Sympathovagal Influences on Heart Rate and Blood Pressure Variability in Highly Trained Endurance Athletes.

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the Degree of Master of Arts.

Department Of Physical Education
Faculty Of Education
McGill University
Montreal, Quebec, Canada
July, 1996
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ACKNOWLEDGMENTS

First, I would like to thank all the subjects which volunteered in this study for their time and cooperation. I would also like to thank the staff at Sacré-Coeur hospital for the use of the equipment and for any technical and scheduling help that was given. I am especially grateful to Dominique and Martin for all their time and effort in teaching me how to use the equipment.

To my thesis advisor, Dr. Helene Perrault, thank you for accepting me to undertake this project and for all your time, effort, and guidance throughout the completion of this thesis. To my parents, brothers, and grandparents for their constant support and encouragement throughout my M.A. program, thank you. Also, my sincerest appreciation to Pat for all her support and understanding throughout my final year. Finally, a special thank you to Paul for his time and continual support, but most of all for his infinite patience without which none of this would have been possible, THANK YOU!
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ABSTRACT

The evidence for an increase in resting vagal tone to explain the lower heart rate of endurance athletes remains controversial. This study used spectral analysis of heart rate (HRV) and blood pressure (BPV) variability to examine the vagal and sympathovagal influences on the sinus node in 12 endurance-trained athletes (A) and 10 age and sex-matched control subjects (C) (age: 26(1.2) yrs; VO2max: A: 68.2(2.1) vs C: 41.4(2.0) ml/kg/min; p<0.05). Continuous ECG and BP recordings were obtained during supine rest, sitting, controlled respiration (12 breaths/min), standing, exercise at heart rates of 100 and 130 beats/min, and after 5 and 15 minutes of seated recovery. No differences were observed between A and C in the vagal or high frequency (HF) components (48.0(4.0) vs 44.2(6.9) nu), in the low frequency (LF) components (55.8(6.9) vs 52.0(4.0) nu), or in the LF/HF ratios (1.72(0.4) vs 1.22(0.2)) of the HRV spectral components at rest or other experimental conditions, despite the lower resting heart rates of the A (53.1(1.8) vs 65.1(2.1) beats/min; p<0.05). Standing and exercise decreased the HF components and increased the LF/HF ratios similarly in both the A and C, controlled respiration induced similar rises in the HF component of HRV. Despite a significantly higher systolic BP in athletes, no difference was observed between A and C in the HF or LF components of either systolic or diastolic BPV. Exercise induced an increase in the HF component of BPV which was associated with the rise in breathing frequency. These results indicate a similar sinoatrial sympathovagal balance in A and C suggesting that training-induced
bradycardia could result from an adaptation in the intrinsic sinoatrial properties.
Also, endurance training does not appear to influence the beat-to-beat BPV.
RÉSUMÉ

L'importance d'une modification du tonus vagal pour expliquer la fréquence cardiaque (FC) basse de l'athlète d'endurance (A) au repos demeure controversée. L'analyse spectrale de l'intervalle R-R de l'ECG ou des variations cycle-à-cycle de la pression artérielle (PA) permet de quantifier les influences vagale (Haute Fréquence: HF; 0.16-0.5 Hz), sympathique (Basse Fréquence: BF; 0.05-0.15 Hz) ou le rapport sympatho-vagal (BF/HF) dans le contrôle de la FC et de la PA. Un ECG et un enregistrement de pression continu (Finapress) ont été obtenus chez 12 A et 10 témoins (T) en position allongée (20 min), en orthostation (10 min) et pendant un effort sur ergocycle à une FC stable de 130 bpm. Pour chaque condition, l'analyse spectrale de 512 intervalles R-R et cycles de PA successifs a été effectuée et les composantes HF et BF ont été déterminées. Malgré une FC basale inférieure chez les A (53 vs 65 bpm; p<0.05), l'analyse spectrale témoigne d'un même BFIHF basal chez les A et les T (1.72 vs 1.22) et de réponses similaires à l'orthostation (A:. 5.72 vs T: 6.31) et à l'effort (A:2.48 vs T:2.65). Une diminution de la composante HF de la variabilité sinusale est observée en orthostation (A:10.83 vs T: 10.77) ainsi qu'à l'effort (A: 25.6 vs T: 25.0) par rapport à la situation basale (A:33.7 vs T:36.2). Malgré une pression artérielle systolique plus élevée chez les A, aucune différence n'a été observée entre les groupes pour les composantes HF et BF de la variabilité cycle-à-cycle de la PA. Une augmentation de la composante HF de la variabilité de la PA systolique en réponse à l'effort qui semble tenir à l'augmentation de la fréquence
respiratoire. Aucune variation de la variabilité de la PA diastolique n'est observée en réponse à l'orthostatisme ou à l'exercice. Ces résultats indiquent des influences vagales identiques chez les A et les T ce qui laisse croire qu'une diminution du rythme sinusal intrinsèque pourrait donc être à l'origine de la bradycardie de l'athlète.
PART I: REVIEW OF RELATED LITERATURE
1.0 Training-Induced Bradycardia: Description of the Phenomenon

Chronic exercise training is responsible for many cardiovascular adaptations. One of the earliest documented adaptations to endurance training is a decrease in heart rate both at rest and during submaximal exercise for any given absolute workload. Using 202 athletes of the 1928 Olympic games, Bramwell and Ellis (1929) demonstrated mean resting heart rates of 66, 63, and 58 beats/min for sprinters, middle distance runners, and marathon runners, respectively. Weight lifters of approximately the same age (22-27 yrs) averaged a resting heart rate of 80 beats/min. Also from the same Olympiad, Hoogerwerf (1929) measured the resting heart rates of 198 contestants using electrocardiography and found an average of 50 beats/min with some marathon runners and cross-country skiers showing values between 30 and 40 beats/min.

Since these early reports, numerous cross-sectional and longitudinal studies have confirmed this observation leading to the common use of the term "training-induced bradycardia". Table 1 summarizes a few cross-sectional studies which have compared resting heart rates in endurance-trained individuals with those of non-trained controls. Athletes involved in endurance type activities such as running, cycling, and soccer consistently demonstrate resting heart rates in the range of 8-17 beats/min (12.9-24.4%) lower than non-trained individuals (Jost et al., 1989; Lewis et al., 1980; Maciel et al., 1985; Mathur & Igbokwe 1988; Smith et al., 1989). From Table 1, it can be seen that the average resting heart rate of mainly endurance athletes (54.2 beats/min) was 20.3% lower than that seen for
Table 1. Comparisons of resting heart rate and \( \text{VO}_2\text{max} \) between endurance athletes and non-athletes.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects</th>
<th>Mean age (yrs)</th>
<th>Resting HR (bpm)</th>
<th>HR (%)</th>
<th>( \text{VO}_2\text{max} ) ml/kg/min</th>
<th>( \text{VO}_2\text{max} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jost et al. (1989)</td>
<td>Sedentary Runners</td>
<td>24.8</td>
<td>62</td>
<td>50.5</td>
<td>73.5</td>
<td>+45.5</td>
</tr>
<tr>
<td></td>
<td>Runners</td>
<td>25.5</td>
<td>54</td>
<td>-12.9</td>
<td>42.8</td>
<td>+52.0</td>
</tr>
<tr>
<td>Smith et al. (1989)</td>
<td>Sedentary Runners</td>
<td>27</td>
<td>70.2</td>
<td>44.8</td>
<td>65.4</td>
<td>+70.0</td>
</tr>
<tr>
<td></td>
<td>Runners</td>
<td>25</td>
<td>54.7</td>
<td>-22.2</td>
<td>76.2</td>
<td>+70.0</td>
</tr>
<tr>
<td>Lewis et al. (1980)</td>
<td>Sedentary Elite cyclist</td>
<td>26</td>
<td>70</td>
<td>44.8</td>
<td>76.2</td>
<td>+70.0</td>
</tr>
<tr>
<td></td>
<td>Runners</td>
<td>21</td>
<td>53</td>
<td>-24.4</td>
<td>76.2</td>
<td>+70.0</td>
</tr>
<tr>
<td>Maciel et al. (1985)</td>
<td>Sedentary Runners</td>
<td>29</td>
<td>70</td>
<td>39.4</td>
<td>53.8</td>
<td>+36.5</td>
</tr>
<tr>
<td>Mathur &amp; Igbokwe (1988)</td>
<td>Sedentary Soccer players</td>
<td>21.7</td>
<td>73.8</td>
<td>35.6</td>
<td>70.9</td>
<td>+71.1</td>
</tr>
<tr>
<td>Katona et al. (1968)</td>
<td>Sedentary Elite rowers</td>
<td>21.9</td>
<td>63</td>
<td>35.6</td>
<td>70.9</td>
<td>+71.1</td>
</tr>
<tr>
<td>Mean for Sedentary</td>
<td></td>
<td>25.1</td>
<td>68.2</td>
<td>42.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean for Athletes</td>
<td></td>
<td>22.7</td>
<td>54.2</td>
<td>66.0</td>
<td>+55.2</td>
<td></td>
</tr>
</tbody>
</table>

\( \% \) = percentage difference from sedentary subjects; HR = heart rate.
sedentary subjects (68.2 beats/min). Additionally, during submaximal exercise, endurance trained athletes consistently display lower heart rates than non-trained individuals for any given absolute workload, with the differences in heart rates being similar to those seen at rest (Dixon et al., 1992; Lewis et al., 1980). When National athletes from a non-endurance type sport, such as weight-lifting were examined, resting heart rates were either not different (Longhurst et al., 1980; Manapace et al., 1982; Pearson et al., 1986) or greater than (74 ±3.1 vs 62 ±5.0 beats/min, mean ± SD) those of non-trained individuals (Jost et al., 1989), suggesting that aerobic type exercise training may be necessary to produce resting bradycardia.

Findings from cross-sectional comparisons of endurance athletes and non-athletes, however, cannot be taken to ascertain that their lower resting and submaximal heart rates are the direct consequences of their chronic exercise training regime. For this reason, longitudinal studies have also been performed in animals and humans to determine if training-induced bradycardia could be achieved following an endurance exercise training program. In rats, reductions in resting heart rates of 16 to 28 beats/min have been observed following 10-16 weeks of treadmill running (Barnard et al., 1976; Hughson et al., 1976; Schaefer et al., 1992) and 10 weeks of swimming (Lin & Horvath, 1972). Similarly, Ordway et al. (1982) and Marsland (1968) reported decreases in resting heart rate from 64 ±11.8 to 51 ±3.2 beats/min (±SD), and from 42 to 29 beats/min following 6 weeks of treadmill running in dogs and 16 weeks of running in
Standardbred horses, respectively. In humans, endurance training programs consisting of either running (Ekblom et al., 1973; Pollock et al., 1977; Wilmore et al., 1970), cycling (Krzeminski et al., 1989; Maciel et al., 1985), or walking (Williams et al., 1981) have typically produced reductions in resting heart rate ranging from 4-13 beats/min (5.8-17.3%) following training of 5 to 20 weeks of training. Similarly, lower heart rates during submaximal exercise are also observed following an endurance training program (Ekblom et al., 1973; Frick et al., 1967; Krzeminski et al., 1989).

Discrepancies in the magnitude of heart rate difference between endurance athletes and non-athletes (8-17 beats/min) and in individuals following training programs (4-13 beats/min) may result from differences in the frequency and total duration of the exercise training. In most training programs, the total duration ranges from a few weeks to several months, while in elite athletes the training has been ongoing for several years or even decades. In addition, traditional training programs use a frequency of 3 or 4 times per week while athletes usually follow a daily training routine. Unfortunately, no studies examining the time course of resting heart rate training adaptations were found. Longitudinal studies compared pre- and post-training resting heart rates, but did not measure daily or weekly changes, hence, the exact duration of endurance training needed to induce resting bradycardia is difficult to assess.

