suggested that endurance training results in a decrease in maximal heart rate (Al-Ani et al, 1996; Seals & Chase, 1989; Pollock & Ayres, 1977). Results from table 2 indicate some decreases in HR max following endurance training in the order of magnitude of 2 to 9 bpm or 1-4.7% (Al-Ani et al, 1996; Seals & Chase, 1989; Pollock & Ayres, 1977). In contrast, there are many studies that have failed to show a significant effect of endurance training on maximal heart rate (Stein et al., 1999; Ahmaidi et al., 1998; Mier et al., 1997; Steinhaus et al., 1990).

In summary, these observations suggest that endurance training results in a consistent decrease in HRr and increase in VO_2max. The effects of endurance training on HR max are inconsistent and controversial making it impossible to draw any conclusions. The results lead to the conclusion of a training induced resting bradycardia, but the physiological basis of this phenomenon remains unexplained.

2.0 Experimental evidence of training-induced bradycardia

Theoretically, an increased parasympathetic influence, a decreased sympathetic tonic activity or a combination of parasympathetic and sympathetic changes or an intrinsic heart rate change or any combination of any of these factors can account for training-induced bradycardia. Several approaches have been used in human and animal research to examine this phenomenon through both cross-sectional comparisons of trained and untrained animals or athletic and non-athletic humans or through the use of longitudinal training studies. One of the main approaches is the use of pharmacological blockage agents. In such cases, the sympathetic nervous activity to the sinus node is inhibited from interacting with post-synaptic receptors through B-adrenergic receptor blocker, while an atropine is generally used to block the cholinergic receptors removing any influence originating from the parasympathetic nervous system. Under these conditions, the magnitude of the difference in heart rate between the baseline condition and the atropine blockade reflects the magnitude of the resting vagal influence. This approach however is limited by the fact that it is difficult to establish to what extent complete blockade is achieved and that there may also be compensatory adaptations to the autonomic segment that was blocked making difficult to clearly establish a true effect of this segment under intact conditions.

2.1 – Pharmacological blockade in trained versus untrained in animals.

Table 3 shown results from five studies conducted over the last 30 years on Spargue-Dawley or Wister rats trained to run on a motor driven treadmill (Barnard et al., 1976; Hughson et al., 1976; Negrao et al., 1992) or submitted to swimming (Hassan, 1991; Yu-Chong & Horvath, 1972) and compared to their untrained counterparts. The training consisted of 5 to 7 bouts per week of 45-90 minutes of exercise for 6 to 13 weeks.

Atropine was used in all studies to block the parasympathetic influences on the sinus node. Sympathetic blockade was achieved using either propranolol or carbachol. These blockades were used either as single or dual approaches leading to concurrent vagal and sympathetic blockade. The resulting heart rate is thought to reflect the intrinsic rate of the SA node independent of autonomic influences. Hassan (1991) and Yu-Chong & Horvath, (1972) found lower sympathetic influences on the heart after training with a concurrent decrease in the parasympathetic value, although the main contributor to training bradycardia in these studies was found to be the sympathetic influence. Hughson et al. (1976) however found an increase in the parasympathetic influence and a decrease in intrinsic heart rate without any changes in sympathetic values in trained animals compared to the untrained rats. Negrao et al. (1992) Barnard et al. (1976) found no changes in the sympathetic or the parasympathetic influences after endurance training, although training-bradycardia was apparent. The overall results are thus far from consistent. A number of factors such as the intensity, length, and type of

Table 3. Longitudinal studies of potential contributing mechanism for training bradycardia in animals.

Author	n Species	Training	HR Δ%	Approach	Results
Negrao et al., 1992	15 Wister rats	13 weeks, 5/week motor driven treadmill 26.8 m/min	-3	Methylatropine and Propranolol blockade	PS: no change S: no change IHR: decreased
Hassan, 1991	30 Sprague-Dawley	30 days, 1/day 1.5 swimming	-13	Propranolol and Atropine blockade	PS: decreased S: decreased
Barnard et al.,1976	8 Sprague-Dawley	12 weeks, 5/week, 60 min motor driven treadmill	-8	Propranolol and Atropine blockade	PS: no change S: no change
Hughson et al., 1976	30 Sprague-Dawley	10 weeks, 5/week, motor driven treadmill, 19 m/min	-4	Carbachol and Atropine blockade	PS: increased S: no change IHR: decreased
Yu-Chong & Horvath, 1972	Sprague-Dawley	42 day, 1/day, 60 min. swimming.	-17	Propranolol and Atropine blockade	PS: decreased S: decreased IHR: no change

HR=resting heart rate; Δ%= difference between pre and post endurance training. PS=Parasympathetic nervous system; S=Sympathetic system. PB=Pharmacological Blockade; IHR=Intrinsic Heart Rate.

exercise as well as drug type, drug dosages and mode of administration which vary from study to study may all account for some discrepancy in the results.

Use of pharmacological blockade in humans.

Experimental data using pharmacological blockade to identify the appropriate explanation for training-induced bradycardia (seen in table 4) have been collected either through comparative studies of athletes and healthy non-athletes (Benedito et al., 1985; Lewis et al., 1980; Katona et al., 1982) or through comparisons of pre- and post-training observations in healthy sedentary humans (Benedito et al., 1985; Ekblom et al., 1973). Between-group comparisons of endurance trained athletes and non-athletes have shown both shown lower resting heart rate during combined sympathetic & parasympathetic blockade in athletes compared to non-athletes. These results suggest that a reduction in the intrinsic rate of the sino-atrial and not an increase in the resting cardiac parasympathetic influence may be responsible for training-induced bradycardia. This could occur as a result of training-induced changes in membrane properties leading to a lower threshold for spontaneous depolarization (Katona et al., 1982). Results from the few longitudinal training studies do not support this suggestion. The first study conducted by Ekblom et al. (1973) examined the effect of an intensive 5-week endurance-training program on the extent of atropine-induced increase in resting heart rate. Results indicated a significant

Table 4 – Cross-sectional and longitudinal studies on the potential contributing mechanism to training-induced bradycardia.

Cross-sectional studies

Author	n M/F	Age yrs	Subjects	HR Δ%	VO_2max Δ%	Approach	Results
Benedito et al; 1985	13 M 7 M	29 20	Sedentary Runners	-24	+36.5	RSA; HR variation in control tidal volume. Atropine blockade	RSA: no difference PS: no difference
Lewis et al., 1980	8 M 8 M	26 21	Sedentary Cyclists	-26	+70.0	Atropine and propranolol blockade	Lower resting HR after Dual-blockade
Katona et al., 1982	10 8	22 21	Oarsman Nonathletes	-32	+70.1	Atropine and propranolol blockade	Lower resting HR after Dual-blockade

Longitudinal studies

			Training	HR Δ%	VO_2max Δ%	Approach	Results
Ishida et al., 1998	4M/8F	37	8 weeks, 2/week	-11		HRV-frequency domain	HF: no change LF: no change LF/HF: no change
Levy et al., 1998	13 M	68	6 months 4-5/week	-9.0	+21	HRV-time domain:SDRR	SDRR: increased
	11 M	28	6 months 4-5/week	-5.0	+17	HRV-time domain: SDRR	SDRR : increased
Shi et al., 1995	8 M	28	8 months, 4/week	-14	+27.2	Atropine & metoprolol blockade	PS: increased S: no change
Benedito et al., 1985	7 M	29	10 weeks, 5/week	-16	+15.6	RSA; HR variation in control tidal volume. Atropine blockade	RSA: no change PS: no change
Ekblom et al., 1973	14 M/F	23-32	5 weeks, 5-6/week	-14	+19.0	Atropine & propranolol blockade	PS: increased S: decreased

M/F=Male/Female; HR=resting heart rate; Δ%=difference between pre and post endurance training/ or trained untrained subjects. RSA=Respiratory Sinus Arrhythmia; PS=Parasympathetic nervous system; S=Sympathetic system. PB=Pharmacological Blockade; IHR=Intrinsic Heart Rate.

training effect on maximal oxygen uptake associated with a significant resting-bradycardia. The extent of atropine-induced increase in heart rate was found to be greater after the training program, suggesting an increase in the cardiac parasympathetic activity.

Later on, Benedito et al. (1985) and Shi et al. (1995) again used pharmacological blockade to examine the effects of endurance training program lasting 10 weeks and 8 months respectively. In both cases, VO_2max also increased (16 and 27%) and training-induced bradycardia was observed. In the study by Benedito et al. (1985) an effect of training on resting cardiac parasympathetic influence was not found while a significant increase in the vagal influence was again found in the study by Shi et al. (1995), however they did not find change in the intrinsic HR following dual blockade. Benedito et al. (1985) did not used concurrent dual blockade, such that a direct effect of training on the intrinsic rate of the sinus node could not be examined. It is thus obvious from this very limited set of experimental data that a clear explanation for the training-induced bradycardia is still lacking.

More recently, the method of heart rate variability analysis has been used to provide a non-invasive appreciation of the cardiac vagal influences as well as the sympatho:vagal balance. The technique has been applied to both sedentary as well as active healthy subjects or trained athletes both in an attempt to provide additional clues to the mechanism underlying training-induced bradycardia as well as to examine the interaction of endurance exercise on heart rate modulations and cardiovascular health.

3.0 The assessment and interpretation of Heart Rate Variability.

The assessment of heart rate variability is based on the analysis of series of consecutive cardiac cycles, typically identified as R-R intervals. The ECG allows for the collection of a series of consecutive R-R or N-N (N=normal) interval time changes and changes in instantaneous HR. Data can be presented in a tachogram and reflects the change in R-R intervals in sec or the number of beats (Malik et al., 1996). ECG recordings for the purpose of examining heart rate variability have typically been performed for durations of 2 minutes up to 48 hours (Jensen-Urstad et al., 1997; Goldsmith et al., 1997; Davy et al., 1996). Twenty-four hour recordings generally allow the fluctuations in heart rate to be assessed over long cyclic periods such as night and daytime variability reflecting the variability associated over the entire circadian variations on HR (Burgess et al., 1997). Five-minute recordings of HR are commonly used to assess responses of heart rate variability to selected physiological stressors (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 heart rate variability (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 the 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.

Frequency Domain Analysis of Heart Rate Variability

The Frequency based analysis of HRV data is depends on initial treatment of the data using a second order statistical method such as an autocorrelation function or Fast Fourier Transform (FFT) algorithm to produce frequency based 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 that must be considered a stationary condition (Malik et al., 1996). Application of these techniques to the series of R-R interval allows decomposing the variability of the signal into that corresponding to different frequencies. The Power Spectral Density 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 application of the HRV assessment techniques to various physiological as well as clinical situations of interests has lead to the need for standardization of this analytical tool in order to provide for comparative and meaningful interpretations (Malik et al., 1996). More specifically, standardization of the range of frequencies of interest has been proposed.

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

consisting of 3 distinguishable peaks. One peak is found to occur at a very low frequency (VLF) of 0 to 0.03 Hz that captures the long period rhythms. A second peak is found to occur in the low frequency range (LF) between 0.03 to 0.15 Hz and is generally centered at about 0.1 Hz (Malik et al., 1996) 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 (Quetelet, 1842; Hutchinson, 1850), which translates into a frequency range of 0.15 to 4.0 Hz. This frequency range is referred to as the high frequency (HF) 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. Findings from pharmacological muscarinic receptor blockade showing a significant decrease or the disappearance of the HF peak indicate that the R-R spectral power in the HF band may be used as a marker of cardiac parasympathetic activity (Malik et al., 1996). Although LF spectral power has been considered to be a marker of sympathetic activity, results from pharmacological studies using B–blockers have not provided clear evidence to support this assumption since 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). The use of an LF: HF ratio has been promoted to describe the interaction and balance of both the sympathetic and parasympathetic influences on the HR and has been referred to as the sympathovagal balance.

Finally, the occurrence of very low frequency (VLF) peak is generally thought by many to be related to circadian rhythmic events such as night and day differences in cortisol secretion and thermoregulation. To effectively determine the physiological importance of this frequency band ECG recording must be prolonged up to forty-eight hours to capture physiological events, which occur every twenty-four to twenty-six hours.