In general, it has been observed that improvements in cardiorespiratory fitness occur when endurance type exercises, such as running, cycling, or skiing, are performed 3-4 times per week for 30-45 min per session at an intensity level
greater than 60% VO$_2$max (Brynteson & Sinning, 1973; Fox et al., 1975; Saltin et al., 1968; Sharkey, 1970). With this intensity and frequency of training, decreases in resting heart rate have been demonstrated in young adults (22-32 yrs old) in as little as 5 to 6 weeks (Ekblom et al., 1973; Williams et al., 1981). However, the intensity and duration needed to produce adaptations in resting heart rate may vary depending upon the age and level of fitness of the individuals. Several studies, using training programs of a lower intensity carried out over a longer period of time (10-32 weeks), have reported decreases of 3 to 11 beats/min (4.6-13.4%) in the resting heart rates of middle-aged (33-53 yrs old) sedentary individuals (Hartley et al., 1969; Ismail et al., 1973; Oscai et al., 1968; Pollock et al., 1971; Pollock et al., 1969; Skinner et al., 1964; Wilmore et al., 1970). The magnitude of bradycardia which occurs with training may also vary with age. Table 2 demonstrates heart rate adaptations to aerobic training programs in a number of selected studies with groups of varying ages. Results indicate that groups with a mean age < 35 years showed an average decrease in resting heart rate of 13.4% following training, whereas groups with a mean age > 35 years displayed an average decrease of only 7.0%. However, some studies which have compared the heart rate response to endurance training between younger (18-32 yrs) and older individuals (50-82 yrs) observed no difference in
Table 2. Changes in resting heart rate and VO$_2$ max following endurance training programs.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Mean Age (yrs)</th>
<th>Training (wks)</th>
<th>Resting HR (beats/min)</th>
<th>VO$_2$max (ml/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Ekblom et al. (1973)</td>
<td>26</td>
<td>5</td>
<td>75</td>
<td>62</td>
</tr>
<tr>
<td>Maciel et al. (1985)</td>
<td>29</td>
<td>10</td>
<td>69</td>
<td>58</td>
</tr>
<tr>
<td>Pollock et al. (1969)</td>
<td>33</td>
<td>20</td>
<td>68</td>
<td>61</td>
</tr>
<tr>
<td>Pollock et al. (1977)</td>
<td>35</td>
<td>20</td>
<td>67</td>
<td>59</td>
</tr>
<tr>
<td>Oscai et al. (1968)</td>
<td>37</td>
<td>20</td>
<td>64</td>
<td>58</td>
</tr>
<tr>
<td>Ismail et al. (1973)</td>
<td>40</td>
<td>32</td>
<td>66</td>
<td>61</td>
</tr>
<tr>
<td>Wilmore et al. (1970)</td>
<td>41</td>
<td>10</td>
<td>69</td>
<td>65</td>
</tr>
<tr>
<td>Hartley et al. (1969)</td>
<td>47</td>
<td>10</td>
<td>66</td>
<td>61</td>
</tr>
<tr>
<td>Pollock et al. (1971)</td>
<td>49</td>
<td>20</td>
<td>65</td>
<td>62</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>68.8</td>
<td>61.7</td>
</tr>
</tbody>
</table>

* L/min; HR = heart rate; %Δ = percentage change between pre and post values.
the level of bradycardia achieved (Hagberg et al., 1985; Seals et al., 1994; Stratton et al., 1994). Even though no change in resting heart rate has been observed with aging in humans (Conway et al., 1971; Fleg et al., 1990; Kostis et al., 1982; Lakatta, 1993; Seals, 1993; Smith et al., 1987), the response to submaximal dynamic exercise has consistently shown a 11-29% smaller tachycardia in older individuals as compared with young control subjects in response to the same relative work loads (Hagberg et al., 1985; Lakatta, 1993; Stratton et al., 1992; Stratton et al., 1994). The attenuated cardiac response to exercise may result from a lesser vagal withdrawal (Lakatta, 1993; Shannon et al., 1987) or a reduced β-adrenergic responsiveness (Conway et al., 1971; Stratton et al., 1992) which have been observed in older individuals compared with younger control subjects in response to dynamic exercise. Overall, it appears that the older individuals can achieve a training-induced bradycardia, however, it remains unclear whether the degree of this bradycardia is comparable to that seen in younger subjects.

All of the studies commonly used to describe the phenomenon of training-induced bradycardia have also documented concurrent increases in VO₂max in the range of 10 to 35% (Table 1). It is uncertain whether or not increases in VO₂max are necessary to elicit bradycardia in response to endurance training. Evidence that training bradycardia is unrelated to VO₂max changes is seen in studies examining the effects of detraining. Ehsani et al. (1978) observed endurance runners after 3 weeks of detraining while Pavlik et al. (1986)
observed cyclists and endurance runners following 8 weeks of detraining. Both authors observed significant decreases in VO₂max within 21-30 days, whereas resting heart rate remained unchanged. Since bradycardia is retained early in the detraining period whereas VO₂max decreases, it would seem that training-induced bradycardia is independent of some of the adaptations associated with VO₂max.

In addition to the increase in maximal aerobic capacity, an increase in the plasma volume (Green et al., 1991a, 1991b; Green et al., 1990; Convertino, 1987; Convertino et al., 1980) may also play an important role in cardiovascular adaptations to prolonged endurance training. Green et al. (1991a), using 10-12 days of cycling (2 hrs/day, 60% VO₂max), reported a significant increase in plasma volume (9.6%), as well as a 10.3% decrease in resting heart rate. However, using a 3 day cycling program (2 hrs/day, 65% VO₂max), Green et al. (1990) reported a 20% increase in plasma volume and an increase in stroke volume, while resting heart rate was unchanged, suggesting that adjustments in plasma volume and stroke volume may occur rapidly with the onset of exercise training, prior to any heart rate adaptations. The elevated stroke volume is suggested to result from an increase in the end diastolic volume and the Frank-Starling mechanism (Green et al., 1989; Green et al., 1990; Stratton et al., 1994). A persistent increase in the end diastolic volume could potentially contribute to training bradycardia by altering intrinsic myocardial frequency by stretching the atrial pacemaker cells. It is still uncertain whether or not the
increased plasma volume and end diastolic volume contribute to the resting bradycardia induced by endurance training.

Studies which have examined the heart rate effects of non-aerobic training programs, such as weight lifting, have shown less consistent results. Several authors have observed no change in resting heart rate following 6-20 weeks of weight training (Lusiani et al., 1986; Harris & Holly, 1987; Ricci et al., 1982; Stone et al., 1983a). These findings support those of the cross-sectional studies which observed similar resting heart rates between national level weight lifters and non-trained individuals. Some authors, however, have observed decreases in resting heart rate of 10-12.7% following weight training programs (Kanakis & Hickson, 1980; Kusinitz & Keeney, 1958; Stone et al., 1983b). These inconsistencies may be due to the intensity, frequency, duration, and amount of muscle mass used in the training programs.

The existence of training-induced bradycardia has been documented for several decades. From the studies that have been performed it appears that the intensity, duration, frequency and type of training program are important determinants of this phenomenon. As well, this adaptation occurs at any age, but the magnitude of change may be less in older individuals. Although training bradycardia has been investigated for many years, the exact mechanisms responsible are still unclear.
Heart rate is regulated by a number of intrinsic and extrinsic mechanisms. The intrinsic cardiac frequency is determined by the depolarization rate of spontaneously firing pacemaker cells of the sinoatrial node of the right atrium and is independent of neural influences. In humans, the intrinsic firing rate of the sinoatrial node is approximately 100 beats/minute (Sherwood, 1993). This rate may however be changed according to the importance of sympathetic and parasympathetic neural inputs. Sympathetic and parasympathetic nervous system activity to the sinoatrial node alters heart rate by increasing and decreasing the rates of cell depolarization, respectively. The control of heart rate is mainly attributed to changes in the rate of diastolic depolarization of the pacemaker cells: vagal stimulation decreasing the rate of depolarization, hence heart rate, while β-adrenergic stimulation causes an increase in this rate. At rest, parasympathetic influences to the heart is predominant, resulting in a normal resting heart rate of approximately 70 beats/minute (Berne & Levy, 1992).

2.0 Experimental Evidence for Mechanisms of Training-Induced Bradycardia

Training bradycardia may be produced by decreasing sympathetic nervous system activity, increasing parasympathetic nervous system activity, lowering intrinsic heart rate, or through a combination of any of these. In humans, investigators have used several approaches to investigate differences in heart rate and its control between trained and non-trained individuals. One of the most widely used approaches is pharmacological blockade, which involves
selectively blocking β-adrenergic or cholinergic receptors of the sympathetic and parasympathetic nervous system respectively, to determine their respective influence over heart rate. A second approach is a non-invasive electrocardiological method that examines the variability in successive R-R intervals from the electrocardiogram (ECG) recordings in order to assess the relative contributions of parasympathetic and sympathetic outflow to the sinus node. A less common approach, used only in animals, is a surgical removal of all autonomic nervous system activity from the heart, to assess intrinsic heart rate.

2.1 Evidence from the Surgical Approach

Various surgical methods have been used to examine whether alterations in intrinsic heart rate play a role in producing training-induced bradycardia. The intrinsic heart rate is defined as the rate of discharge of the sinus node pacemaker cells in the right atrium in the absence of parasympathetic and sympathetic influences. Three methods have been used to investigate intrinsic heart rate including total surgical sympathectomy and vagotomy (Ordway et al., 1982; Sigvardsson et al., 1977), isolation of whole heart (Tipton 1968; Tipton et al., 1977), and isolation of atrial tissue (Bolter et al., 1973; Schaefer et al., 1992). The studies using isolated hearts and atria necessitate cross-sectional designs, comparing the intrinsic heart rates of trained animals with those of non-trained, because the procedures are irreversible.
Ordway et al. (1982) surgically removed all of the afferent and efferent autonomic innervation to the hearts of dogs by cutting the nerves coursing to the heart via the great vessels. Three to five weeks following denervation, the dogs were treadmill-trained for six weeks (1 hr/day, 5 days/wk). No difference was observed between pre- and post-training heart rates (95 ± 3.5 vs 96 ± 5.3 beats/min; ± SD). A sham-operated group, however, did show significant resting bradycardia (65 ± 4.8 vs 51 ± 3.2 beats/min) following training. Sigvardsson et al. (1977) used chemical sympathectomy (6-Hydroxy-dopamine) and surgical vagotomy to achieve cardiac denervation in rats that had been previously trained for 12 weeks on a treadmill (1 hr/day, 5 days/wk). Trained rats displayed significantly lower resting heart rates following denervation compared with non-trained controls, suggesting a decrease in intrinsic heart rate. These authors also trained a group of rats which were sympathectomized prior to training, and observed no bradycardia compared with controls. This finding is similar to that shown by Ordway et al. and suggests that an intact autonomic influence is essential throughout the training period to produce resting bradycardia.

Tipton (1968) and Tipton et al. (1977) assessed intrinsic heart rate by surgically removing whole hearts from rats and placing them in a Krebs-Henseleit (95% O₂, 5% CO₂, NaCl, KCl, NaHCO₃, CaCl₂, MgSO₄, KH₂PO₄, and glucose) solution to measure heart contraction frequency following 10-12 weeks of treadmill running (1-1½ hrs/day, 5 days/wk). Isolated hearts from trained rats showed no difference in beating frequency when compared with those of non-
trained rats, suggesting that intrinsic heart rate was not altered by training. On the other hand, Schaefer et al. (1992) and Bolter et al. (1973) using a similar Krebs-Henseleit solution isolated the atrial tissue of rat hearts to measure the intrinsic heart rate. The training programs consisted of running on a treadmill (2 hrs/day, 5 days/wk) for 12 weeks (Schaefer et al., 1992) and swimming (1 hr/day, 5 days/wk) for 15 weeks (Bolter et al., 1973). In both studies, significantly lower atrial contraction frequencies were observed in trained compared with non-trained rats, indicating that intrinsic heart rate per se, i.e. independent of autonomic influences, was decreased with endurance training.

From these studies, therefore, it is not clear to what extent training-induced bradycardia can be explained by a change in intrinsic heart rate. Differences in the experimental methods used to measure intrinsic heart rate, or in the training programs employed could contribute to the discrepancies in the findings. One problem with the "in situ" denervation method to assess intrinsic heart rate is that humoral factors are not eliminated and therefore, cannot be controlled. With isolation studies, confounding humoral factors are eliminated, however the influence of local paracrine and autocrine mechanisms cannot be overlooked (Dzau, 1992). In addition, differences in the type and method of tissue preparation may account for discrepancies in the results.
2.2 Evidence from the Pharmacological Blockade Approach

By blocking a branch of the autonomic nervous system with a pharmacological agent, the influence of that branch over heart rate can be assessed by comparing the pre- and post-blocking heart rates. Following administration of a muscarinic antagonist such as atropine, the influence of the parasympathetic or vagal influence on the sinus node can be calculated in beats per minute as the difference between the heart rates in the blocked and non-blocked conditions. Similarly, by administering a β-adrenergic blocker such as propranolol, sympathetic influences on the sinus node can be determined as the amount of cardiac deceleration from the non-blocked to the blocked condition. When both atropine and propranolol are given simultaneously, the resultant heart rate is assumed to be the intrinsic heart rate since all autonomic influence should have been removed (Jose, 1966; Jose & Taylor, 1969; Lin & Horvath, 1972; Nordenfelt, 1971). This pharmacological approach has been used in both animals and humans to investigate the mechanisms inherent to training bradycardia.

Most animal studies are cross-sectional because significant changes in heart rate occur over the span of the training period due to aging. Hughson et al. (1976), Barnard et al. (1976), Negrao et al. (1992), and Lin & Horvath (1972) administered atropine (atropine: 1-3 mg/kg or methylatropine: 3-5 mg/kg) and propranolol (4-8 mg/kg) to trained rats to examine the effect of training on intrinsic heart rate. Training programs consisted of either treadmill running for
10-13 weeks for 45-60 min/day, 5 days/wk (Hughson et al., 1976; Barnard et al., 1976; Negrao et al., 1992) or swimming for 10 weeks for 1 hr/day, 5 days/wk (Lin & Horvath, 1972). All training programs were sufficient to produce significant resting bradycardia. Following double blockade, all four studies showed significantly lower heart rates (eg. 312 vs 359 beats/min) in the trained rats compared with non-trained controls. The magnitude of decrease in the presumed intrinsic heart rate of trained rats ranged from 20-54 beats/min. In addition to double blockade, Barnard et al. (1976), Lin & Horvath (1972), and Negrao et al. (1992) also performed single blockades with atropine and/or propranolol to assess the relative contributions of parasympathetic and sympathetic activity on the sinus node, respectively, to training bradycardia. Lin & Horvath (1972) and Negrao et al. (1992) observed smaller cardiac accelerations in trained rats (89 ± 10.5 and 59 ± 5 beats/min, respectively) compared with non-trained (109 ±5.5 and 112 ±6 beats/min, respectively) following atropine injection. Similarly Tipton & Taylor (1965), using only a single blockade with atropine (1 mg/kg), observed a smaller cardiac acceleration in trained rats (41 beats/min) compared with non-trained (59 beats/min) following 6-9 weeks of treadmill training. The lower level of atropine-induced cardiac acceleration seen in these studies suggests that parasympathetic activity was decreased in the trained resting animals. Barnard et al. (1976), however, found no difference in the magnitude of cardiac acceleration between trained and non-
trained animals at rest, indicating that no alteration in the level of vagal tone on the sinus node had occurred with training.

The three studies which examined the influence of sympathetic activity through β-adrenergic blockade in producing training bradycardia (Barnard et al., 1976; Lin & Horvath, 1972; Negrao et al., 1992) all observed no significant difference in cardiac deceleration between trained and non-trained groups. Results for selective sympathetic blockade, therefore, concur that a change in the resting sympathetic influence on the sinus node does not contribute to resting bradycardia following training.

Because results from dual blockade suggest a reduction and not an increase in resting vagal tone after training, and because results for single sympathetic blockade do not show any significant changes in the sympathetic influence, it is logical from these observations to assume that training-induced bradycardia may also result from a change in intrinsic heart rate. A reduction in vagal activity would act to increase heart rate in trained animals, not decrease it, yet all the trained groups showed lower resting heart rates than control animals following endurance training. This suggests that the induced reduction in intrinsic heart rate may be sufficient to overcome any change in vagal tone to the sinus node and still result in a bradycardia.

The benefit of using animal models is that the experiments have a greater level of intrinsic control, since feeding, training, and other extraneous factors can be more tightly controlled. However, one problem with using animal models is
that the findings may not accurately represent what is occurring in humans because of species differences; rats for example, have resting heart rates which are 5-6 times those of humans. In addition, the sinus nodes of rats are under different relative sympathetic and parasympathetic influences at rest (Schaefer et al., 1992).

Pharmacological blockade studies in humans use the same approach as those done in animals. Most of the human studies performed have been cross-sectional, comparing athletes with non-athletes, presumably due to the extensive time requirement needed to conduct longitudinal training studies.

Cross-sectional studies: Using cross-sectional designs, Katona et al. (1982), Smith et al. (1989), Lewis et al. (1980), and Frick et al. (1967) compared sedentary individuals with elite rowers, marathon runners, elite cyclists, and endurance trained athletes from various sports, respectively. All four studies used a similar combined pharmacological blockade with atropine (0.03-0.04 mg/kg) and propranolol (0.1-0.25 mg/kg) to evaluate the difference in intrinsic heart rate between athletes and non-athletes at rest. In all of these studies, the heart rates following blockade were significantly lower in athletes compared with non-athletes. The range of decreases in these intrinsic heart rates for the athletic groups was 7 to 20 beats/min. Reasoning that the order of administration of the blocking agents could alter the overall heart rate responses, Katona et al. observed similar end-results in resting heart rates during double
blockade whether propranolol or atropine was given first. This observation was therefore taken to confirm the completeness of the autonomic blockade in both situations. In addition, both Katona et al. (1982) and Smith et al. (1989) performed single blockade experiments to evaluate parasympathetic and sympathetic nervous system influences on the sinus node under resting conditions. There were no differences in the extent of cardiac deceleration between athletes and non-athletes following propranolol administration in any of these studies. This indicates a similar level of sympathetic influence on the sinus node in athletes and non-athletes and may be taken to suggest that the observed lower heart rates of athletes are not related to a lower resting sympathetic influence on the sinus node.

When the parasympathetic influence was blocked through atropine administration, Smith et al. (1989) showed a significantly greater cardiac acceleration in athletes (45 beats/min) compared with non-athletes (38 beats/min), suggesting a greater resting vagal tone in the athletes. On the other hand, Raab et al. (1960) as well as Katona et al. (1982) also used atropine in similar dosages and observed no difference between athletes and non-athletes in the degree of cardiac acceleration. Considering these controversial findings it is surprising that a higher resting vagal tone in athletes is the commonly provided textbook explanation (Astrand & Rodahl, 1986; Clarke, 1975; Lamb, 1984; McArdle et al., 1991; Shephard, 1994) for the lower resting heart rates of endurance athletes.
A possible explanation for the inconsistency in vagal influence on the sinus node may be due to the nature of cross-sectional studies. By selecting a previously endurance trained athletic population, the exercise program is not controlled by the investigator and thus, subject to variability. Also, by comparing pre-selected athletic and sedentary groups, it is not known if differences are due solely to training or are inherent to the athletic population. By conducting longitudinal studies, in which sedentary individuals are trained over a certain length of time, any differences observed between the pre- and post-training periods are more likely due to that specific training program.

**Longitudinal studies:** Ekblom et al. (1973) trained subjects with cross-country running 5-6 times/wk for 5 weeks and Shi et al. (1995) trained subjects with a running program 4 times/wk for 8 months. Following double blockade with atropine (2-3 mg) and propranolol (1 mg) or metoprolol (0.05 mg/kg) no difference in heart rate was observed between the pre- and post-training conditions, indicating no change in intrinsic heart rate. Similarly, when propranolol or metoprolol was administered alone, no difference was seen between the pre- and post-training conditions in the level of cardiac deceleration. This suggests that the sympathetic influence on the sinus node was also unaltered following the training programs. In response to atropine, however, both Ekblom et al. (1973) and Shi et al. (1995) observed a significantly greater cardiac acceleration in the post-training condition (46 and 44 beats/min) compared with the pre-
trained (40 and 34 beats/min) respectively, indicating that the resting vagal influence on the sinus node was greater in subjects following training. On the contrary, Maciel et al. (1985) trained subjects by cycling for 25 min/day, 5 times/wk for 10 weeks and observed an 11 beats/min reduction in resting heart rate. Following the training period, results from single atropine blockade (0.04 mg/kg) showed no change in cardiac acceleration, suggesting that the vagal influence on the sinus node was unaffected by this training program.

Overall, results from both cross-sectional and longitudinal studies of humans, failed to observe any differences in the sympathetic responses to blockade between the trained and untrained groups, suggesting that the sympathetic influence on the sinus node does not play a role in producing resting training bradycardia. Results from combined parasympathetic and sympathetic blockade showed that the intrinsic heart rate is significantly lower in the endurance trained state. Ekblom et al. (1973) were the only authors who didn’t observe a decrease in the intrinsic heart rate following training, even though a significant resting training bradycardia had been achieved. This may be due to the relatively short training period of only 5 weeks as compared with the highly trained endurance athletes who trained on a regular basis. This short training duration may have been long enough to elicit changes in vagal tone to the sinus node, but not sufficient to induce changes in sinoatrial node pacemaker cells responsible for the intrinsic heart rate.
There are no consistent findings with regards to the effects of training on resting vagal tone. Some studies showed increased parasympathetic influence on the sinus node (Ekblom et al., 1973; Smith et al., 1989) in the trained state, while other studies showed no change (Katona et al., 1982; Maciel et al., 1985; Raab et al., 1960). The reason for this inconsistency is unclear. Blockades were similar in all studies, and were therefore, probably not a confounding factor. The level and type of training varied for all the studies and may account for some of the differences observed. An additional problem may be the approach used to assess autonomic activity. Pharmacological agents used in blockade studies may also exert non-selected effects on physiological functions such as reflex control mechanisms. For example, by blocking the parasympathetic branch of the autonomic nervous system with atropine, it is not certain if other physiological changes occur to counteract this block, such as a decrease in sympathetic activity. Also, some of the pharmacological agents used (i.e., atropine) cross the blood brain barrier and may exert central nervous system effects which differ between the trained and non-trained states (Longo, 1966; Meyers & Abreu, 1952).

2.3 Heart Rate Variability Analysis Approach

Heart rate variability can be examined through a non-invasive approach using the heart rate variability signal obtained from successive R-R intervals of a continuous ECG recording. Oscillations in R-R intervals result from normal
rhythmical variations associated primarily with respiratory sinus arrhythmia due to fluctuations in vagal efferent activity but also with sympathetic nervous activity (Eckberg, 1983; Katona et al., 1975; Pagani et al., 1986; Saul et al., 1991). The frequency power spectrum of the variation in successive R-R intervals is obtained through the application of a Fast Fourier Transform function or an autoregressive technique. The resulting power spectra contain three main peaks. The high frequency (HF) variations (>0.20 Hz) are produced by respiratory sinus arrhythmia and are essentially due to the rapidly varying vagal influence on the sinus node (Akselrod et al., 1981; Pomeranz et al., 1985). The low frequency (LF) variations (0.05 - 0.15 Hz) can be attributed to a combination of both sympathetic and parasympathetic influence on the sinus node (Akselrod et al., 1981; Cowan, 1995; Pomeranz et al., 1985; Sayers, 1973). An additional, very low frequency (VLF) variation (<0.04 Hz) has been attributed to peripheral vasomotor regulation (Saul et al., 1990; Stein et al., 1994), thermoregulation, and the renin-angiotensin system (Stein et al., 1994). Spectral components of the heart rate variability signal may be expressed in the following forms:

\[ \text{Power}_{\text{peak}} \; (\text{ms}^2 \cdot \text{Hz}^{-1} \text{ or } \text{bpm}^2 \cdot \text{Hz}^{-1}) , \; \text{Power}_{\text{area}} \; (\text{ms}^2 \text{ or } \text{bpm}^2) , \; \% \text{ of total power} \; (\text{ms}^2 / \text{total power} \; (\text{ms}^2)) , \; \text{or Normalized units} \; (\text{ms}^2 / \text{total power} \; (\text{ms}^2) - \text{VLF} \; (\text{ms}^2)) \].