The sum of the spectral power within each band represents total spectral density or power. This power is generally reported either in absolute units of beats2/Hz or s2/Hz or in normalized units HF = HF X 100/ (TP – VLF) and LF = LF X 100 (TP –VLF) as suggested by Malik et al. (1996). 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 (Malik et al. 1996). For example, total spectral power may be increased during night versus day or conversely decrease upon standing compared to lying or sitting (Gagnon, 1996).

3.1 The role of respiratory sinus arrhythmia in HRV.

A number of factors constantly modulate heart rate such that the measured heart rate at rest is the outcome of the tonic sympathetic and parasympathetic influences on the sinus node as well as of rhythmic modulations resulting from hormonal influences, the arterial baroreflex, chemoreflexes, or as a result of respiration. Under resting conditions, heart rate oscillations are primarily related to the movements of breathing (Donders, 1868; Davies et al., 1967; Hirsch and Bishop, 1981; Horner et al, 1995).

Respiratory Sinus Arrhythmia

Respiratory sinus arrhythmia (RSA) describes the periodic cardiac interval changes associated with the respiratory cycle. In resting humans, RSA essentially accounts for all the variability of heart rate. Typically, heart rate may be seen to accelerate during inspiration as a result of the breathing-induced inhibition of the vagal influence on the sinus node and decelerates during expiration as the vagal inhibition is lifted. The extent of such changes in HR throughout a breathing cycle has been commonly used as an index of the magnitude of RSA and is termed RSA amplitude. Investigations of RSA have helped to provide more information on the integrative mechanisms influencing the vagal cardiac center.

Mechanism of RSA

The cycle of respiration, as it is understood, is initiated by phrenic nerve stimulation via the respiratory medullary center causing the diaphragm

and external intercostals to contract. These events in turn may trigger inter-central communication between the respiratory center and the cardiac vagal center, leading to an inhibition of vagal flow. As a result of 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.

Assessing RSA

Typical investigation methods for the determination of RSA in animals consist of anesthesia, 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 BF on RSA (Haggenmiller et al., 1996; Hirsch & Bishop, 1981). 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 centered 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 (Haggenmiller et al, 1996). Two general approaches have been used to explore the effects of breathing frequency and tidal volume on RSA amplitude. Using the first approach, RSA amplitude is determined in healthy subjects selected at random and is reported for various individual spontaneous breathing rates. This approach reveals that the breathing patterns of the general population are diversified and include BF, which can range from 6-31 in adults (Quetelet, 1842; Hutchinson, 1850) and Vt that range between 442 to 1549 ml (Dejours et al., 1961). Similarly, RSA amplitude in healthy humans may be found to vary between one and twelve times the lowest RSA amplitude value that has been measured (Hirsch and Bishop; 1981).

The second approach uses an imposed breathing rate to examine RSA amplitude in a random sample of subjects repeatedly assessed under different breathing rates given by an auditory or visual cue (Haggenmiller et al., 1996; Brown et al., 1993). Similarly, for tidal volume, the subject can voluntarily control tidal volume following a visual tracing of his/her breathing pattern or tidal volume can be altered using passive ventilation (Brown et al., 1993; Selman et al., 1982).

Results from measurements of RSA amplitude when Tidal volume is changed indicate a greater RSA with higher Tidal volume. Also, a significant

correlation has been found between RSA and tidal volume (Hirsch and Bishop, 1981; Haggenmiller et al., 1996; Calabrese et al., 2000). According to Grossman & Kollai (1993), variations in breathing pattern mainly or solely appear to influence the amount of residual inspiratory vagal tone (and as a consequence, reciprocal changes in RSA amplitude). Thus, the more rapid and shallow the breathing, the greater the amount of parasympathetic influence upon the heart and the smaller the RSA. Rapid, shallow respiration results in a relatively ineffective gating of vagal effects whereas slow, deep breathing produces more complete inspiratory gating. Saul et al (1991 p 627 Berntson) suggested that at slow breathing frequencies, the phasic vagal cholinergic influence on the sinus atrial node has sufficient time to achieve full effect and dissipate. At more rapid breathing frequencies, however, responses to successive cycles of cholinergic action begin to merge and phasic sinus atrial responses decrease in amplitude.

Significance of HRV and RSA.

In order to gain insight as to the significance of the R-R spectrum pattern, investigators have 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; Pagani et al., 1991; Vybiral et al., 1989; Montano et al., 1994; Hayano et al., 1994; Goldberger, 1999; Bloomfield & Sweibel, 1998; Strano et al., 1998; Freitas, 2000).

Head-up tilt typically results in a decrease in total spectral power with a shift in power density distribution from a predominant HF towards a predominant LF band. 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; Pagani et al., 1991; Vybiral et al., 1989; Hayano et al., 1994; Goldberger, 1999; Bloomfield & Sweibel, 1998; Strano et al., 1998). 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 active standing helps to facilitate venous return and maintain blood pressure. Finally, HRV as been examined during dynamic and static exercise (Dixon et al., 1992; Furlan et al., 1993; Bernardi et al., 1990; Yamamoto et al., 1991; Casadei et al., 1995; Lellamo et al; 1999; Gonzàlez-Camarena et al., 2000). Typically during exercise HR increases as the result of vagal inhibition and sympathetic stimulation. Because there is less parasympathetic activity, the extent of its modulation by breathing or other mechanisms will also be reduced. With increased levels of exercise intensity vagal tone is almost completely abolished and thus heart rate variability nearly completely disappears. Results from analysis of HRV during an acute bout of steady-state exercise generally indicate a decrease in total spectral power with concomitant decreases in the absolute values of LF and HF components (Dixon et al, 1992; Kamath et al, 1991). Although a decrease in the overall

extent of HRV is found during dynamic exercise, an increase in the HF power spectral density expressed in normalized units has been reported in some studies in response to moderate and intensive dynamic exercise.(Casadei et al., 1994; Bernardi et al, 1990). Inasmuch as the heart rate variability in the HF band has typically been ascribed to parasympathetic influences, it has been difficult to reconcile this observation with the well-known inhibition of cardiac vagal influence typical of moderate and intensive exercise. This conflicting finding has led Casedei et al. (1994) to propose an alternate explanation for the interpretation of HRV during dynamic or static exercise such that the increase in the HF spectral density may be a reflection of an increase in the breathing rate inherent to the exercise protocol and its mechanical influence on heart rate modulation.

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 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% (Lellamo et al., 1999; 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 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.

4.0 Influences of endurance training on time domain and frequency domain of heart rate variability.

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 or following exercise rehabilitation in diseased population. Tables 5-10 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.

4.1 Results from 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 to as indices of parasympathetic and sympathetic modulatory influences on the SA node. Table 5 summarizes nine studies published over the last 8

Table 5. Time domain heart rate variability in trained subjects compare to untrained subjects.

Authors	n F/M	Age (Yrs)	Subjects	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 & Okada, 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†
Gregoire 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 ($p<0.05$).

years on a total of 339 athletic (Jensen-Urstad et al., 1997; Ishida et al., 1997; Lazoglu et al., 1996) or fit subjects (Tulppo et al., 1998; 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 & Okada, 1997; Goldsmith et al., 1997; Gregorie et al., 1996; Davy et al., 1996; Sacknoff et al., 1994). As seen in table 5, 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; 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 6. 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 those studies 165 males and 88 females between 21 and 69 years old were evaluated under standard resting conditions. VO_2max was measured in 5 of 8 studies and attests to an

Table 6. Frequency domain heart rate variability in trained subjects compare to untrained subjects.

Authors	n F/M	Age (Yrs)	Subjects	VO_2max Δ%	HR Δ%	TP Δ%	LF Δ%	HF Δ%
Yataco et al; 1997	14 M	69 ± 4	Sedentary					
	15 M	69 ± 7	Athletes	+70†		+8.9†	+8.1†	+24.9†
Jensen-Urstad et al.,	13 M	25 ± 1	Sedentary					
1997	16 M	25 ± 3	Elite runners		-33.6†	+117.4†	+67.1†	+90.0†
Ishida et al., 1997	16	27 ± 12	Sedentary					
	8	27 ± 12	Athletes		-12.7†		- 33.3†	+66.7†
Goldsmith et al., 1997	8 M	29 ± 3	Sedentary					
	8 M	28 ± 4	Endurance	+87.6	-25.4†	+118.9†	+177.2†	+339.9†
Gregoire et al., 1996	10 M	21 ± 2	Untrained					
	10 M	27 ± 4	trained	+20.7	-12.9†	+17.4		
	9 F	22 ± 2	Untrained					
	10 F	22 ± 3	trained	+23.0	-5.1†	+7.7		
	8 M	44 ± 8	Untrained					
	9 M	43 ± 5	trained		-23.3†	+48.9		
	7 F	44 ± 8	Untrained					
	10 F	44 ± 7	Trained		-16.9†	+119.1†		
Davy et al., 1996	11 F	56 ± 2	Sedentary					
	9 F	53 ± 1	Runners	+88.4†	-19.1†	+156	+112.9†	+353.5†
Lazoglu et al., 1996	10	28 ± 3.	Sedentary					
	10	27 ± 2	Weight lifters	+30.7†	-7.4	+27.7	+52.1	+119.8
	12	29 ± 3	Cyclists	+46.5†	-3.9	+28.5	-18.0	-13.5
Sacknoff et al., 1994	3F/9M	26 ± 2	Sedentary					
	1F/17M	30 ± 0	Endurance		-22.7†	-52.3†	-43.6†	-188.0†
Furlan et al., 1993	16 M/13 F	29 21	Control Swimmers		-10		+31.5†	26.9†
	14 M/ 7 F							
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 the trained and less trained group; TP=total power; LF=low frequency; HF=high frequency; † Significant difference (P<0.05). †= significant difference compare to the group above (p<0.05).

average 61% higher value in athletes than non-athletes with differences ranging from 20 to 88% (Yataco et al., 1997; Goldshmith 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 & Okada, 1997; Goldsmith et a., 1997; Gregoire et al., 1996; Davy et al; 1996; Lazoglu et al., 1996). Frequency domain variables generally appeared higher in highly trained individuals compare 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% Yataco et al. (1997), Jensen-Urstad et al. (1997), Ishida and Okada (1997), Goldsmith et al., 1997, and Davy et al. (1996)). 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; Davy et al., 1996). On the other hand, Sacknoff et al. (1994) and Ishida & Okada (1997) found significant lower values of the HF component, 33% to 47 %, in athletes compare to sedentary controls. A clear explanation for this discrepancy remains can not provided, since the same method has been used in these studies. In addition, a close look at overall results in cross-sectional studies

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 component of HRV in highly endurance trained individuals, which may be suggestive of a higher vagal influence. Few studies (Kenney, 1985; Benedito et al., 1985) have however examined the more specific vagally- mediated respiratory component of HRV, RSA. In the study by Kenney (1985) subjects were classified with respect to their physical activity status, from sedentary subjects, recreational runners and competitive distance runners and 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 come to any conclusion as to the exercise training status and extent of RSA.

4.2 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.

Heart Disease Patients

Several longitudinal studies have been conducted on patients undergoing rehabilitation following a myocardial infraction or after revascularization to examine time domain and frequency domain heart rate variability following exercise rehabilitation programs (Lellamo et al., 2000; Pardo et al., 2000; Duru et al., 2000; Malfatto et al., 1997; La Rovera et al., 1992). These programs were generally in-hospital based initiated some 2-4 weeks after myocardial infarction or revascularization and lasted for between 2 and 12 weeks. The changes in functional status or fitness were measured using symptom limited VO_2 peak tests or other endurance-type tests on bicycle ergometers or treadmills. These studies are summarized in table 7. As can be seen, VO_2 peak increased significantly by up to 30% in three studies compared to pre training values (Lellamo et al., 2000; Pardo et al., 2000; Duru et al., 2000) while a decrease between 4 to 17 % was found for resting heart rate in all 5 studies (from lower compare to pre training) with the difference from pre-training values reaching statistical significance in four of these studies. SDRR intervals increased by as much as 50% in all studies although a statistical significant increase of approximately 30-35% was only found in two studies of these studies (Lellamo et al., 2000; Malfatto et al., 1997). In table 8 are shown results from four studies examining the influence of physical rehabilitation on frequency domain heart rate variability.

Table 7. The effect of physical rehabilitation on time domain heart rate variability in heart disease patients.

Authors	n F/M	Age (Years)	Disease characteristics	Training (wks)	VO_2 peak Δ%	HR Δ%	R-R Δ%	SDRR Δ%
Lellamo et al., 2000	45 M	59 ± 8	With (22)or without MI (23) after revascularization	2	+14.9 †	-7†	+7.4†	+26.2 †
Pardo et al., 2000	5 F/15 M	71 ± 13	Cardiac, phase 2.	12	+57.1 †	-7.4†	+8.0†	+9.2
Duru et al., 2000	12 M	56 ± 5	Recent MI	8	+30.0 †	-17.1†	+20.6 †	+50.0
Malfatto et al., 1997	22 (M/F)	52 ± 7	Recent uncompleted MI	8		-7.9†	+9.0†	+35.0 †
La Rovera et al., 1992	11 M	47 ± 6	Recent MI	4		-3.6	+1.8	
Mean	**94 M 16 F**	**58**		**2-12**	**28.2**	**-8.0**	**+8.7**	**+27.6**

M/F=male/female; Δ%=different between pre and post physical rehabilitation; HR=heart rate; R-R=mean R-R intervals; SDRR=standard deviation between R-R intervals.

Table 8. The effect of physical rehabilitation on frequency domain HRV in heart disease patients.

Authors	Subjects N F/M	Age (Years)	Disease	Training (weeks)	VO_2peak Δ%	HR Δ%	TP Δ%	LF Δ%	HF Δ%
Pardo et al; 2000	5 F/15M	71 ± 13	Cardiac, phase 2.	12	+57.1 †	-7.4†	+5.0	+8.7	+12.8†
Duru et al., 2000	12 M	56 ± 5	MI	8	+30.0 †	-17.1†		-10.0	-38.5
Malfatto et al., 1997	22 (M/F)	52 ± 7	Recent uncompleted MI	8		+7.9†		-10.7†	+54.5†
La Rovera et al., 1992	11 M	47 ± 6	Recent MI	4		-3.6		+28.6	+3.6
Mean	**49 M 16 F**	**57**		**4-12**	**+46.9**	**-8.7**	**+5.0**	**+2.0**	**+15.9**

M/F=male/female; Δ%=different between pre and post physical rehabilitation; HR=heart rate; TP=total power; LF=low frequency; HF=high frequency.

Total power of heart rate variability was only reported in one study while the distribution into HF and LF HRV bands were presented in all four studies. In general, there appeared to be a trend towards increasing the HF or vagal related HRV after rehabilitation. A statistically significant increase in HF ranging between 12.8 and 54.5 %was reported in two studies (Pardo et al., 2000; Malfatto et al., 1997), a slight but non-significant increase being observed in a third study of La Rovera et al. (1997). A statistically significant decrease in the LF band of HRV (-10.7 %) was only seen in one study (Malfatto et al., 1997), other reports indicating non-significant LF component increases in two or LF component decrease in one. It would thus appear from these observations that exercise rehabilitation may be associated with some increase in the HF or vagal component of HRV. A decrease in the vagal or HF component of HRV is however a hallmark of coronary heart disease (Malik et al., 1996). A gradual return towards higher HF component values is generally observed with healing and recovery from disease. Considering that the present studies were conducted at different stages following myocardial infarction it is difficult to dissociate direct exercise-training influences from those related to the normal heart disease recovery process. A better understanding of the influence of endurance training on HRV thus requires a more thorough review of observations on healthy sedentary subjects.

Healthy population

Tables 9 and 10 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; Seals & Chase, 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; Seals & Chase, 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 a significant increase in SDRR was found in 3 studies (Levy et al., 1998; Stein et al., 1999; Seals & chase, 1989). As seen in table 10, a statistically significant increase in the power of the HF or vagal component of HRV was only seen in one study of young subjects 20 ± 1 yrs old, undergoing 25 min cycling sessions every day for a period of 6 weeks (Al-Ani et al., 1996).

Table 9. The Effect of endurance training on time domain HRV during rest in sedentary healthy population.

Authors	n F/M	Age (Yrs)	Training (wks)	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 10. The effect of endurance training on resting values of frequency domain of HRV in healthy population.

Authors	n F/M	Age (Yrs)	Training (wks)	VO_2max Δ%	HR Δ%	TP Δ%	LF Δ%	HF Δ%
Yamamoto et al; 2000	7 M	21 ± 1	6	11.6 †	-22			
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	**109 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 ($p<0.05$).

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 increases ranging between 2.7% and 5.1% (Loimaala et al., 2000; 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. (1996) 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). Overall, these observations indicate controversy and inconsistency in findings. The significance of theses observations is difficult to establish.

4.3 Significance of frequency domain analysis HRV for training-induced bradycardia

Results from cross-sectional and longitudinal studies of the interaction between exercise training and heart rate variability have also been examined in an attempt to bring more insight as to the etiology of training-induced bradycardia. As previously discussed, a significantly lower resting heart rate is generally seen in the comparison of highly trained or trained healthy individuals compared to untrained subjects, as can be a higher component of HF or vagal index of HRV tables 1, 5, and 6. In contrast, Furlan et al. (1993) found a lower HF power in athletes with higher LF and higher LF: HF ratio. Moreover, Benedito et al. (1985) and Kenney (1985) examining the more

specific vagal index of respiratory sinus arrhythmias found either no difference in the amplitude of RSA between runners and sedentary subjects (Benedito et al., 1985) or higher amplitude in high trained athletes (Kenney, 1985). Results from longitudinal studies using HRV analysis generally indicate increases in total heart rate variability as expressed either in the time domain as SDRR or in total spectral power density which may be ascribed to an increase in the HF component (Shi et al., 1995; Levy et al., 1998; Bonaduce et al., 1998; Yamamoto et al., 2000) although an absence of change in the vagal spectral component of HRV has also been reported (Ishida & Okada, 1998). Overall, results from the study of HRV in athletes or in association with endurance training reflect a higher HF or vagal component. In contrast to pharmacological approaches, which presumably block all cholinergic post-synaptic effects and thus both the tonic and the modulatory vagal activity to the sinus node, it is not clear that the HF component of HRV reflects vagal tone. Indeed, changes in the HF component may be seen simply as the result of changing breathing rate while the heart rate and thus presumably the tonic vagal activity remains unchanged (Calabrese et al., 2000). Thus, an increase in the HF band of HRV while reflecting greater cardiac vagal modulatory influences does not exclude the concurrent possibility of a decrease in the intrinsic rate of depolarization of the sinus node.

5.0. Position of the problem

Experimental evidence obtained both in animals and in humans concurs to establish that endurance training exerts a direct influence on the heart to decrease resting sinus rhythm. An exception to this general observation may be found in older humans in whom training-induced bradycardia is not systematically reported. A clear explanation for the phenomenon of training-induced bradycardia has not yet been provided. Results from most investigations using a pharmacological blockade approach indicate an enhanced cardiac vagal influence in the endurance-trained state that may or may not be associated with a decrease in sinus node sympathetic activity or in the intrinsic rate of depolarization of the sinus node. The recent use of heart rate variability analysis introduces a new dimension to the picture of heart rate regulation. In most applications of HRV to athletes and/or trained subjects a higher HF component is observed which may be interpreted as a greater vagal influence. It must be recognized however that HRV assesses the combined influences of both tonic vagal activity and its modulation resulting from breathing and other physiological influences. There exists limited information if any, as to the influence of endurance training on the characteristics of breathing-induced modulatory influences on the heart. For a given heart rate or tonic vagal activity, the amplitude of heart rate modulation throughout a breathing cycle is generally proportional to the duration of the breathing cycle. In the present study we examined characteristics of respiratory sinus arrhythmia in healthy sedentary subjects

prior to and following 4 weeks of intensive endurance training. Because we were interested in the time course of these adaptations we also repeated the assessments each week until the end of the program. We also wished to examine if training would influence the relationship between breathing frequency and RSA amplitude. We reasoned that training-induced bradycardia would result in a larger RSA for a given spontaneous breathing frequency. In turn, a training-induced upward shift in the breathing frequency-RSA amplitude relationship would reflect a similar extent of breathing-induced vagal modulation with a higher tonic vagal activity. On the other hand, a change in the slope or sensitivity of the breathing frequency-RSA amplitude relationship would reflect combined effects of training both on the breathing-induced vagal modulatory influences and tonic vagal activity.

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PAET II: EXPERIMENTAL STUDY

Introduction:

Endurance exercise training is known to induce resting bradycardia both in humans and animals. (Lewis et al., 1980; Jensen-Urstad et al., 1997; Smith et al., 1989) However, a clear explanation for this phenomenon still remains to be provided. Results from studies in rats using single or combined parasympathetic and sympathetic blockade (Negrao et al., 1992; Hassan, 1991; Barnard et al., 1976; Hughson et al., 1976 and Yu-Chong & Horvath, 1972) indicate either a higher (Hughson et al., 1976), a lower (Hassan, 1991) parasympathetic influence or no change in vagal influences (Barnard et al., 1976), a reduced sympathetic influence (Yu-Chong & Horvath, 1972 & Hassan, 1991) or a decrease in the intrinsic rate of the SA node (Negrao et al., 1992 & Hughson et al., 1976) in trained versus untrained animals.

Results from pharmacological blockade in humans similarly appear to be controversial. Results from three cross-sectional comparisons of endurance athletes and non-athletes indicate similar levels of parasympathetic influence between athletes and non-athletes in two studies (Benedito et al., 1985; Lewis et al., 1980), while a lower heart rate during combined parasympathetic and sympathetic blockade was found in two studies (Katona et al., 1982; Lewis et al., 1980), even being associated with a decrease in the parasympathetic component in the study by Katona et al. (1982). These observations may be taken to reflect that the intrinsic rate of sino-atrial node depolarization may be decreased in the endurance trained state but not necessarily the level of parasympathetic activity. Results from

longitudinal studies have reported either an increase in parasympathetic influences to the sinus node in two studies after 5 weeks and 8 months of training respectively, with (Ekblom et al., 1973) and without (Shi et al., 1995) an associated decreases in the sympathetic influence. In a third longitudinal study, no change in parasympathetic influence was found after 10 weeks of endurance training.

More recently, the investigation of heart rate variability (HRV) has been used in both cross-sectional comparisons of endurance athletes and non-athletes (Tulppo et al., 1998, Yataco et al., 1997, Jensen-Urstad et al., 1997 & Ishida & Okada, 1997) as well as in longitudinal investigations of the effects of endurance training (Lomaala et al., 2000; Schuit et al., 1999; Levy et al., 1998 & Stein et al., 1999). Markers of HRV are reported as standard deviation or variance around a mean steady-state heart rate (time domain) or as the power of frequency components of the heart rate variance obtained using a Fast Fourier Transform function or autoregressive analysis (Malik et al., 1996). Respiratory sinus arrhythmia (RSA) is the single most important determinant of 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. Indices of HRV and more specifically those related to the effect of breathing on heart rate such as the power of the High Frequency (HF) band of spectral analysis of heart rate or the amplitude of RSA thus provide a non-invasive assessment

of the vagal influence on the sinus node. Several studies have compared HRV of athletes and non-athletes (Yataco et al., 1997; Goldsmith et al., 1997, Davy et al., 1996). Results expressed in s^2/Hz generally reveal a larger HF component in athletes (Jensen-Urstad et al., 1997 and Shin et al., 1996), which may be taken to suggest a greater parasympathetic influence. Because the index is not standardized for the R-R interval duration, an alternate explanation may be that the extent of breathing-induced modulation is of the same magnitude in athletes and non-athletes but because the former have a lower resting heart rate the resulting change translates into larger fluctuations. A small number of studies on the effects of endurance training on resting heart rate modulations have also been conducted using HRV (Ishida et al; 1998 and Bonaduce et al., 1998; Levy et al., 1998). Healthy subjects ranging in age from 20 to 67 years were submitted to endurance training programs consisting of cycling and running 3 to 4 X/week for 6 and 8 weeks duration in two studies (Al-Ani et al., 1996; Boutcher & stein, 1995) and of more than 20 up to 52 weeks in the other studies (Loimaala et al., 2000; Schuit et al., 1999; Ishida & Okada, 1998; Stein et al., 1999). Results from these studies again appear controversial.