Percentage of total power (%power) and normalized units (nu) take into account changes in overall variability by expressing the LF and HF components relative to the total power or the total power minus the VLF component, respectively.
2.3.1 Validation of the Heart Rate Variability Components

In order to validate the interpretation of the HF and LF components of R-R variation, selective blockade or enhancement of either the sympathetic or parasympathetic branches of the autonomic nervous system have been used to measure the R-R fluctuations and their power spectra. Parasympathetic blockade in animal studies have demonstrated that atropine decreases overall heart rate variability with reductions in both the LF and HF components (Ferrari et al., 1987; Jansen & Dellinger 1989; Zwiener et al., 1990). Likewise, in human studies, Hayano et al. (1991), Parati et al. (1987), and Tapp et al. (1990) all observed reductions in the total power (91, 65.3, and 41.1%, respectively) using atropine (23 µg/kg, 0.04 mg/kg, 2-4 mg, respectively), with decreases in both the LF and HF spectral components, indicating that the vagal influence on the sinus node is reflected in both these spectral frequencies. To enhance the vagal component of the heart rate variability, controlled respiration at frequencies in the physiological range may be utilized to produce an increase in the HF component. Several studies have shown that this HF respiratory peak can be utilized as a measure of vagal control (Alcalay et al., 1992; Katona et al., 1975). Katona et al. (1975) using bilateral cooling of the vagus nerve in anesthetized dogs observed that the respiratory variations in heart rate were eliminated suggesting that the HF component is due only to vagal activity. Alcalay et al. (1992) using a dose-dependent parasympathetic blockade with atropine (0.1 to 2.5 mg) demonstrated a 95% decrease in the respiration component suggesting that the
respiration peak can be used as a quantitative measure of vagal efferent influence on the sinus node. In addition, parasympathetic blockade with 1-4 mg of atropine in humans (Pomeranz et al., 1985; Selman et al., 1982; Tapp et al., 1990, respectively) or 0.01-0.02 mg/kg of glycopyrrolate in dogs (Akselrod et al., 1981; Akselrod et al., 1985) nearly abolished the HF or respiratory peak, decreasing it by 92% and 93% suggesting that this peak represents solely vagal activity (Selman et al., 1982; Pomeranz et al., 1985). Furthermore, the magnitude of the respiratory peak is reduced in a dose-dependent manner following atropine administration as shown by results from several studies in humans using administration of atropine in doses varying between 0.1 to 2.5 mg (Alcalay et al., 1992; Izraeli et al., 1991; Selman et al., 1982). These authors all observed dose-dependent reductions in the respiratory peak up to as much as 90-95%. It is interesting however that administration of low doses of atropine (0.05 to 1.0 mg, or < 5.2 μg/kg) have been seen to result in an increase in the respiration peak suggesting an increase in vagal stimulation to the sinus node (Alcalay et al., 1992; Izraeli et al., 1991; Kamath et al., 1993; Raczkoska et al., 1983). Authors have suggested that the increase in vagal stimulation to the SA node is the result of central vagal stimulation, while the diminished parasympathetic activity observed at higher doses results from the blockade of postsynaptic muscarinic receptors at the sinus node (Alcalay et al., 1992; Izraeli et al., 1991).
The contribution of sympathetic activity on the SA node is more difficult to isolate than the vagal activity during resting conditions. When total vagal blockade, with 0.1 mg/kg glycopyrrolate, was achieved in dogs (Akselrod et al., 1981; Akselrod et al., 1985) the residual LF component of the power spectrum could be eliminated using beta-sympathetic blockade of 0.1 mg/kg propranolol. Similarly, in rats, beta-blockade using atenolol (1.0 mg/kg) considerably reduced the LF fluctuations (Japundzic et al., 1990). In humans, however, several studies using beta-blockade produced either an increase, a decrease, or no effect on the heart rate variability spectral component. The total variance increased with β-adrenergic blockade (Coker et al., 1984; Cook et al., 1991) using atenolol (100 mg and 200 mg, respectively) or decreased (Coumel et al., 1991) using acebutolol (10 mg/kg) or did not change (Hayano et al., 1991; Hopt et al., 1995) using propranolol (0.2 mg/kg) and thoracic epidural anesthetic, respectively. It is difficult to compare these studies since differences in the effects of various adrenergic blockers on heart rate variability may result from their different specificities. Atenolol is mainly a β₁-selective adrenergic blocker which would inhibit mainly cardiac adrenergic receptors, whereas acebutolol has intrinsic sympathomimetic activity and is more selective for β₂-receptors. Propranolol, on the other hand, is a non-selective β-blocker and therefore antagonises β-adrenergic receptors in both the heart and the peripheral vasculature (Hoffman, 1995). Since the adrenergic receptors of the SA node are
mainly $\beta_1$-type receptors atenolol may inhibit the sympathetic activity to the sinus node to a greater extent than acebutolol and propranolol.

Although the LF spectral component does not represent solely sympathetic activity, the magnitude of the LF peak increases during states of enhanced sympathetic tone such as when changing from a supine to an upright tilt position. Pagani et al. (1986) observed a reduction in the HF component (26.3(4.3) to 11.4(2.8) nu, (SEM)) with a marked increase in the LF component (62.2(6.3) to 83.7(4.6) nu, (SEM)) of the power spectrum because the upright tilt position activates the sympathetic response in order to prevent orthostatic hypotension. A quantitative measure of sympathetic activity within the LF component is still difficult to assess since the LF band also reflects vagal influence. Pomeranz et al. (1985) observed that when respiration was controlled and atropine (0.03 mg/kg) was administered in a supine position the LF component decreased by 84%. These authors concluded that the LF component was mediated almost entirely by the parasympathetic system during supine rest and therefore, quantification of the sympathetic activity on the sinus node may be difficult in the supine position, due to the low levels of sympathetic tone (Pomeranz et al., 1985). Hence, a ratio of power between the low and high frequency bands (LF/HF) has been taken as a measure of sympathovagal balance on the sinus node (Pagani et al., 1986; Malliani et al., 1991). Pagani et al. (1986) using controlled respiration to enhance the vagal influence, observed a reduction in the LF/HF ratio from $2.5 \pm 0.3$ to $0.7 \pm 1.0$, thus suggesting that the
ratio of LF/HF power is a useful index of sympathovagal balance. In addition, postural changes which enhance sympathetic activity, such as passive upright tilt or standing, will greatly augment the LF/HF ratio when compared to supine rest (Hopt et al., 1995; Pagani et al., 1986). Pagani et al. (1986) using β-adrenergic blockade with propranolol (0.6 mg/kg) observed a greatly attenuated rise in the LF/HF ratio after blockade (1.74 ±0.31 to 3.78 ±0.93) compared with controls (3.62 ±1.23 to 20.79 ±3.68) in response to a 90° head up tilt. This indicates that whether the vagal or sympathetic activity is enhanced the LF/HF ratio will either decrease or increase, respectively, and therefore the LF/HF ratio is a good index of sympathovagal balance.

2.3.2 Applications of Heart Rate Variability in Normal Healthy Individuals

Many studies have used heart rate variability analysis in healthy normal subjects to characterize the control of heart rate. Since the mid 1980's over 50 studies have been published to describe the spectral function of the R-R interval under resting conditions and orthostatic challenges. In resting healthy subjects, power spectral analysis reveals two main frequency components: LF(0.05-0.15 Hz) and HF(0.16-0.5 Hz) components in R-R variability (Dixon et al., 1992; Furlan et al., 1993; Kamath et al., 1991; Pagani et al., 1986; Pagani et al., 1988; Perini et al., 1990). Orthostatic challenges such as postural changes from supine to upright tilt, standing or exercise are utilized in order to elicit changes in autonomic activity via the arterial baroreflex mechanisms. These reflexes should
modify the heart rate variability power spectra to one of sympathetic dominance (Kamath et al., 1993). A sample of some of these studies conducted in normal healthy subjects at rest, in response to an orthostatic challenge, and during dynamic exercise appear in Table 3. In most of these studies the subjects were volunteers, ranging in age from 16 to 64 years. In general subjects were submitted to an on line Lead II ECG recording of at least 256 cycles under specific experimental conditions for subsequent analysis of heart rate variability. Results of the heart rate variability analysis from these experimental conditions are shown in Table 3 as mean ± SD or mean(SEM). The R-R variance at rest is shown in Table 3A. The mean values ranged between 1222.0 and 6054 ms² in the supine position and between 8064.6 and 8221.2 ms² in the seated position. The highest R-R variances in the supine position were reported by Furlan et al., (1993) and appear to be due to age since these subjects were 16 years old as compared to 30-52 years reported by the other authors. The higher R-R variances seen in the seated subjects may be partially due to the younger age (21-22 yrs) groups selected, thus suggesting a higher R-R variance in younger individuals.
Table 3. Total R-R Variance and Heart Rate Variability Spectral Components in Normal Healthy Subjects during Rest, Tilt, and Exercise Conditions.

A) R-R Variance

<table>
<thead>
<tr>
<th>Authors</th>
<th>n</th>
<th>Age (yrs)</th>
<th>L/S</th>
<th>Rest (ms²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lombardi et al., (1987)</td>
<td>26</td>
<td>52 (3)</td>
<td>L</td>
<td>1222 (188)</td>
</tr>
<tr>
<td>Furlan et al., (1993)</td>
<td>29</td>
<td>16 (0.4)</td>
<td>L</td>
<td>6054 (1226)</td>
</tr>
<tr>
<td>Pagani et al., (1986)</td>
<td>30</td>
<td>30-45</td>
<td>L</td>
<td>2581 (356)</td>
</tr>
<tr>
<td>Pagani et al., (1988b)</td>
<td>40</td>
<td>38 (2)</td>
<td>L</td>
<td>2722 (300)</td>
</tr>
<tr>
<td>Yamamoto et al., (1991)</td>
<td>8</td>
<td>22 (3)</td>
<td>S</td>
<td>8221.2</td>
</tr>
<tr>
<td>Casadei et al., (1995)</td>
<td>11</td>
<td>21.1 (0.4)</td>
<td>S</td>
<td>8064.6 (2396)</td>
</tr>
</tbody>
</table>

L= lying; S= seated; Mean (SEM)

B) High Frequency Component of Heart Rate Variability Spectral Analysis

<table>
<thead>
<tr>
<th>Authors</th>
<th>n</th>
<th>Age (yrs)</th>
<th>Rest (nu)</th>
<th>Orthostatic stressor</th>
<th>Exercise (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayano et al., (1991)</td>
<td>15</td>
<td>21-24</td>
<td>L 58(4.6)</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Lombardi et al., (1987)</td>
<td>26</td>
<td>52(3)</td>
<td>L 35(3)</td>
<td>T 14 (2)</td>
<td>-60</td>
</tr>
<tr>
<td>Furlan et al., (1993)</td>
<td>29</td>
<td>16(0.4)</td>
<td>L 53.1(2.6)</td>
<td>T 21.5 (1.9)</td>
<td>-59.5</td>
</tr>
<tr>
<td>Pagani et al., (1986)</td>
<td>30</td>
<td>30-45</td>
<td>L 26.3(4.3)</td>
<td>T 11.4 (2.8)</td>
<td>-56.7</td>
</tr>
<tr>
<td>Pagani et al., (1988b)</td>
<td>40</td>
<td>38(2)</td>
<td>L 38(3.0)</td>
<td>T 10.0 (2)</td>
<td>-73.7</td>
</tr>
<tr>
<td>Dixon et al., (1992)</td>
<td>14</td>
<td>27(0.7)</td>
<td>L 43.7(6.0)</td>
<td>St 23.8 (3.0)</td>
<td>50¹ 21.8 (2.8)¹</td>
</tr>
<tr>
<td>Kamath et al., (1991)</td>
<td>19</td>
<td>20-32</td>
<td>L 43.7(2.4)</td>
<td>St 29.5 (2.8)</td>
<td>50¹ 31.2 (2.9)²</td>
</tr>
<tr>
<td>Yamamoto et al., (1991)</td>
<td>8</td>
<td>22(3)</td>
<td>S 4230(211)</td>
<td>——</td>
<td>20 855 (134)³</td>
</tr>
<tr>
<td>Arai et al., (1989)</td>
<td>36</td>
<td>15-64</td>
<td>S 2.99(0.1)</td>
<td>——</td>
<td>70 1.08 (0.03)²</td>
</tr>
<tr>
<td>Bernardi et al., (1990)</td>
<td>9</td>
<td>23.1(0.7)</td>
<td>S 44.0(4.0)</td>
<td>——</td>
<td>160 0.3 (0.0)²</td>
</tr>
<tr>
<td>Casadei et al., (1995)</td>
<td>11</td>
<td>21.1(0.4)</td>
<td>S 37.6</td>
<td>——</td>
<td>120 37.0 (6.3)</td>
</tr>
</tbody>
</table>

L= lying; S= seated; T= 90⁰ tilt; St= standing; ¹= bpm²•Hz⁻¹; ²= bpm²; ³= ms²; t= %VO₂max; * % change from rest; Mean (SEM)
Table 3. Total R-R Variance and Heart Rate Variability Spectral Components in Normal Healthy Subjects during Rest, Tilt, and Exercise Conditions.