Significant increases in VO_2max were found in all the studies (Yamamoto et al., 2000; Loimaala et al., 2000; Schuit et al., 1999; Ishida & Okada, 1998; Stein et al., 1999, Al-Ani et al., 1996; Boutcher et al., 1995) as was a significant decrease in resting heart rate (Yamamoto et al., 2000; Loimaala et al., 2000; Ishida & Okada, 1998; Al-Ani et al., 1996; Boutcher et

al., 1995). Nonetheless, a significant increase in the HF or parasympathetic index of HRV was only found in two studies (Al-Ani et al; 1996 and Yamamoto; 2000); results from other investigations showing non-significant decreases (Ishida et al 1998; Schuit 1999), slight increases (Loimaala et al 2000; Stein et al. 1999) or no change (Boutcher et al., 1995) of HF power component of HRV. The discrepancy in findings is difficult to explain. To ensure that the breathing influence on heart rate is indeed found within the standard HF band (0.15-0.40 Hz) subjects are often asked to breathe at a target pace above 0.15 Hz or 9 breaths/minute (generally 0.20Hz) given by an auditory cue chosen to fall within the standard HF band. This was seldom in these studies, which may contribute to explain the discrepancy.

The more specific assessment of breathing-induced HRV is to measure the amplitude of RSA. We are not aware of any investigations of the effects of training on RSA characteristics such as RSA amplitude defining the maximum change in heart rate throughout a breathing cycle and RSA phase describing the timing of this maximum change within the breathing cycle. It is generally accepted that RSA amplitude increases when heart rate is reduced and inversely, decreases when heart rate increases (Katona & Jih, 1975; Eckberg, 2000). In addition, for a given steady-state heart rate, RSA amplitude decreases with shorter breathing cycles i.e. faster breathing rates and increases with slower breathing rates reaching a plateau (Hirsch & Bishop, 1981).

In the present study we examined the effects of a short intensive endurance training program on the breathing-induced vagal modulations of heart rate using both frequency spectrum analysis of HRV as well as measurement of RSA characteristics. Because we were interested in the time course of these adaptations we also repeated the assessments of HRV and RSA each week until the end of the program. We also wished to examine if training would influence the relationship between breathing frequency and RSA amplitude. We reasoned that training-induced bradycardia would result in a larger RSA for a given spontaneous breathing frequency. In turn, a training-induced upward shift in the breathing frequency-RSA amplitude relationship would reflect a similar extent of breathing-induced vagal modulation with a higher tonic vagal activity. On the other hand, a change in the slope or sensitivity of the breathing frequency-RSA amplitude relationship would reflect combined effects of training both on the breathing-induced vagal modulatory influences and tonic vagal activity.

Methodology

Subjects:

Thirty one, healthy untrained male and female subjects, 25-55 years old, non-smokers, volunteered to participate in this study by responding to an advertisement in McGill university campus newspaper requesting subjects to take part in a physical training study. Subjects were considered to be untrained if they had not been participating in more than two sessions of exercise per week for 6 months prior to the beginning of the study. After a description of the experimental design and the protocol, all subjects signed an informed consent form approved by the institutional Ethics Review Board.

Training Group

Twenty-five subjects agreed to participate in 3-5 sessions per week of intensive aerobic exercise. Subjects could choose from a choice of 12 sessions per week including: an aerobics class, a Tai-Bo class, fast walking/jogging or spin class (stationery cycling with music). Each session included three parts: Warm-up (10-12 min, 50-60 % HR reserve), main set (40 minutes @ 50-85% HR reserve) and cool down (10 minutes @ 50-60 % of HR reserve). HR was monitored regularly throughout the class by palpation of the left artery. Two subjects, chosen randomly in each training session wore a HR monitor (Sport tester (Polar™) during the entire session, to ensure that the intensity of exercise corresponded to the targeted heart rates.

Five subjects dropped out of the study. Three subjects did not complete the minimum requirements for completion of 3 exercise sessions per week and their data was excluded from the study. Seventeen subjects (11 Females and 6 males) 34.4 ± 10.6 years old finished 12-20 sessions of exercise training during the 4 weeks of training. Six subjects who had dropped out of the training program after one week and remained untrained thereafter accepted to be tested again after 4 weeks of the initial test and were used to form the control group.

Control Group

Six subjects (5 females and one male) 35.3 ± 12.7 years old agreed to be evaluated before and after a 4-week period during which they maintained their standard lifestyle pattern and did not participate in any formal physical activity program.

Experimental Protocol

Subjects in the training group were asked to perform two progressive maximal exercise tests, one before and one after the training program. For HRV and RSA measurements, subjects were asked to report to the cardio-respiratory laboratory on four different occasions, namely: before the training program, twice during the second and the third week of training and after having completed the training program.

Subjects in the control group were asked to report to the laboratory only before and after the four weeks. Subjects in the control group did not perform a maximal exercise test however they reported not being physically active and did not change their pattern of physical activity during the 4-week period of observation.

VO_2 max determination:

VO_2 max was estimated using the Leger-Boucher track test (Leger et al; 1988) performed on the first day of the first week of training, and again within three days of their last training session. The end-point of the test was defined as being unable to maintain the required running speed as for at least to consecutive markers separated by a distance of 50 meters.

Assessment of Heart Rate Variability and respiratory sinus arrhythmia:

On the day prior to VO_2 max testing, subjects reported to the laboratory for assessment of heart rate variability. Subjects were asked to avoid consumption of caffeine 2 to 3 hours prior to testing and were asked to refrain from talking and to remain as motionless as possible during testing while the laboratory was dimly lit and all visual and auditory distractions were minimized. 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, subjects were asked to follow a breathing rate given by an auditory cue at 1) four breathing cycles lower and 2) four breathing cycles higher than their spontaneous rate. Subject's blood pressure was determined before the first recording and during the last minute of each recording using an electronic arm sphygmomanometer (Sunbeam model 7650) placed on the left arm.

Heart rate variability data acquisition and analysis

The recording of data was performed on an IBM Computer 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 Cantered 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; Calabrese et al., 2000).

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 for breathing rate.

Statistical Analysis

Values are reported as means ± standard deviation. Changes in VO_2max before and after the training were evaluated using a t-test for dependent variables. Mean comparison of pre-training to post-training variables was obtained using a MANOVA for repeated measurements in time.

The MANOVA (repeated measures) analysis for respiratory variables included: tidal volume (TV), inspiratory time (IT), Total respiratory time (Ttot), ratio IT: Ttot and ratio TV: IT. Analysis of HRV variables from the time domain included: Mean RR intervals and SD RR intervals while those from the spectral analysis included: Total Power (s), Very Low Frequency (s), Low Frequency (s), High Frequency (s), LF (nu), HF (nu), ratio LF:HF and Respiratory center frequency. For analysis of RSA variables the MANOVA included: RSA Phase, Delay, Amplitude and Slope of the breathing frequency-RSA amplitude relationship. The influence of different breathing conditions (sp, sp-4 b/m, sp + breath /minute) was evaluated using a 3 x 4 ANOVA for repeated measures. Following findings of significant main effects of group (Ctrl vs Training) and time (weeks 1 to 4), post-hoc analyses were achieved using Tukey honest significant (HSD) difference. All analyses were performed using the commercially available statistical software package "STATISTICA"® version 5.0. Statistical significance was set at a level of $P \leq 0.05$.

Results:

As shown in table 1 subjects from the control and training groups exhibited similar personal characteristics such as age, height and weight.

Table 1. Subjects characteristic.

Group	n	Age (yrs)	Weight (kg)	Height (cm)
Training	11F/ 6M	33 ± 11	67 ± 15	169 ± 10
Control	5F/ 1M	35 ± 13	70 ± 19	168 ± 15

F/M=female/male; results are in mean ± sd.

Pre and Post training cardiorespiratory baseline parameters are shown for both the control group and the experimental group in table 2.

Table 2. Results from cardiorespiratory parameters during spontaneous breathing at rest pre and post training.

	Control		Training	
	Pre	**Post**	**Pre**	**Post**
HR (bpm)	70.6 ± 7.0	3.6 ± 7.1	4.0 ± 13.7	74.1 ± 7.1
SBP (mmHg)	117.7 ± 19.0	116.2 ± 13.9	121.1 ± 11.2	115.4 ± 11.1
DBP (mmHg)	78.3 ± 10.6	77.5 ± 15.2	80.9 ± 8.5	72.9 ± 8.3*
Vt (L)	0.60 ± 0.18.	0.34 ± 0.20	0.41 ± 0.18	0.36 ± 0.17
T tot (s)	4.6 ± 1.3	4.6 ± 1.2	4.7 ± 1.5	4.5 ± 1.4

Results presents in mean ± sd; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; Vt: Tidal volume; Ttot: Total breath duration.

* Statistical significant difference from pre training ($p < 0.001$).

Pre-training results indicate no significant difference between groups for resting heart rate, systolic or diastolic blood pressure, and tidal volume or total breath duration. The training group exhibited a significant decrease in DBP ($p < 0.001$) while no change was noted within the control group subjects. A slight decrease in SBP was observed in the training group after 4 weeks (121 ± 11.2 mmHg versus 115.4 ± 11.1 mmHg), which however did not reach statistical significance ($p = 0.08$). No significant difference was observed following training in resting HR, Vt, and Ttot in either the experimental or the control group. Table 3 summarizes the effects of training on maximal exercise characteristics. As expected, maximal workload as well as VO_2max increased significantly ($p<0.01$) after the training program while slight decrease was found in HR max ($p<0.06$).

Table 3 Results from cardiorespiratory and workload responses to training program.

	Pre	Post
HR (bpm)	192 ± 15.6	187 ± 13.5
Vo_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	37.3 ± 4.4	40.0 ± 5.4 †
Maximal work (MET)	10.7 ± 1.3	11.4 ± 1.5 †

Results are present in mean ± sd; HR: heart rate; $V0_2$max: maximal oxygen consume in a min; † Statistically significant difference between pre training ($p<0.01$) and post training.

The Effects of endurance training on heart rate variability and respiratory sinus arrhythmia under spontaneous breathing conditions.

Table 4 summarizes the effects of endurance training on respiratory and time domain Heart rate variability parameters at rest throughout the four weeks of training.

Table 4. Effects of endurance training on cardiorespiratory parameters during the test of HRV.

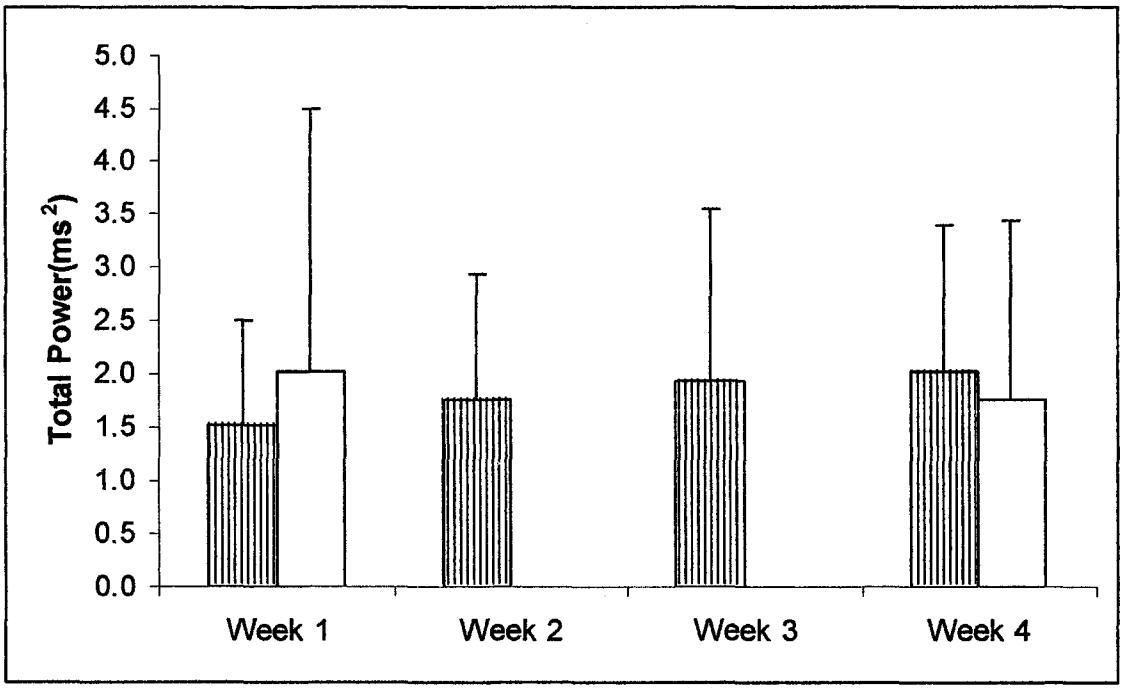
	Week 1	Week 2	Week 3	Week 4
RR (ms)	820 ± 180	790 ± 70	800 ± 80	810 ± 60
SDRR (ms)	51.7 ± 19.4	55.4 ± 21.8	57.1 ± 26.3	59.9 ± 22.2
Vt (L)	0.37 ± 0.20	0.44 ± 0.25	0.39 ± 0.16	0.33 ± 0.15
Ti/Ttot (s)	0.40 ± 0.055	0.39 ± 0.032	0.38 ± 0.026	0.39 ± 0.030
Vt/Ti (ml/s)	0.23 ± 0.12	0.28 ± 0.16	0.25 ± 0.13	0.21 ± 0.10*
T tot (s)	4.4 ± 1.2	4.2 ± 1.0	4.3 ± 1.0	4.4 ± 0.8
SD T tot (s)	0.60 ± 0.27	0.51 ± 0.27	0.51 ± 0.28	0.58 ± 0.28

Results are present as mean ± sd; T – training group; C - control group; RR: mean R-R interval; SDRR: standard deviation of R-R intervals; Vt: tidal volume; Vt/Ti: tidal volume inspiration time ratio; Ttot: total breath duration; SD Ttot: standard deviation of total respiration time; * Statistically significant difference between week 4 and week 2 ($p < 0.01$)

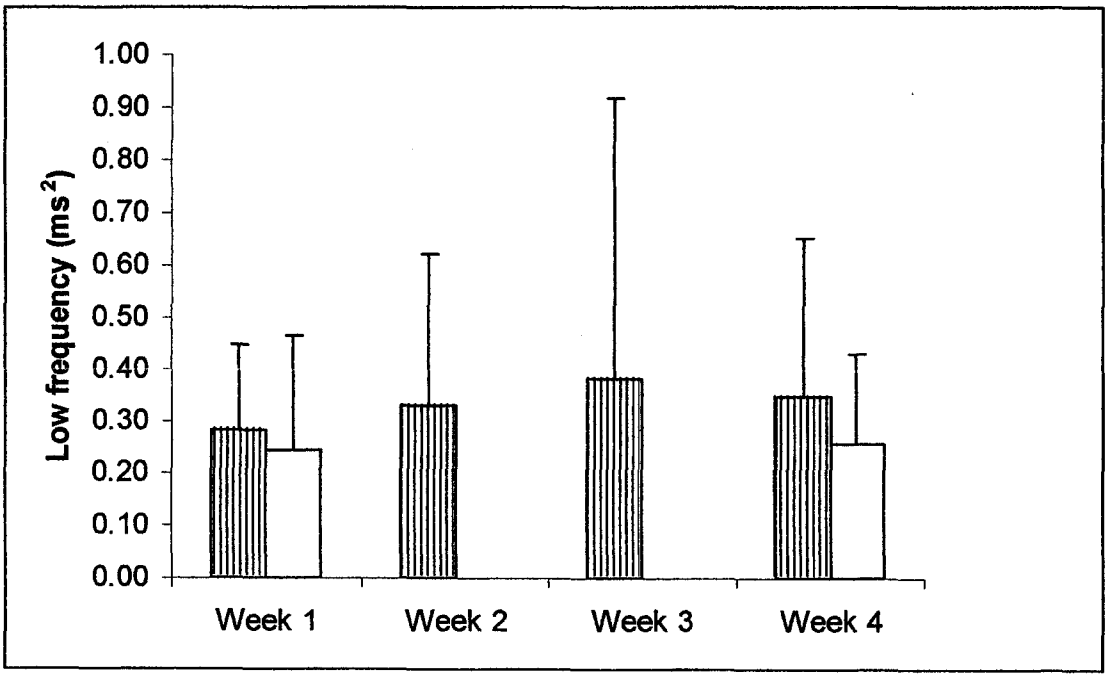
As can be seen, no significant differences were found in the respiratory parameters, Ttot, SD Ttot, Vt, Vt:Ti ratio, and Ti:Ttot ratio before and after the 4 weeks of training. The Vt:Ti ratio was found to be significantly lower at week

4 compare to week two, although no significant different was measured between week four and one. Similarly, no significant changes were found in time domain parameters of HRV (RR and SDRR) over the four-week training period.

The effects of training on the frequency spectrum of HRV are presented in Figures 1.1 and 1.2 under spontaneous breathing conditions. Results from the control group are shown at weeks 1 and 4. Results from the training group are shown every week for the total 4 weeks. Total Power (ms^2), Power of the LF component (ms^2), power of the HF component (ms^2) as well as RCF (ms^2) were not significantly different between the control and the training group at week 1. Comparison at weeks 1 and 4 in the control group indicate no significant change in any of the parameters. In the training group, a slight increase in Total power (ms^2) as well as LF (ms^2) and HF (ms^2) are observed from week 1 to week 3, which however did not reach statistical significance. Thus, no significant change is observed for any of the frequency spectral heart rate variability components.

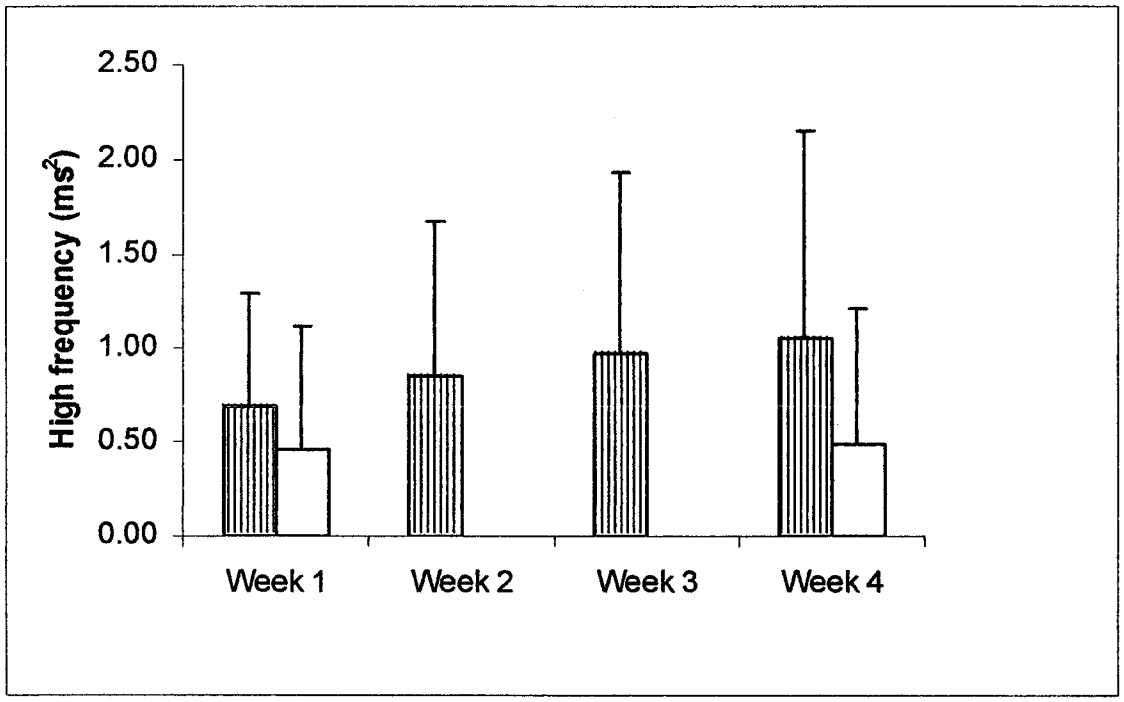


A.

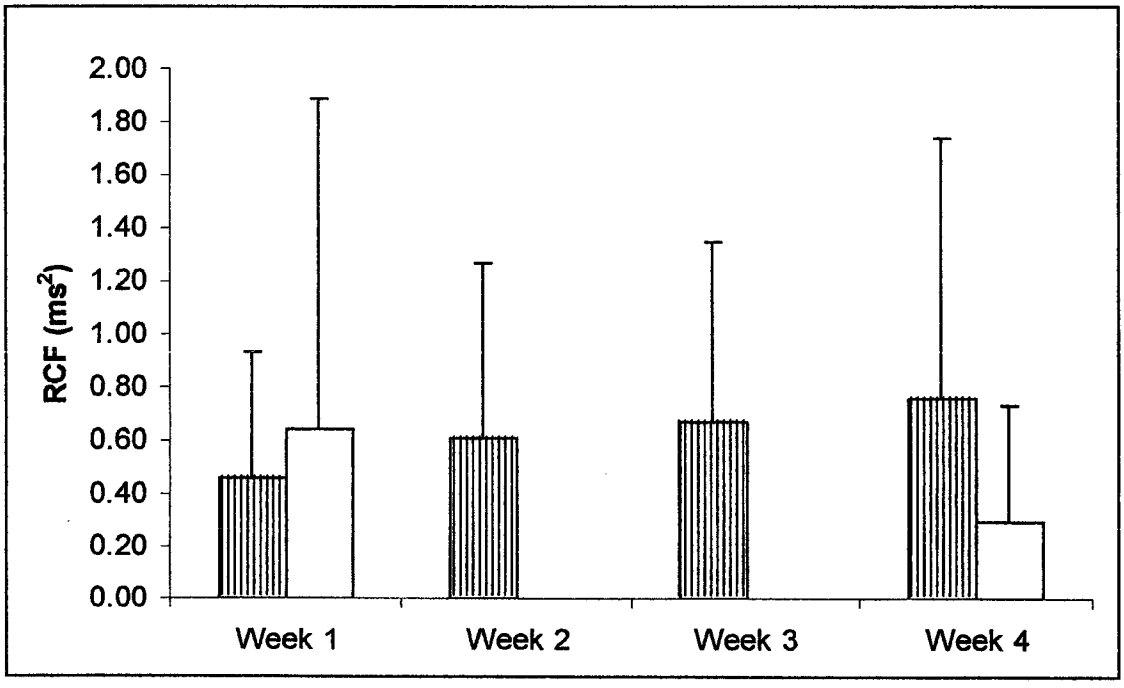


B.

Figure 1.1 TP and LF responds to four weeks of training.



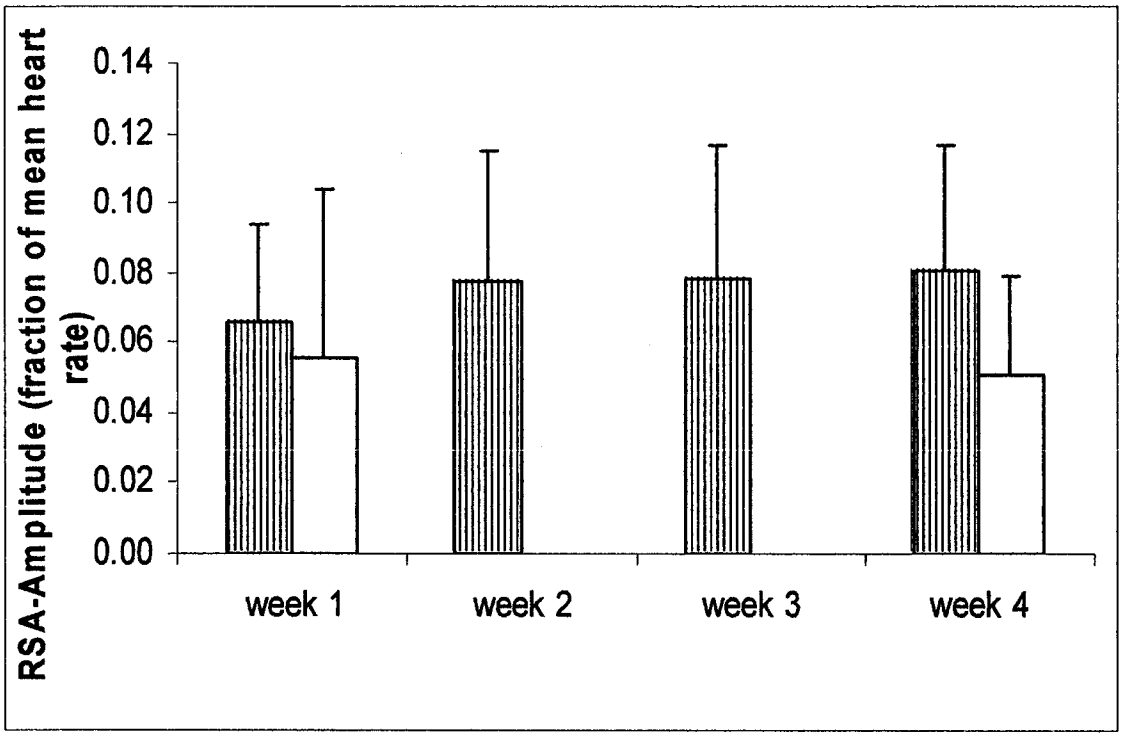
A.