C) Low Frequency Component of Heart Rate Variability Spectral Analysis

<table>
<thead>
<tr>
<th>Authors</th>
<th>n</th>
<th>Age</th>
<th>Rest (nu)</th>
<th>Orhtostatic stressor (nu)</th>
<th>Exercise (W) (nu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayano et al., (1991)</td>
<td>15</td>
<td>21-24</td>
<td>L 29 (4.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lombardi et al., (1987)</td>
<td>26</td>
<td>52(3)</td>
<td>L 53 (3)</td>
<td>T 78 (3)</td>
<td>+47.0</td>
</tr>
<tr>
<td>Furlan et al., (1993)</td>
<td>29</td>
<td>15 (0.4)</td>
<td>L 37.41 (2.9)</td>
<td>T 71.68 (2.7)</td>
<td>+91.6</td>
</tr>
<tr>
<td>Pagani et al., (1986)</td>
<td>30</td>
<td>30-45</td>
<td>L 62.2 (5.3)</td>
<td>T 83.7 (4.6)</td>
<td>+34.6</td>
</tr>
<tr>
<td>Pagani et al., (1988b)</td>
<td>40</td>
<td>38 (2)</td>
<td>L 50 (3)</td>
<td>T 83 (2)</td>
<td>+66</td>
</tr>
<tr>
<td>Dixon et al., (1992)</td>
<td>14</td>
<td>27(0.7)</td>
<td>L 69.6 (5.2)</td>
<td>St 82.9 (4.7)</td>
<td>+19 50 67.6 (5.8)</td>
</tr>
<tr>
<td>Kamath et al., (1991)</td>
<td>19</td>
<td>20-32</td>
<td>L 56.3 (2.4)</td>
<td>St 64.6 (4.3)</td>
<td>+15 50 63.8 (5.0)</td>
</tr>
<tr>
<td>Yamamoto et al., (1991)</td>
<td>8</td>
<td>22 (3)</td>
<td>S 2286(901)</td>
<td></td>
<td>20 467(216)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>185 5.57(2.1)</td>
</tr>
<tr>
<td>Arai et al., (1989)</td>
<td>36</td>
<td>15-64</td>
<td>S 12.0 (0.3)</td>
<td></td>
<td>70 4.47(0.08)</td>
</tr>
<tr>
<td>Bemardi et al., (1990)</td>
<td>9</td>
<td>23.1(0.7)</td>
<td>S 56.0(4.0)</td>
<td></td>
<td>160 0.55(0.02)</td>
</tr>
<tr>
<td>Casadei et al., (1995)</td>
<td>11</td>
<td>21.1(0.4)</td>
<td>S 53.1</td>
<td></td>
<td>120 11.1(5.8)</td>
</tr>
</tbody>
</table>

L= lying; S= seated; T= 90° tilt; St= standing; 1= bpm²*Hz⁻¹ ; 2= bpm²; 3= ms² ; t= %VO₂max; * % change from rest; Mean (SEM)
Results from power spectral analysis of heart rate variability with the HF and LF components (nu) are shown in Tables 3B and 3C, respectively. The power of the HF component ranges between 26.3 and 58.0 nu during supine rest and between 37.6 and 44.0 nu, and 2.99 bpm$^2$ and 4230 ms$^2$ (absolute units) during seated rest. The supine HF values appear to be influenced slightly by age with the higher HF components observed in the younger subjects. The seated values are difficult to compare since several different units were utilized. The LF components (Table 3C) range from 29.0 to 62.2 nu in the supine position and from 53.1 to 56.0 nu, and from 12.0 bpm$^2$ to 2286 ms$^2$ in the seated position. The results reported by Arai et al. (1989) during the seated condition appear puzzling since considerable higher absolute HF and LF values would be expected. The LF/HF ratios were similar in both the supine (0.5 and 3.7) and seated (0.5 and 4.0) conditions. As can be seen in Table 3B and 3C, only three authors (Furlan et al., 1993; Hayano et al., 1991; Yamamoto et al., 1991) observed greater power in the HF spectral components as compared with the LF component during supine and seated rest. The remaining authors all observed lower HF components as compared with the LF components. Overall, the majority of these studies reported lower HF components than LF spectral components during either supine or seated rest. As mentioned earlier, it has been shown that the vagal influence to the sinus node is predominant at rest. Since the LF component represents both sympathetic and parasympathetic activity it would appear that the higher resting levels of the LF component
observed in the above studies most likely represent more vagal activity than sympathetic activity to the SA node.

*Response to Orthostatic Stress Tests:* In response to a passive tilt, or a standing position, studies in healthy normal subjects have consistently demonstrated a decrease in the HF component with an increase in the LF component of the power spectrum in all individuals (Lombardi et al., 1987; Lindqvist et al., 1990; Pomeranz et al., 1985; Dixon et al., 1992; Guzzetti et al., 1988; Kamath et al., 1991; Fallen et al., 1988; Pagani et al., 1986; Furlan et al., 1993). Summaries of selected authors are presented in Tables 3B and 3C which have examined the HF and LF spectral components in response to an orthostatic challenge. All of these authors reported reductions in the HF component with large increases in the LF component from the resting condition. The range of decrease in the HF component from rest was from 56.7 to 73.7% during 90° passive upright tilt and from 32.5 to 45.5% during standing. The range of increase in the LF component from rest was between 34.6 to 91.6% during 90° passive upright tilt and 15 to 19% during standing. The LF/HF ratio increased by 5.8 to 10 times during tilt and by 0.5 to 2.0 times during standing compared with the LF/HF ratio at rest. The large increase in the LF/HF ratio indicates a significant shift towards greater sympathetic influence in the tilt position. Passive tilt appears to elicit a much greater sympathetic response than active standing, as evidenced by the larger increases in LF/HF ratios. This may be explained by
the fact that standing does not produce as great an orthostatic stress as passive
tilting because active leg muscle contractions during standing facilitate venous
return to maintain cardiac output and blood pressure.

Exercise: Tables 3B and 3C are summaries of the HF and LF spectral
components reported during rest and dynamic exercise. All the exercise
protocols were performed on a cycle ergometer at various intensities under
metabolic steady state conditions, thus at a constant heart rate. In the transition
from a seated or supine resting condition to an initial level of exercise (20-110
W) the HF component has been consistently shown to decrease (Arai et al.,
1989; Bernardi et al., 1990; Casadei et al., 1995; Dixon et al., 1992; Kamath et
al., 1991; Yamamoto et al., 1991). This finding is consistent with the theory of
vagal withdrawal during the onset of exercise. Arai et al. (1989), Yamamoto et
al. (1991), and Casadei et al. (1995) all observed decreases in the absolute LF
power (12.0 to 4.5 bpm², 2285.7 to 467.0 ms², and 2140.9 to 257.8 ms²,
respectively) between resting and exercising conditions. However, Casadei et
al. and Bernardi et al. reporting the LF component in normalized units observed
no significant differences in going from rest to initial exercise, be it 30W or 110W.
Dixon et al. (1992), and Kamath et al. (1991) also did not observe any significant
difference between the LF components in the resting and exercising conditions,
however, this may be due to the order of the experimental protocol since both
Dixon et al. and Kamath et al. had a standing condition prior to the exercise
phase. Considering that standing enhances the sympathetic activity to the sinus node, it can be assumed that in going from a standing to an exercising condition the LF component would already be enhanced and may have remained the same or actually decreased at the onset of exercise. In addition, the initial level of exercise was different in all the studies (20 to 110W). Therefore, conclusions concerning the LF trend between rest and exercise would have to be specific for the resting condition and exercise intensity level used. The LF/HF ratio, indicating the sympathovagal balance, has not been widely utilized. Dixon et al. (1992) and Arai et al. (1989) observed increases in this indicator between rest and exercise (1.6 to 3.2 and 4.8 to 6.7, respectively), however, the differences were not significant. Since few studies have used the LF/HF ratio, the trend of the spectral components of heart rate variability reflecting sympathovagal balance between resting and exercising conditions is still uncertain. Additional studies are needed to define the shifts in sympathovagal balance in going from rest to different positions.

Tables 3B and 3C show some studies which have evaluated the heart rate variability response during successive levels of increasing exercise intensities. The exercise intensities varied from 20W to 221W depending upon the study. During graded exercise, Casadei et al. (1995) and Bernardi et al. (1990) observed increases in the HF component (from 32.0 to 80.4 nu and from 37.0 to 88.9 nu) during graded exercise (37 watts/5 minutes and 30 Watts/5 minutes, respectively), suggesting that as exercise intensity progresses, vagal
influence is enhanced, contrary to what might be expected. On the other hand, Arai et al. (1989) and Yamamoto et al. (1991) both reported decreases in the HF component from 1.08 to 0.30 bpm² and from 855.1 to 1.06 ms², as the exercise intensity increased by 30W every 2 minutes and 2.0W every minute, respectively. These two authors however, did not use a steady state exercise protocol which is necessary when using the heart rate variability analysis technique. Furthermore these authors expressed their results in absolute units and therefore, as exercise intensity increases the R-R interval variability would be expected to decrease. The LF component decreased by 68-99% in all studies as exercise intensity increased from a low workload to 60-100% VO₂max (Arai et al., 1989; Bernardi et al., 1990; Casadei et al., 1995; Yamamoto et al., 1991). The observations of an increase in the HF component and a decrease in the LF component with increasing exercise intensity would lead to the unlikely conclusion of enhanced vagal tone during moderate to high levels of exercise. Casadei et al. (1995) addressed the difficulties associated with using heart rate variability during exercise. Their findings suggest that the observed variations in the LF/HF ratio are the result of confounding factors rather than a reflection of true physiological phenomena.

**Confounding Factors:**

One of the major reasons for the inconsistencies when examining the autonomic nervous system responses to exercise may be the units used to
express the power of the heart rate variability spectral components. The five studies which reported these results in absolute units (ms² or bpm²) may be misleading (Arai et al., 1989; Casadei et al., 1995; Kamath et al., 1991; Yamamoto et al., 1991). Since the R-R intervals and the total variance decrease progressively with increasing intensity of exercise, the absolute area under the LF and HF curves is also seen to decrease (Casadei et al., 1995). The use of absolute units (ms² or bpm²), therefore, does not give any indication of the relative contribution of the LF or HF curves to the total variation in heart rate. Normalized units [ms²/total power(ms²) - VLF(ms²)], on the other hand, reflect the relative distribution of the spectral power within the LF and HF bands. This can be shown by Casadei et al. (1995) who reported decreases from 201.9 to 128.2 ms² in the HF component using absolute values, whereas they showed increases from 32 to 80.4 nu using normalized units during progressive exercise. In fact, the use of normalized units is also a recommendation of the Task force committee of the European society of cardiology and the North American society of pacing and electrophysiology (1996).

There may also be some other methodological confounding factors. A proper sampling rate must be chosen since a low sampling rate may produce a jitter in the estimation of the R-wave reference point thereby altering the spectrum (Malik et al., 1996). In most studies the sampling rate error is fixed. For example, if the sampling rate is set at 250 Hz the program can localize a specific R-peak with a 4 ms accuracy. Therefore, with increasing exercise
intensity the R-R interval (ms) becomes increasingly smaller leading to an increase in the relative importance of the sampling error, thus becoming a greater contributor to the total R-R variance. In addition, discrepancies in the LF and HF responses to exercise, may result from the different exercise protocols used. Arai et al. (1989) and Yamamoto et al. (1992) used incremental exercise protocols in which the work loads were increased every 1-2 minutes and therefore, their subjects did not achieve steady state exercise at each stage. Steady state exercise is important since the heart rate variability technique requires stationary points in order to obtain frequency distributions because the heart rate variability is calculated as fluctuations around a stable baseline. Furthermore, it is difficult to make comparisons between studies which employ different protocols. For example, exercise levels varied from 20-110 W initially to 60%-100% \( V_O_2_{max} \) at peak exercise. Therefore, any comparisons made with the initial or peak exercise were not necessarily comparing the same level or intensities of exercise.