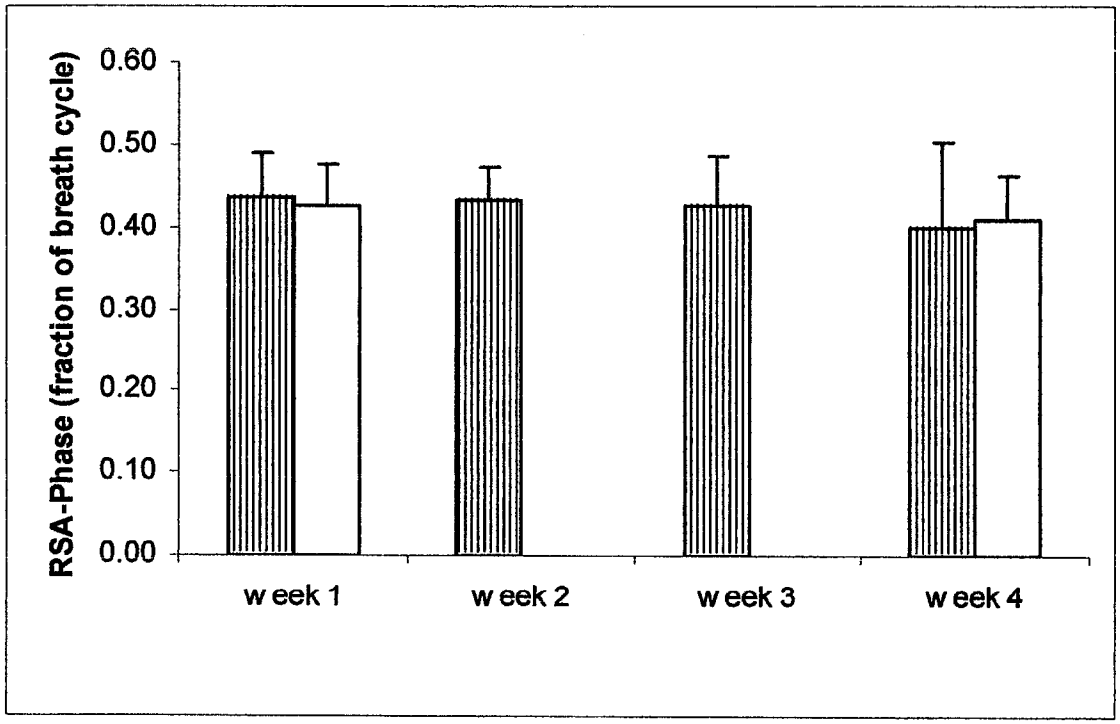
B.

Figure 1.2 HF and RCF responds to four weeks of training.

The effects of endurance training on Respiratory Sinus Arrhythmia variables are shown in Figures 2.1 and 2.2. Figure 2.1A shows RSA amplitude under freely breathing conditions expressed in fraction of mean heart rate. Values for the control groups are shown at weeks 1 and 4 while those for the experimental group are shown at every week throughout the 4-week training program. Controls results indicate no change in RSA at week 1 compare to week 4, In addition Results from the experiment group indicate a slight increase in RSA at week 2 compared to baseline, which however did not reach statistical significance. No further increase was observed at weeks 3 and 4. Figure 2.1B illustrates RSA phase expressed in fraction of breath duration under spontaneous breathing conditions in control subjects at weeks 1 and 4 and at each week for the training group. A slight but non-statistically significant decrease in RSA phase is seen at week 4 compared to weeks 1, 2, and 3 in the experimental group. Similarly, Figure 2.2 shows no significant changes in RSA delay (s) at any time over the four weeks of training.



A.



B.

Figure 2.1 The effect of four weeks of training on RSA amplitude and RSA phase.

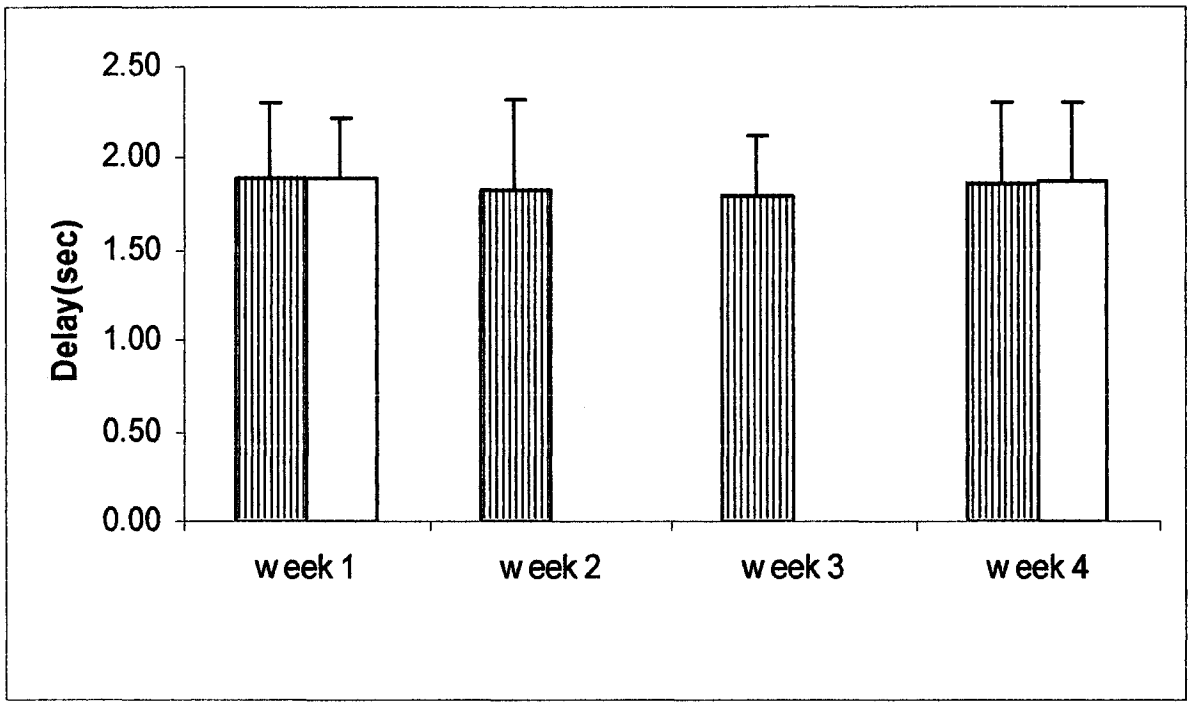


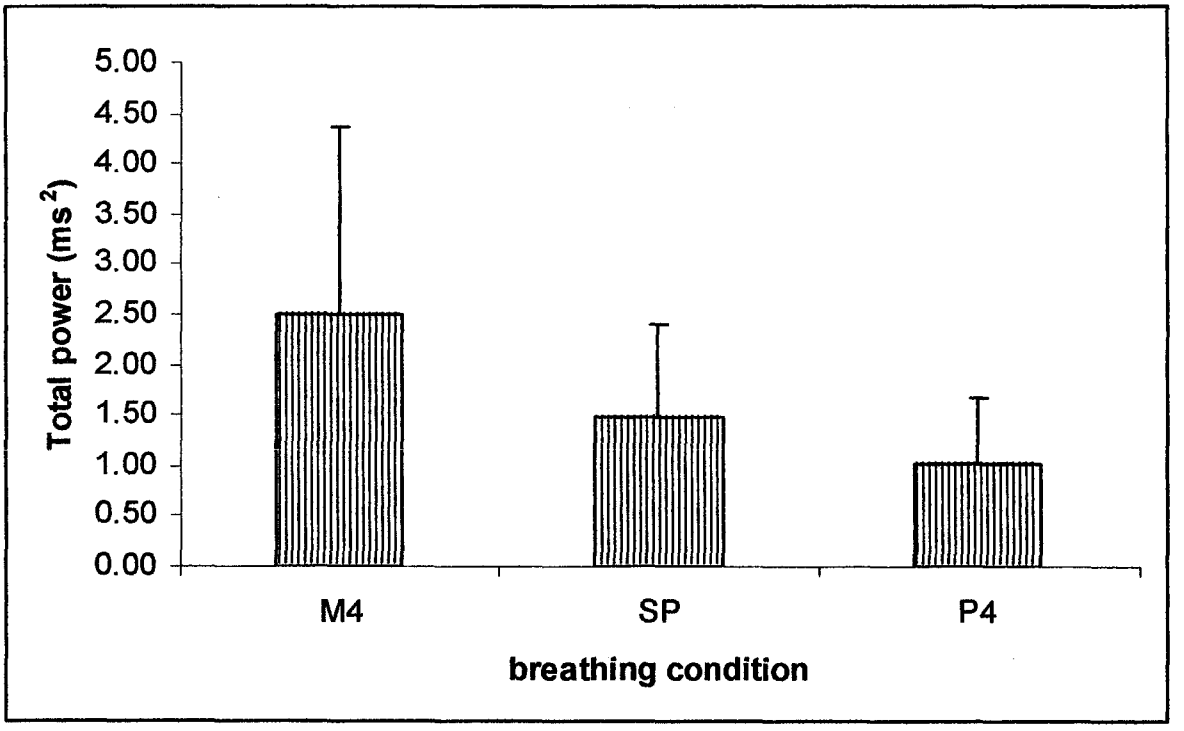
Figure 2.2 The effect of four weeks of training on delay.

Changes in Heart rate variability and respiratory sinus arrhythmia with imposed breathing rates.

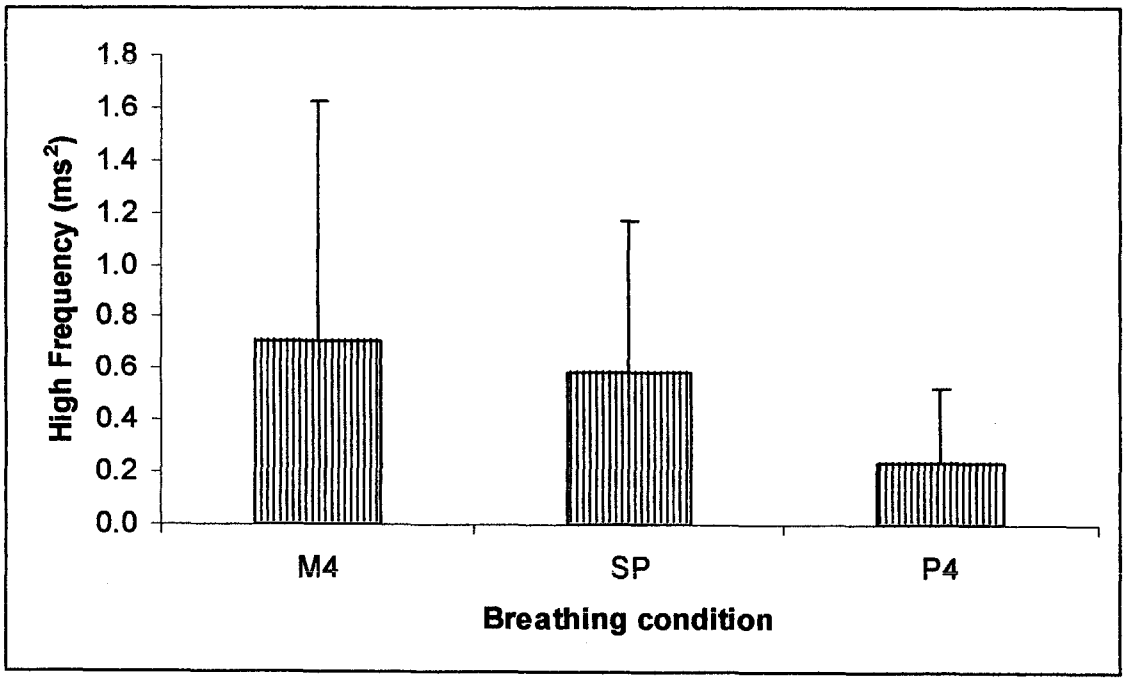
Responses of frequency spectral components of heart rate variability variables in relation to increasing (P4) and decreasing (M4) breathing frequency by 4 breath/minute from baseline are shown in figures 3.1 and 3.2 As seen in Figure 3.1A, total spectral power (ms^2) was significantly higher at M4 compared to Sp and lower at P4 compare to M4 and. This was mainly accounted for by a concurrent fall in the HF power component at P4 and increase at M4 compared to Sp (Figure 3.1B). RCF (ms^2) as seen in figure 3.2 was significant higher in M4 compare to SP and P4.