**Recovery:** The heart rate variability power spectra have also been examined by four authors at various stages of recovery. It would be expected from the previous understanding of vagal predominance at rest that vagal activity should reappear during the recovery from exercise phase. Three of these authors used short recovery time frames of 2-15 minutes (Arai et al., 1989; Bernardi et al., 1990; Dixon et al., 1992) while the fourth author (Furlan et al., 1993) examined
the heart rate variability power spectrum at 1, 24, and 48 hours of recovery. Arai et al. (1989) and Bernardi et al. (1990) observed increases of 68 and 95%, respectively, in the LF/HF ratio from peak exercise to immediate recovery (2-4 minutes) from exercise. As recovery progressed (8-9 minutes) both these authors observed further increases in the LF/HF ratio (from 8.7 to 11.1 and from 2.2 to 3.8, respectively). Dixon et al. (1990) on the other hand, observed no changes in the LF/HF ratio from immediate recovery (5 minutes) to early recovery (15 minutes). This discrepancy may be due to the fact that both Arai et al. and Bernardi et al. utilized exercise protocols to exhaustion, while Dixon et al. only utilized 50% VO2max. Therefore, recovery from exhaustive exercise could be more pronounced than those of lower exercise intensities. Both Arai et al. and Dixon et al., however, used absolute units and therefore, due to an increase in overall heart rate variations after exercise, an increase in absolute value would be expected. Furlan et al. (1993) using a much longer recovery time frame of 1, 24, 48 hours, observed decreases in the LF/HF ratio of 9.0, 5.4, and 1.3 respectively. None of the recovery LF/HF ratios reported by these authors (Arai et al., 1989; Bernardi et al., 1990; Dixon et al., 1992) had quite reached their resting values, except for the 48 hour recovery values reported by Furlan et al. (1993). Overall, it appears that the LF/HF ratio increases between exercise and immediate recovery and continues to increase at least until the ninth minute (Arai et al., 1989). Beyond one hour, however, the LF/HF ratio declines progressively.
but is still elevated above baseline values at 24 hours recovery (Furlan et al., 1993).

2.3.3 Applications of Heart Rate Variability in the Patient Population

Over the last 10-15 years, heart rate variability has also been used to assess autonomic function in patients with chronic diseases which may alter autonomic nervous system activity, such as in hypertension (Furlan et al., 1990; Guzzetti et al., 1988), congestive heart failure (Malliani et al., 1991; Murakawa et al., 1993; Sands et al., 1989; Saul et al., 1988), myocardial infarction (Lombardi et al., 1987; Buchanan et al., 1993), diabetes (Ewing et al., 1985; Masaoka et al., 1985; Thomaseth et al., 1990), and other autonomic dysfunctions (Akselrod et al., 1987; Bernardi et al., 1989; Sands et al., 1989). A sample of several studies which have examined the heart rate variability in patients with different types of autonomic disorders are shown in Table 4. The patients sampled have an average age range between 29 and 64 years. The patients were submitted to either a 24 hour ECG recording (Casolo et al., 1991; Furlan et al., 1990; Saul et al., 1988) or a 10-30 minute ECG recording for later heart rate variability analysis. The majority of the studies used 512 consecutive R-R intervals for the analysis. The results of the heart rate variability analysis in patients during rest and/or orthostatic challenge are shown in Table 4 as mean ± SD or mean(SEM). The results show that in the resting condition, the patient population has a lower total R-R variance than healthy control subjects. Furlan et al. (1990) in
Table 4. Total R-R Variance and Heart Rate Variability Spectral Components in the Patient Population during Rest and Orthostatic Stress Tests.

A) Total R-R Variance

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects/n</th>
<th>Age (yrs)</th>
<th>Rest (ms²)</th>
<th>°%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furlan et al., (1990)</td>
<td>C/28</td>
<td>37(2)</td>
<td>24186(3172)</td>
<td>-70</td>
</tr>
<tr>
<td></td>
<td>HT/18</td>
<td>45(2)</td>
<td>7347(842)</td>
<td></td>
</tr>
<tr>
<td>Guzzetti et al., (1988)</td>
<td>C/35</td>
<td>42 (2)</td>
<td>2546 (315)</td>
<td>-24.3</td>
</tr>
<tr>
<td></td>
<td>HT/40</td>
<td>45 (1)</td>
<td>1927 (244)</td>
<td></td>
</tr>
<tr>
<td>Casolo et al., (1991)</td>
<td>C/15</td>
<td>61 (3.9)</td>
<td>539 (25)</td>
<td>-82</td>
</tr>
<tr>
<td></td>
<td>CHF/15</td>
<td>64 (2.3)</td>
<td>98 (3.9)</td>
<td></td>
</tr>
<tr>
<td>Lombardi et al., (1987)</td>
<td>C/26</td>
<td>52(3)</td>
<td>1222 (36.9)</td>
<td>-8.9</td>
</tr>
<tr>
<td></td>
<td>MI/26: 2 weeks</td>
<td>54(2)</td>
<td>1113 (19.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MI/29: 1 year</td>
<td></td>
<td>1237 (33.8)</td>
<td></td>
</tr>
</tbody>
</table>

C= control subjects; HT= hypertensive patient; CHF= congestive heart failure patients; MI= myocardial infarction patients; °% change from control subjects.

Mean (SEM)

B) High Frequency Component of Heart Rate Variability Spectral Analysis

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects/n</th>
<th>Age (yrs)</th>
<th>Rest (nu)</th>
<th>Orthostatic stressor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>° %</td>
</tr>
<tr>
<td>Furlan et al., (1990)</td>
<td>C/28</td>
<td>37(2)</td>
<td>21.09(1.8)</td>
<td>T 11.6 (1.6)</td>
</tr>
<tr>
<td></td>
<td>HT/18</td>
<td>45(2)</td>
<td>19.1(2.5)</td>
<td>T 16.5 (1.8)</td>
</tr>
<tr>
<td>Guzzetti et al., (1988)</td>
<td>C/35</td>
<td>42 (2)</td>
<td>33.1 (2.4)</td>
<td>T 16.5 (1.8)</td>
</tr>
<tr>
<td></td>
<td>HT/40</td>
<td>45 (1)</td>
<td>24.0 (2.7)</td>
<td>T 16.5 (1.8)</td>
</tr>
<tr>
<td>Binkley et al., (1991)</td>
<td>C/15</td>
<td>28.9(1.8)</td>
<td>23.9¹</td>
<td>T 16.5 (1.8)</td>
</tr>
<tr>
<td></td>
<td>HT/10</td>
<td>49.2(3.4)</td>
<td>6.1¹</td>
<td>T 16.5 (1.8)</td>
</tr>
<tr>
<td>Saul et al., (1988)</td>
<td>C/21</td>
<td>54</td>
<td>2.3 (0.5)²</td>
<td>T 16.5 (1.8)</td>
</tr>
<tr>
<td></td>
<td>CHF/25</td>
<td>56</td>
<td>0.5 (0.2)²</td>
<td>T 16.5 (1.8)</td>
</tr>
<tr>
<td>Casolo et al., (1991)</td>
<td>C/15</td>
<td>61 (3.9)</td>
<td>77 (12.4³)</td>
<td>T 16.5 (1.8)</td>
</tr>
<tr>
<td></td>
<td>CHF/15</td>
<td>64 (2,3)</td>
<td>5 (1.5³)³</td>
<td>T 16.5 (1.8)</td>
</tr>
<tr>
<td>Lombardi et al., (1987)</td>
<td>C/26</td>
<td>52(3)</td>
<td>35 (3)</td>
<td>T 14 (2)</td>
</tr>
<tr>
<td></td>
<td>MI/26: 2 weeks</td>
<td>54(2)</td>
<td>19 (3)</td>
<td>T 13 (2)</td>
</tr>
<tr>
<td></td>
<td>MI/29: 1 year</td>
<td></td>
<td>28 (2)</td>
<td>T 11(2)</td>
</tr>
<tr>
<td>Thomaseth et al.,(1990)</td>
<td>C/10</td>
<td>36.1</td>
<td>347(72)⁴</td>
<td>St 161(35)⁵</td>
</tr>
<tr>
<td></td>
<td>D/10</td>
<td>42.6</td>
<td>12(2)⁴</td>
<td>St 7(1)⁴</td>
</tr>
</tbody>
</table>

C= control subjects; HT= hypertensive patient; CHF= congestive heart failure patients; MI= myocardial infarction patients; D= diabetic patients; T= 90° tilt; St= standing; ° %; ²= bpm; ³= m²; ⁴= EHz; °% difference from control subjects; **% change from rest.

Mean (SEM)
Table 4. Total R-R Variance and Heart Rate Variability Spectral Components in the Patient Population during Rest and Orthostatic Stress Tests.

C) Low Frequency Component of Heart Rate Variability Spectral Analysis

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects/n</th>
<th>Age (yrs)</th>
<th>Rest (nu)</th>
<th>Rest Orthostatic stressor (nu)</th>
<th>* %</th>
<th>Orthostatic stressor (nu)</th>
<th>* %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furlan et al. (1990)</td>
<td>C/28</td>
<td>37 (2)</td>
<td>57.7 (2.6)</td>
<td>T 80.7 (2.2)</td>
<td>-5.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HT/18</td>
<td>45 (2)</td>
<td>54.3 (2.5)</td>
<td>T 74.3 (2.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guzzetti et al. (1988)</td>
<td>C/35</td>
<td>42 (2)</td>
<td>54.4 (2.6)</td>
<td>+25</td>
<td>48.3</td>
<td></td>
<td>+9.3</td>
</tr>
<tr>
<td></td>
<td>HT/40</td>
<td>45 (1)</td>
<td>68.0 (2.9)</td>
<td>T 74.3 (2.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binkley et al. (1991)</td>
<td>C/15</td>
<td>28.9 (1.8)</td>
<td>77 (3.4)</td>
<td>+13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HT/10</td>
<td>49.2 (3.4)</td>
<td>87 (4.4)</td>
<td>T 78 (3)</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saul et al. (1988)</td>
<td>C/21</td>
<td>54</td>
<td>15.1 (2.4)</td>
<td>-80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHF/25</td>
<td>56</td>
<td>3.0 (0.6)</td>
<td>T 78 (3)</td>
<td>-90</td>
<td></td>
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</tr>
<tr>
<td>Casolo et al. (1991)</td>
<td>C/15</td>
<td>61 (3.9)</td>
<td>230 (46)</td>
<td>T 78 (3)</td>
<td>+47.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHF/15</td>
<td>64 (2.3)</td>
<td>22 (4.1)</td>
<td>T 78 (3)</td>
<td>+5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lombardi et al. (1987)</td>
<td>C/26</td>
<td>52 (3)</td>
<td>53 (3)</td>
<td>T 78 (3)</td>
<td>+45.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MI/28:2 weeks</td>
<td>54 (2)</td>
<td>74 (3)</td>
<td>+39.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MI/29:1 year</td>
<td>53 (3)</td>
<td>0</td>
<td>T 77 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomaseth et al. (1990)</td>
<td>C/10</td>
<td>36.1</td>
<td>1024 (218)</td>
<td>St 1190 (187)</td>
<td>+16.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D/10</td>
<td>42.6</td>
<td>18 (8)</td>
<td>St 24 (6)</td>
<td>+33.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C= control subjects; HT= hypertensive patient; CHF= congestive heart failure patients; MI= myocardial infarction patients; D= diabetic patients; T= 90° tilt; St= standing; 1= %; 2= bpm; 3= m²; 4= EHz; * % difference from control subjects; ** % change from rest.

Mean (SEM)
hypertensive patients and Casolo et al. (1991) in CHF patients have reported decreases in the R-R variance of as much as 70 and 80% in the patient population compared with healthy individuals. The frequency spectral components of the heart rate variability are also significantly different in the patient population when compared to healthy subjects. Guzzetti et al. (1988) compared hypertensive patients (diastolic BP >95mmHg) with a control group and found a 27.5% lower HF component and a 25% higher LF component in hypertensive patients at rest. This suggests that reduced vagal influence and enhanced sympathetic activity on the sinus node is present in hypertensive patients. The degree of hypertension, however, may be important since the power spectral components failed to detect significance differences between subjects with mild hypertension and normotensive subjects (Furlan et al., 1990).