Changes in RSA amplitude expressed in fraction of mean heart rate are shown in Figure 4A. RSA-amplitude is higher during M4 condition compare to Sp and P4 condition 0.1 ± 0.05, 0.06 ± 0.03, and 0.05 ± 0.02, respectively. RSA-phase was not affected by changes in breathing frequency (Figure 4B).

Increasing breathing frequency by 4 breaths decreased Ttot from 4.7 ± 1.5 sec to 3.5 ± 0.8 sec while decreasing breathing rate increased Ttot from 4.7 ± 1.5 sec to 7.5 ± 4.1 sec. No significant changes in R-R interval were found with changes in breathing frequency.



A.



B.

Figure 3.1 Changes in total power and high frequency heart rate variability with imposed breathing rates.

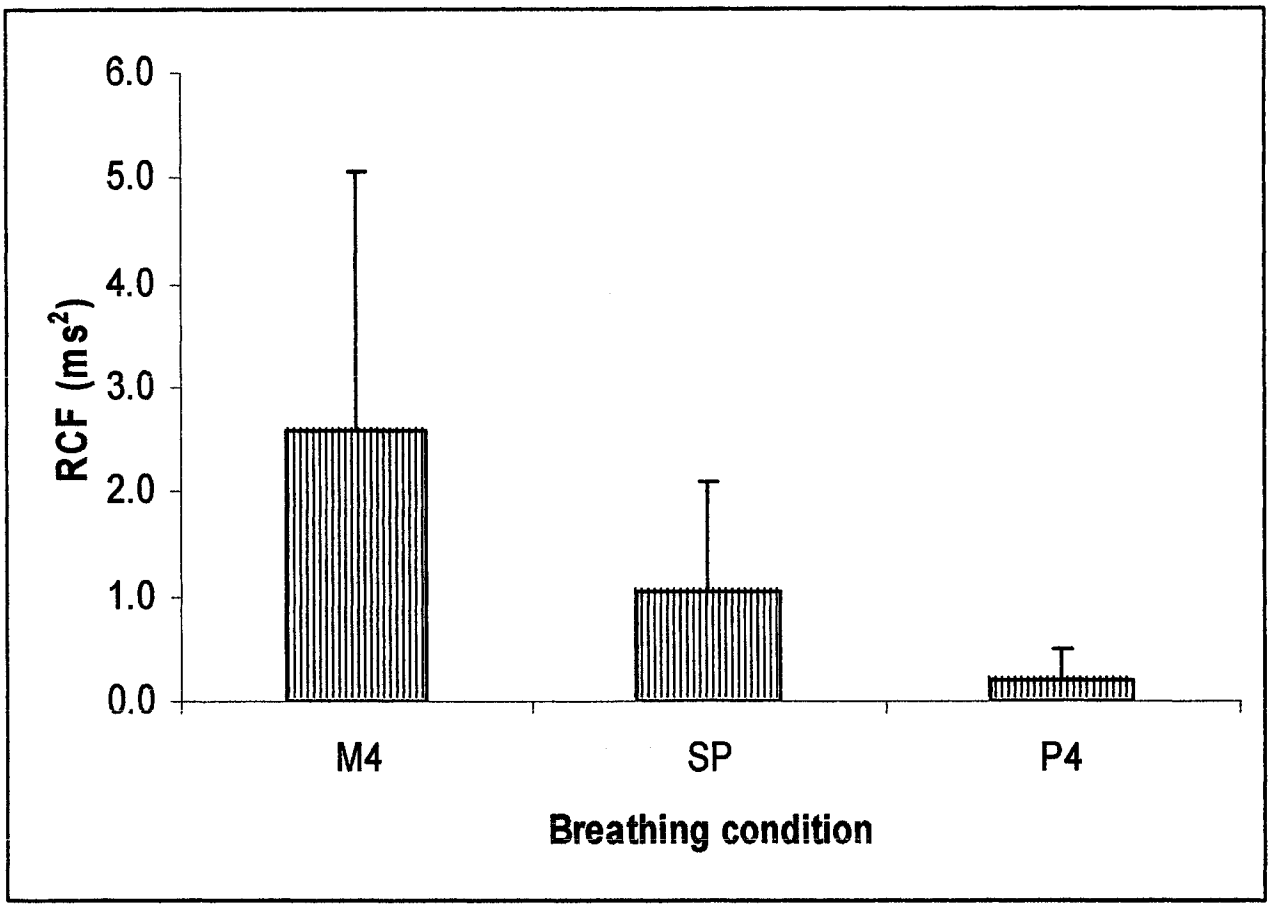
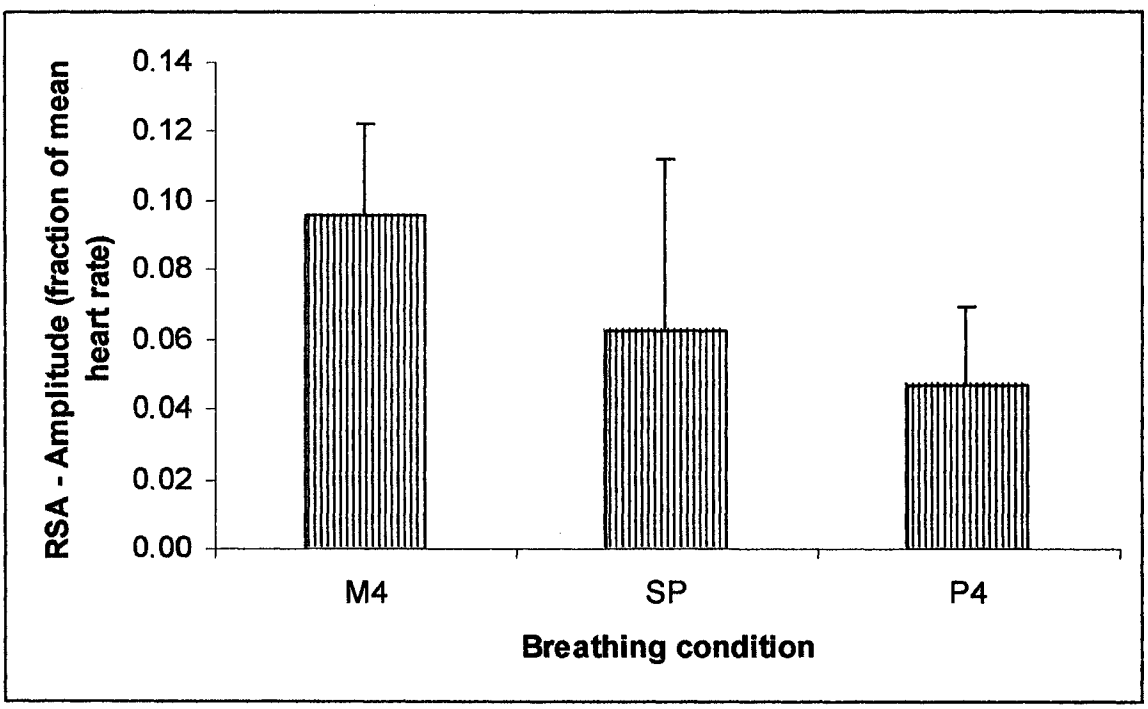
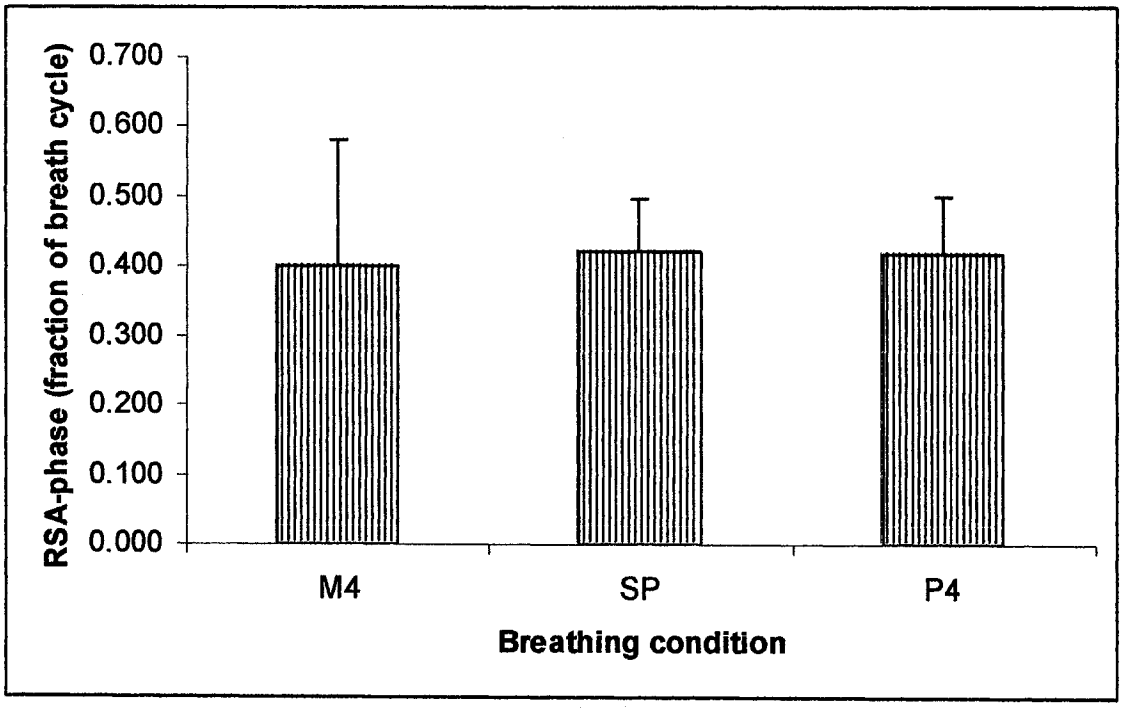


Figure 3.2 Changes in RCF heart rate variability with imposed breathing rates.



A.



B..

Figure 4. Changes in respiratory sinus arrhythmia with imposed breathing rates.

Effects of training on the RSA amplitude response to changes in breathing frequency.

Figure 5 illustrates the scatter plot of individual RSA amplitude in response to changing breathing frequencies in the experimental group before and after the 4 weeks of intensive training. Results indicate a significant negative linear relationship between RSA amplitude and breathing frequency. A slight increase in the slope of the RSA amplitude versus breathing frequency relationship is observed after 4 weeks of endurance training. However, mean comparison of the average of individual slopes did not reveal statistical significance.