Myocardial infarction (MI) is known to elicit autonomic reflexes depending on the site of the infarction (Kamath et al., 1993). Lombardi et al. (1987) reported that within two weeks after an acute MI, the power spectra of the HF component was significantly smaller (46.0%) and the LF component was significantly greater (39.6%) than in control subjects at rest. This difference may reflect a change in the sympathovagal balance with a shift towards sympathetic predominance. Within one year after the MI, the HF component was only reduced by 20% and the LF component was similar to the control values, suggesting a normalization of the sympathovagal balance. Patients with congestive heart failure (CHF) have been shown to have markedly diminished heart rate variability compared with
normal subjects over a 24 hour period (Malliani et al., 1991a; Murakawa et al., 1993; Sands et al., 1989). Casolo et al. (1991) observed an 82% lower total R-R variance in the CHF patients as compared with control subjects. In addition, Saul et al. (1988) and Casolo et al. reported decreases in the HF (76 and 94%, respectively) and LF (80% and 90%, respectively) power spectra components in CHF patients compared with the control subjects. Binkley et al. (1991) also reported reduced HF components but in contrast to others, reported an increase in the LF power component. Authors which have examined patients with diabetes, particularly insulin-dependent diabetes, have observed decreases in heart rate variability, with the degree of the decrease associated with the extent of the disease and the age of the subject (Ewing et al., 1985; Masaoka et al., 1985). Both the HF and LF power components were reduced by 96 and 98%, respectively (Thomaseth et al., 1990) in diabetic patients, diagnosed to be affected by severe autonomic neuropathy. A decrease in the total heart rate variability is attributed to a reduction of the parasympathetic and sympathetic inputs to the sinus node with the reduction in the LF component related to the degeneration and slow conduction of the sympathetic fibers that accompanies diabetic autonomic neuropathy (Van Den Akker et al., 1983).

Response to Orthostatic Tests: Very few studies have used orthostatic challenges in the patient population. Three studies which utilized orthostatic stress tests include Guzzetti et al. (1988) in hypertensive patients using a
passive 90° upright tilt, Lombardi et al. (1987) in patients who have had an MI using a 90° upright tilt, and Thomaseth et al. (1990) which used a standing position in diabetic patients. Guzzetti et al. (1988) in hypertensive patients reported less of a decrease in the HF component (31.3%) as compared with control subjects (65.0%) and a smaller increase in the LF component (9.3%) as compared with the control subjects (48.3%), in going from rest to a 90° tilt. This probably reflects a higher level of sympathetic influence seen in hypertensive patients at rest. In patients, two weeks following an MI, Lombardi et al. (1987) using upright tilting observed only half the decrease in the HF component and no change in the LF component as compared to the control group in going from a resting condition to a tilt position. The HF component only decreased by 31.6% as compared to 60% in the control subjects presumably because the HF component had already been depressed at rest, while no modification of the LF component (+5%) was observed because it had already been elevated in the resting condition. One year after an MI, an upright tilt produced a decrease of 60.7% in the HF component, a marked increase of 47.2% in the LF component, and an increase in the LF/HF ratio (from 3.0(0.9) to 14(3.5)) from resting conditions. These findings were similar to those observed in healthy controls. This suggests that the sympathetic predominance seen two weeks after an MI is followed by recovery of the vagal influence and a normalization of the sympathovagal balance in response to an upright tilt. Thomaseth et al. (1990) in diabetic patients, observed a lesser decrease in the HF component (41.7 vs
53.6%) and a greater increase in the LF component (33.0 vs 16.2%) as compared with the control group in going from rest to a standing position. However, the absolute changes in HF and LF components were much less in the diabetic group since the resting HF and LF components were lower than those of the control group by 96 and 98%, respectively. The lower absolute levels of HF and LF spectral components are consistent with impairment of both the parasympathetic and sympathetic pathways in the diabetic population. Overall, in the patient populations investigated, the disease state was associated with a significant reduction in the HF component of the heart rate variability spectra, with an increase in the LF/HF ratio, suggesting an alteration in the resting sympathovagal balance towards greater sympathetic tone. In response to orthostatic stress, patients consistently demonstrated an attenuated decrease in HF and increase in LF spectral components, indicating a perturbation of the arterial baroreflex system. These findings correlate with the pathologies of various diseases and demonstrate that the heart rate variability technique is a valuable tool for assessing autonomic dysfunction in chronic disease.

2.3.4 Investigation of Training Bradycardia using Heart Rate Variability Spectral Analysis

It is well known that endurance training results in training bradycardia. The more commonly accepted explanation is that vagal activity to the sinus node is chronically enhanced in trained individuals to yield a lower heart rate (McArdle et al., 1991). Very few studies have used the heart rate variability analysis
method to investigate resting training bradycardia, and of these studies all of them have been cross-sectional, comparing athletes with non-athletes. Tables 5A and 5B summarize four studies which have compared the heart rate variability analysis of non-athletes with those of athletes under different experimental conditions. The non-athletes were healthy normal volunteers and the athletes were either distance runners, cyclists, or swimmers. The age of the non-athletes and athletes were similar with the average age between studies ranging from 16 to 29.3 years. Both the control and athletic groups were submitted to on line Lead II ECG recordings for a period of 256 successive R-R intervals, except for the study conducted by Goldsmith et al. (1992) who used 24 hour recordings. The HF and LF components are summarized in Table 5A and 5B, respectively. Furlan et al. (1993) observed a 27% lower HF component, a 46% higher LF component, with a higher LF/HF ratio (1.62 vs 0.78) in the swimmers than the sedentary subjects at rest. These results suggest that vagal influence to the sinus node is actually decreased, while sympathetic influence to the sinus node is increased in athletes compared with non-athletic controls. Dixon et al. (1992) and Goldsmith et al. (1992), on the other hand, observed significantly higher HF components (42.3% and 340%, respectively) and lower LF/HF ratios (0.86 vs 1.60 and 3.6 vs 2.3, respectively) in distance runners and cyclists at rest, suggesting that the athletes had greater vagal influence on the sinus node and a lesser relative contribution from the sympathetic nervous system to the sinus node. These authors however, did not use normalized units
Table 5. Heart Rate Variability Spectral Components in Athletes and Non-athletes during Rest, Exercise, and Recovery Conditions.

A) High Frequency Component of Heart Rate Variability Spectral Analysis

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects/n</th>
<th>Age (yrs)</th>
<th>Rest (nu)</th>
<th>Exercise (W)</th>
<th>Recovery min (nu)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>%</td>
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</tr>
<tr>
<td>Furlan et al.</td>
<td>C/29</td>
<td>16 (0.4)</td>
<td>53.12 (2.6)</td>
<td>50 (2.8)</td>
<td>35.8 (2.2)</td>
</tr>
<tr>
<td>(1993)</td>
<td>Sw/21</td>
<td>16 (0.4)</td>
<td>38.81 (2.5)</td>
<td>-26.9</td>
<td></td>
</tr>
<tr>
<td>Dixon et al.</td>
<td>C/14</td>
<td>27.4 (0.7)</td>
<td>43.7 (6.0)</td>
<td>50 (2.8)</td>
<td>35.8 (2.2)</td>
</tr>
<tr>
<td>(1992)</td>
<td>R/10</td>
<td>28.4 (1.1)</td>
<td>62.2 (3.4)</td>
<td>27.9 (3.9)</td>
<td>51.7 (4.2)</td>
</tr>
<tr>
<td>Goldsmith et al.</td>
<td>C/8</td>
<td>29.3 (1.0)</td>
<td>318 (68.2)</td>
<td>370 (6.3)</td>
<td>31.7 (9.1)</td>
</tr>
<tr>
<td>(1992)</td>
<td>R,Cy/8</td>
<td>28.5 (1.4)</td>
<td>1399 (274)</td>
<td>+340</td>
<td></td>
</tr>
<tr>
<td>Bernardi et al.</td>
<td>C/9</td>
<td>23.1 (0.7)</td>
<td>44.0 (4.0)</td>
<td>37.0 (6.3)</td>
<td>31.7 (9.1)</td>
</tr>
<tr>
<td>(1990)</td>
<td>Cy/6</td>
<td>17.8 (1.1)</td>
<td>32.1 (4.9)</td>
<td>31.5 (5.3)</td>
<td>23.9 (5.2)</td>
</tr>
</tbody>
</table>

C= control subjects; Sw= swimmers; R= runners; Cy= cyclists; 1= bpm²·Hz⁻¹; 2= ms²; t= %VO₂max; % difference from controls; % Change from rest; % % Change from exercise.

Mean(SEM)

B) Low Frequency Component of Heart Rate Variability Spectral Analysis

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects/n</th>
<th>Age (yrs)</th>
<th>Rest (nu)</th>
<th>Exercise (W)</th>
<th>Recovery min (nu)</th>
</tr>
</thead>
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<tr>
<td></td>
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<td></td>
<td>%</td>
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<td></td>
<td></td>
<td>(nu)</td>
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</tr>
<tr>
<td>Furlan et al.</td>
<td>C/29</td>
<td>16 (0.4)</td>
<td>37.41 (2.9)</td>
<td>67.6 (5.8)</td>
<td>69.9 (7.4)</td>
</tr>
<tr>
<td>(1993)</td>
<td>Sw/21</td>
<td>16 (0.4)</td>
<td>54.61 (2.6)</td>
<td>60.2 (10.1)</td>
<td>67.3 (9.4)</td>
</tr>
<tr>
<td>Dixon et al.</td>
<td>C/14</td>
<td>27.4 (0.7)</td>
<td>69.6 (5.2)</td>
<td>67.6 (5.8)</td>
<td>69.9 (7.4)</td>
</tr>
<tr>
<td>(1992)</td>
<td>R/10</td>
<td>28.4 (1.0)</td>
<td>53.6 (3.1)</td>
<td>60.2 (10.1)</td>
<td>67.3 (9.4)</td>
</tr>
<tr>
<td>Goldsmith et al.</td>
<td>C/8</td>
<td>29.3 (1.0)</td>
<td>1169 (137)</td>
<td>63.0 (6.3)</td>
<td>68.2 (9.1)</td>
</tr>
<tr>
<td>(1992)</td>
<td>R,Cy/8</td>
<td>28.5 (1.4)</td>
<td>3240 (455)</td>
<td>+177</td>
<td></td>
</tr>
<tr>
<td>Bernardi et al.</td>
<td>C/9</td>
<td>23.1 (0.7)</td>
<td>56.0 (4.0)</td>
<td>63.0 (6.3)</td>
<td>68.2 (9.1)</td>
</tr>
<tr>
<td>(1990)</td>
<td>Cy/6</td>
<td>17.8 (1.1)</td>
<td>67.9 (4.9)</td>
<td>68.5 (5.3)</td>
<td>76.1 (5.2)</td>
</tr>
</tbody>
</table>

C= control subjects; Sw= swimmers; R= runners; Cy= cyclists; 1= bpm²·Hz⁻¹; 2= ms²; t= %VO₂max; % difference from controls; % % Change from rest; % % Change from exercise.

Mean(SEM)
to report their findings, and therefore a higher R-R interval (ms) would be expected in response to endurance training, resulting in higher standard deviations and hence a higher total variance (ms²). Consequently, these results do not reflect the proportions of HF and LF spectral components with respect to the total variance. Bernardi et al. (1990) observed no significant differences in the HF component, LF component, and the LF/HF ratio of R-R interval variability between the athletes and non-athletes at rest, suggesting similar autonomic influence to the sinus node at rest between the two groups. Meanwhile, the differences in autonomic function suggested by Furlan et al. would result in a higher resting heart rate and not bradycardia in athletes.