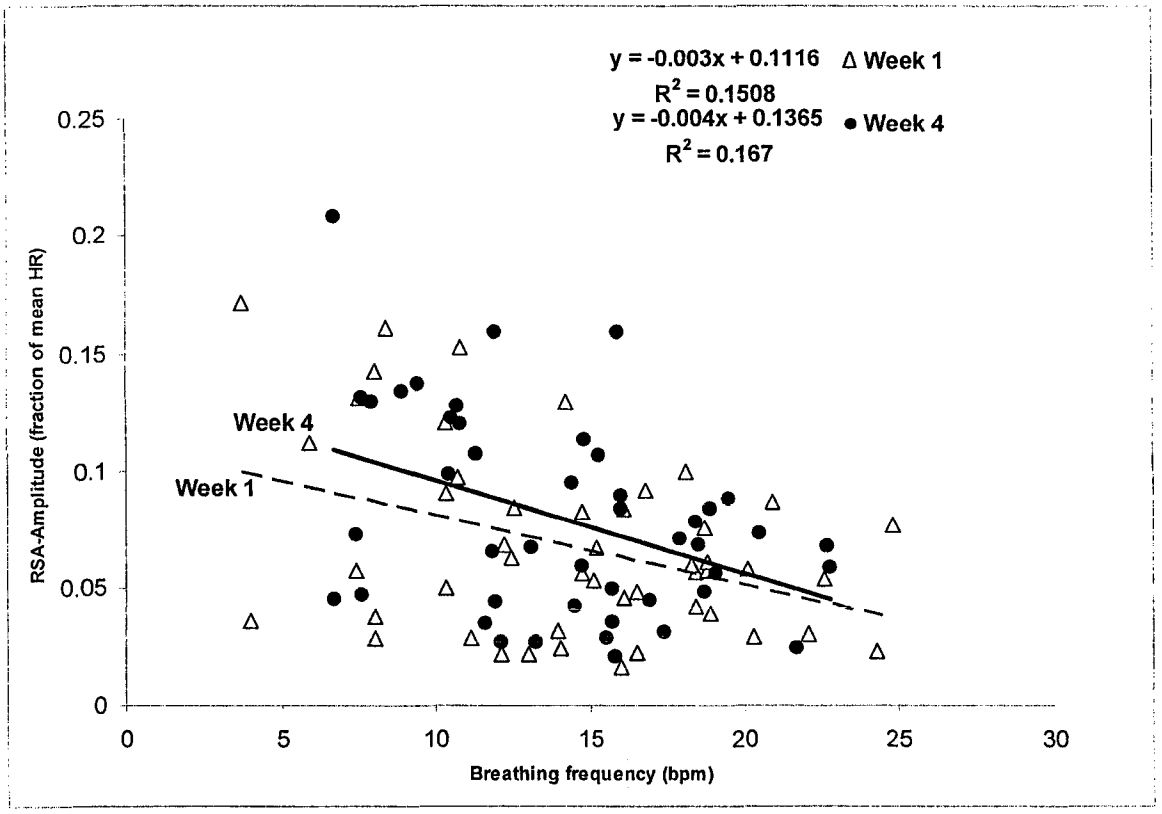


Figure 5. Endurance training effect on the changes in RSA-amplitude, due to changes in breathing frequency.

Discussion

Results from the present study indicate significant improvements in VO_2max and a significant decrease in resting diastolic blood pressure after four weeks of intensive endurance training in healthy sedentary middle-age subjects. This however was not associated with significant effects on vagal markers of heart rate variability and respiratory sinus arrhythmia. Results confirmed a significant relationship between breathing frequency and the amplitude of respiratory sinus arrhythmia with a tendency towards a greater gain in RSA amplitude after 4 weeks of training.

Effect of training on VO_2max, and blood pressure.

Significant changes in VO_2max are generally found in healthy sedentary populations following long-term endurance training. Results from the present study are in agreement with previous reports on short term endurance training performed over the last 30 years indicating significant increases in VO_2 max after as little as 3 to 6 weeks of training (Mier et al.,1997; Al-Ani et al., 1996, Torii et al., 1992; Dart et al., 1992; & Steinhaus et al., 1990). No change was found in the control group attesting to a true effect of training. Our results also indicate a slight decrease in maximal heart rate ($p<0.06$). Such an effect of endurance training ranging in magnitude between 2 to 9 bpm or 1- 4.7% has previously been reported following some 6 to 30 weeks of endurance training (Al-Ani et al., 1996; Seals & Chase, 1989; Pollock & Ayres, 1977) but not with a shorter periods (Mier et al., 1997;

Steinhaus et al., 1990). Our subjects did appear to show true maximal effort as seen from the increase in maximal aerobic power suggesting that the observed effect is not the effect of poor exercise motivation but may be related to a true exercise-induced adaptation occurring as early as after 4 weeks of intensive training. In the present study, four subjects did less than 14 seesion while another four did more than 16 session, most of the group achieved between fourteen and sixteen sessions. However, we found no significant relationship between the number of completed sessions and the change in VO2 max. suggesting that

In addition to the improvement in aerobic capacity a significant decrease in diastolic arterial blood pressure was observed following the endurance training protocol. Results from the time course analysis indicate this not to be present after 3 weeks but to be definitely observed after 4 weeks of training. A chronic decrease in systolic blood pressure has also been reported after endurance training in a number of studies of healthy and hypertensive sedentary subjects (Choquette & Ferguson, 1973; Hagberg et al., 1983; Krotkiewski et al., 1979) often found concurrently with a decrease in body mass. In the present study, subjects did not report significant changes in body mass over the course of training and only a slight effect was seen of systolic BP. Since in the majority of the previously cited studies training duration ranged between 1 and 27 months (Seals and Hagberg, 1984), it is possible that a longer training period be needed to induce a reduction in systolic blood pressure.

The adaptation in diastolic blood pressure may be related to a decrease in peripheral vascular resistance, which has been reported following both acute bouts of endurance exercise in healthy and hypertensive subjects (Kingwell et al., 1992; Rosolova et al., 1991 and Van Hoof et al., 1989) as well as after endurance training in hypertensive subjects (Cade et al., 1984). It has been previously suggested that the effect of endurance training on resting arterial blood pressure may be the result of an exercise-induced hypotension, which may last for some 24 hours after the last exercise bout (Van Hoof et al., 1989). In the present study however, subjects were evaluated more than 24-hours after their last exercise session, which makes a post-exercise hypotension mechanism unlikely. In addition, such an effect would presumably have been noticeable as soon as exercise training was initiated and not only after three weeks of training. It would thus appear that our intensive short-term endurance training protocol was sufficient to induce a resting change in peripheral resistance. Such changes have been related to changes in cardiopulmonary baroreflex function in both healthy and hypertensive individuals (Kingwell et al., 1992) as well as to changes in insulin sensitivity (Doering et al., 1998). These functions were however not measured in the present study. A clear explanation for our observation can thus not be provided.

Effect of training on heart rate and heart rate variability.

Our results did not show any significant change in resting heart rate following training. A decrease in resting heart rate is generally expected after endurance training but this is normally reported after at least 8 weeks of training (Stein et al., 1999; Ishida & Okada, 1998, Al-Ani et al., 1996; Sheldahl et al., 1994) with training-induced bradycardia not being reported after short term training (Mier et al., 1997; Torii et al., 1992; Steinhaus et al., 1990). Similarly, effects of training on either time-domain or frequency domain indices of HRV were not seen after 4 wks of training. On the other hand, the present results indicate resting HRV and RSA to be highly reproducible from one week to the next given similar breathing and heart rate characteristics. Indeed, for most indices of HRV or RSA we found correlations coefficients > 0.90 from one week to the next with no change in tidal volume, total breath duration or heart rate.

There exist only a limited number of longitudinal studies examining the influence of training using HRV analysis. Results from these studies generally indicate increases in total heart rate variability which may be ascribed to an increase in the HF or vagal component (Shi et al., 1995; Levy et al., 1998; Bonaduce et al., 1998; Yamamoto et al., 2000) although an absence of change in the vagal spectral component of HRV has also been reported (Ishida & Okada, 1998; Schuit et al., 1999; Loimaala et al., 2000). Little correlation if any was found between the change in VO_2 max following

training and the index of HRV. Similarly, our results indicate no significant change in either of the characteristics of RSA, the specific breathing-related influence of HRV mediated by transient inhibitions of the cardiac vagal activity. A slight increase in the second week of exercise was seen in the frequency domain HRV and RSA amplitude; however this increased did not reach a significant difference. There exist to our knowledge only a handful of studies examining the influence of exercise on respiratory sinus arrhythmia (Benedito et al., 1985; Kenney, 1985). Benedito et al. (1985) examined RSA amplitude in athletes and non-athletes as well as before and after endurance training and did not find any significant difference. More recently, Yamamoto et al. (2000) found a significant increase SDRR and HF in 7 untrained 22 ± 1 year old after the first week which then remained unchanged until the end of the training program some 5 weeks later.

The discrepancy in finding may be related to a difference in the age of the subjects. It has been previously suggested that endurance training results in a more modest bradycardia in middle-age and older individuals compared to younger subjects (Levy et al., 1998) and a review of the literature on the exercise training interactions with HRV indicate only three out of the eight studies of in older population (38-67 yrs old) to show significant changes as a result of training (Levy et al., 1998; Stein et al., 1999; Seals & Chase, 1989). We therefore examined whether pre and post-training differences in resting heart rate and HRV would be seen in the present study

if we separated subjects according to age. Results from subjects aged between 25 and 35 yrs (N=14) were therefore examined separately before and after training. Result still indicated no pre and post training difference in heart rate or in total power or the HF or vagal component of HRV. The breathing pattern is also known to affect the amplitude of RSA and thus total HRV (Brown et al., 1993; Haggenmiller et al., 1996) results are in agreement with this, showing a significant relationship between the amplitude of RSA and breathing frequency ($r= 0.40$; $p< 0.05$). Comparisons of the slopes of this relationship before and after the 4 weeks of endurance training indicate an upward shift in the response curve suggesting a greater gain after training. Statistical analysis on these slopes did however not reach statistical significance.

Differences in HRV between athletic and non-athletic populations have generally been found concurrent with differences in maximal aerobic capacity and/or in resting heart rate. Investigations of HRV in healthy active populations including runners, cyclists, and swimmers generally indicate a greater HF component of HRV in athletes versus non-athletes (Yataco et al., 1997; Ishida & Okada, 1997) with a correlation coefficient of 0.79 and 0.70 being measured between VO_2max and total power or the HF component frequency of HRV, respectively. Similarly, a correlation coefficient of 0.46 may be calculated between the change in resting HR and the change in the HF component of HRV (Yataco et al; 1997, Jensen-Urstad et al., 1997 and

Ishida & Okada, 1997. Nonetheless, changes in HRV have also been reported in the absence of changes in resting heart rate (Stein et al., 1999). The significance of these changes remains to be clearly established from both a health-related perspective and a physiological mechanistic perspective. The interest in determining HRV has been mainly inspired by clinical evidence showing a relationship between reduced total heart rate variability and/or the HF spectral power of HRV and an increased risk of arrhythmic event and sudden death (Bigger et al., 1992; Farrell et al., 1991; Malik et al., 1996).

As seen by the decrease in arterial blood pressure, the present findings suggest that health-related benefits of endurance training may occur without changes in HRV. Thus, while a reduced HRV may signal an adverse health prognosis, an increase in HRV in healthy individuals is not required for health-related benefits. The physiological significance of endurance training-related changes in the HF component of HRV also needs to be carefully considered. It is quite clear that changes in the HF component of HR may be seen simply as the result of changing breathing rate while the heart rate and thus presumably the tonic vagal activity remains unchanged (Calabrese et al., 2000) Thus, an increase in the HF band of HRV while reflecting greater cardiac vagal modulatory influences is not a measure of cardiac vagal tonic activity. The possibility of concurrent changes in the intrinsic rate of the sinus node, in the tonic vagal influences as well as in modulatory influences must all be recognized as part of the HF band. On the other hand, considering that RSA amplitude in the present study is standardized for the resting heart rate,

it may be interpreted as a "pure" index of respiratory-induced vagal modulations independent of tonic influences. Inasmuch, our results suggest a trend towards an increased gain in the respiratory-induced modulation of heart rate by breathing after training although at the spontaneous breathing rate, pre and post-training differences were not statistically significant.

In conclusion, results from the present study suggest that while four weeks of intensive endurance training may significantly increase maximal aerobic power and provide some health related benefit such as a decrease in diastolic pressure, concurrent changes in resting heart rate or its modulation are not observed. This suggests that resting heart rate adaptation to training may require longer duration for it to occur, although an absence of change in resting heart rate or HRV has also been reported after 22 and 27 weeks of training in 46-68 years old healthy subjects. It is also possible that autonomic adaptations of the sinus node as a result of endurance training may be limited in older individuals. Future studies are needed to examine the significance of adaptations in heart rate and its modulation in response to endurance training in healthy sedentary middle-age subjects.

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