Response to Orthostatic Stress tests: Two studies have compared the orthostatic responses of athletes with those of sedentary controls. Dixon et al. (1992) compared the heart rate variability spectra of distance runners and sedentary controls during active standing and observed similar changes in HF and LF components and in the LF/HF ratio between athletes and control subjects. Furlan et al. (1993) used a passive 90° tilt protocol to compare the autonomic responses of competitive swimmers with those of normal controls, and demonstrated a 42% lower HF component in athletes, a 17% greater LF component, and a greater LF/HF ratio (6.7 vs 4.4) in going from rest to tilt in the swimmers. The greater LF/HF ratio in athletes suggests a shift in the
sympathovagal balance towards one of greater sympathetic predominance in response to a tilt position.

Exercise: Bernardi et al. (1990) and Dixon et al. (1992) have compared the heart rate variability responses of athletes with non-athletes going from a resting to an exercising condition as seen in Tables 5A and 5B. Dixon et al. observed similar decreases of 50% in the HF component of control subjects and 55.1% in the HF component of athletes between rest and moderate level (50% Vo2 max) exercise. Bernardi et al., on the other hand, observed no significant change in the HF component between rest and low level exercise (30W) in either control subjects (-15.9%) or athletes (-2%). Neither study observed any changes in the LF component between rest and exercise in either the athletes or control subjects. Dixon et al. were the only authors who compared the LF/HF ratio between athletes and control subjects. In the transition from rest to exercise, control subjects showed a two fold increase in the LF/HF ratio (1.6 to 3.2), while athletes displayed a similar increase of 2.44 fold (0.86 to 2.1).

Bernardi et al. (1990) also compared the heart rate variability responses of non-athletes with athletes during progressive exercise. As seen in Table 3A, large increases in the HF component were observed in both control subjects (37.0 to 88.9 nu) and athletes (31.5 to 99.9 nu) as exercise progressed from 30 W to 120 W (30W/5 min) and from 30 W to 210 W, respectively, with the increase in the HF component being twice as large in the athletes (217 vs
104%). These authors also observed similar decreases in LF component (Table 3B) of both the control subjects and athletes (63.0 to 11.1 vs 68.5 to 0.1 nu, respectively) with the decrease in the LF component being larger in the athletes (99.9 vs 82.4%) as compared to the non-athletes.

Recovery: Dixon et al. (1992) and Bernardi et al. (1990) also compared peak exercise and the first recovery period between controls and athletes, however, these authors reported opposite trends. Dixon et al. observed similar increases in the HF component in both the athletes (46.0%) and control subjects (39.1%) whereas Bernardi et al. observed a greater decrease in the athletes (318.0%) compared to the control subjects (180.4%). The results reported by Dixon et al. are in absolute units and therefore, would be expected to increase because the R-R variations of the heart rate should be increasing during the recovery. Bernardi et al. reported very elevated HF components as exercise progressively increases to exhaustion, therefore, the recovery values would have to decrease in order to return to pre-exercise values. With respect to the LF spectral component, Bernardi et al. (1990) observed greater increases in athletes (99.9%) compared with non-athletes (83.7%) between peak exercise and recovery. Dixon et al. (1992), however, found no difference between controls (3.3%) and athletes (2.4%) in going from exercise to recovery. This may be because these authors only used 50% VO₂max exercise prior to the recovery recordings, as compared to 100% VO₂max (exhaustion) protocol utilized by
Bernardi et al. The only authors who examined the LF/HF ratio observed small reductions in both control subjects (3.2 to 2.9) and athletes (2.1 to 1.1) in going from peak exercise to the first stage of recovery (Dixon et al., 1992).

Bernardi et al. (1990) and Dixon et al. (1992) also compared the heart rate variability power spectra of athletes and controls during various stages of recovery. Bernardi et al. observed a decrease of 34.7% in the HF component of the control group and no change in the HF component of the athletes (-10%) between 4 and 8 minutes of recovery. Meanwhile, Dixon et al. observed no differences in the change of HF fluctuations between the athletic (+1.2%) and control (-2.8%) groups between 5 and 15 minutes of recovery. All the HF recovery values were lower than the resting HF values indicating that vagal activity to the SA node during recovery had not returned to baseline levels. Increases in the LF component were observed in the non-athletes by both Dixon et al. (+22.3%) and Bernardi et al. (+16.2%) during recovery. However, Dixon et al. and Bernardi et al. showed no differences in the LF component (+9.1% and +3.2%, respectively) of the athletic groups, between the first and last stage of the recovery period, suggesting that the athletes have lesser shifts towards sympathetic predominance during recovery.

In summary, the studies using the heart rate variability technique to compare autonomic nervous system responses of sedentary controls with those of athletes, have demonstrated very inconsistent results. The reason for these
discrepancies may result from the many confounding factors observed when comparing studies. One possible explanation for the discrepancy between the studies may be the subject selection. The athletic group used by Dixon et al. (1992) demonstrated a significant resting bradycardia compared with controls, while the athletic groups used by Bernardi et al. (1990) and Furlan et al. (1993) showed no significant difference in resting heart rate when compared with controls, and therefore any differences observed cannot be related to training-induced bradycardia. An explanation for the greater LF variation observed in athletes at rest by Bernardi et al. and Furlan et al. may be due to the time interval between their last training bout and the testing session. Furlan et al. demonstrated an elevated LF/HF ratio immediately following maximal dynamic exercise, which remained elevated for 24 hours following cessation of exercise but had returned to baseline values within 48 hours. If the athletes had exercised in the 24 hours prior to testing their baseline recordings may have been affected and therefore, may explain the elevated LF component observed by Bernardi et al. and Furlan et al. at rest. Furthermore, as mentioned earlier, the units used to express the spectral components of heart rate variability may be misleading, as results reported in absolute units will change with the total R-R variance (Dixon et al., 1992). In addition, differences in the type and intensity of exercise may affect the heart rate variability response during exercise and recovery. The exercise intensity was reported differently in the studies (%V0_{2\text{max}} or absolute power (W)) and the intensities chosen by the authors
was not always similar. Also, by using absolute workloads, non-athletic and athletic subjects may not be exercising at similar relative (%VO\textsubscript{2} max) levels. By using different initial and peak exercise intensities, the change seen in going from rest to initial exercise or from peak exercise to recovery may be affected. Therefore, due to these inconsistencies, comparisons of heart rate variability response between studies is difficult.
REFERENCES


PART II: EXPERIMENTAL STUDY
One of the adaptations to chronic endurance training in both animals and humans is a decrease in resting heart rate. This phenomenon has been observed in both cross-sectional and longitudinal studies. Studies in which endurance-trained individuals were compared with non-trained individuals showed an 8-17 beats/min lower resting heart rate in the trained group (Jost et al., 1989; Katona et al., 1968; Lewis et al., 1980; Smith et al., 1989). Authors using a 5-12 week endurance training program have typically observed reductions in resting heart rate ranging from 4-13 beats/min after training (Ekblom et al., 1973; Krzeminski et al., 1989; Maciel et al., 1985; Pollock et al., 1977; Wilmore et al., 1970). A lower resting heart rate may result from either an increase in the parasympathetic activity to the sinus node, a decrease in the sympathetic nervous activity, a reduction in the intrinsic heart rate, or through a combination of any of these. Although a common belief is that training-induced bradycardia results from an increase in parasympathetic tone, the mechanism responsible remains controversial. In fact, authors using pharmacological blockades in animals have observed either decreases (Lin & Horvath, 1972; Negrao et al., 1992; Tipton & Taylor, 1977) or no change (Barnard et al., 1976) in parasympathetic activity to the sinus node following 6-13 weeks of endurance training. In humans, authors using pharmacological blockades have found that parasympathetic activity to the sinus node in endurance-trained individuals has been shown to be either greater than (Ekblom et al., 1973; Smith et al., 1989) or not different (Katona et al., 1982; Maciel et al., 1985; Raab et al., 1960) than that
of non-trained individuals. All authors who examined the effects of β-adrenergic blockade observed no difference in sympathetic activity on the sinus node in animals (Barnard et al., 1976; Lin & Horvath, 1972; Negrao et al., 1992) or in endurance-trained athletes compared with sedentary controls (Katona et al., 1982; Smith et al., 1989). When various surgical techniques (sympathectomy + vagotomy, isolated whole hearts, isolated atrial tissues) were used to investigate the intrinsic heart rate in animal studies, authors found either decreases (Bolter et al., 1972; Schaefer et al., 1992; Sigvardsson et al., 1977) or no change (Ordway et al., 1982; Tipton, 1968; Tipton et al., 1977) following 6-15 weeks of endurance training. When a pharmacological blockade approach was used, authors reported a decrease in the intrinsic heart rate of animals (Barnard et al., 1976; Hughson et al., 1976; Lin & Horvath, 1972; Negrao et al., 1992) and in endurance athletes as compared with non-athletes (Frick et al., 1967; Katona et al., 1982; Lewis et al., 1980; Smith et al., 1989). Ekblom et al. (1973), however, has shown no difference in the intrinsic heart rate between athletes and non-athletes. Problems with the pharmacological blockade approach is that it is invasive and the pharmacological agents used in the blockade studies may also exert non-selective effects on physiological functions such as reflex control mechanisms.

A more recently developed, non-invasive technique quantifying autonomic control to the sinus node by taking the duration of successive R-R intervals is heart rate variability. By decomposing the heart rate variability analysis into its
frequency components, two different frequency peaks may be identified. High
frequency (HF) variations (>0.16 Hz) are produced by respiratory sinus
arrhythmia and are essentially due to vagal influence on the sinus node
(Akselrod et al., 1981; Pomeranz et al., 1985). The low frequency (LF)
variations, occurring between 0.05-0.15 Hz, have been attributed to a
combination of sympathetic and parasympathetic influence on the sinus node
(Akselrod et al., 1981; Cowan, 1995; Pomeranz et al., 1985). The LF/HF ratio
has been suggested to be a useful index for expressing overall autonomic
balance to the sinoatrial node (Malliani et al., 1991a; Pagani et al., 1986).

Only a few authors have used the heart rate variability technique on the
athletic population including runners, cyclists, and swimmers. The purposes of
these studies, however, were not necessarily to examine training-induced
bradycardia, but to examine the sympathovagal response to different stressors
by using the heart rate variability technique on different subject populations. For
this reason the athletic population did not always exhibit training-induced
bradycardia. The results, thus far, are conflicting and are contrary to the
generalized popular statement that there is an enhancement of vagal tone in
response to chronic exercise training. In one case, Furlan et al. (1993) reported
lower HF components and higher LF components in the athletes compared with
the non-athletes at rest. In another case, Dixon et al. (1992) and Goldsmith et
al. (1992) both reported higher HF and lower LF components in the athletic
groups, while in a third case Bernardi et al. (1990) reported no differences in the
HF and LF components between the athletic and non-athletic groups at rest. One reason for these discrepancies may lie in the methodology used. A non-stationary approach would make it difficult to apply the Fourier or autoregressive methods to assess R-R interval variability. Another reason may be due to the type of units (absolute or normalized units) used to express the power spectral components. Also, the level of aerobic fitness of the athletes and the time since the last bout of exercise were not controlled for in many of the studies.

The purpose of this study is to examine in endurance-trained athletes, selected for their high level of aerobic fitness and showing documented resting bradycardia, the heart rate variability at rest, during orthostatic stress, exercise, and recovery, with respect to responses in healthy non-trained, age and sex matched control subjects.
METHODOLOGY

Subjects

The study population consisted of two groups of healthy volunteers. The athletic group consisted of 12 (10 males, 2 females) highly trained endurance athletes (mean $V_{O_2\text{max}} = 68.2 \text{ ml/kg/min}; \text{SEM} 2.1$) with an average age of 25.7 years ($\text{SEM} 1.2$) who trained an average of 12-20 hours per week. The sedentary control group consisted of 10 age (25.3 yrs, $\text{SEM} 1.1$) and sex matched (8 males, 2 females) healthy subjects who had not engaged in any regular physical activity (less than three times per week), for a period of at least one year. All subjects were normotensive and were not taking any medication. The subjects were asked to refrain from taking any alcohol or caffeine on the days of testing and from exercising for a period of 12 hours prior to testing. All subjects had breakfast prior to the heart rate variability testing. Written informed consent was obtained from all participants prior to the testing session. Approval for this study was obtained from the Ethics Committee of McGill University, Faculty of Education as well as from the ethics board of Sacré-Coeur Hospital.

Testing Conditions

The experiment was carried out in the morning in a semi-dark room at a constant temperature (19-22°C). Care was taken to ensure that the subjects were as comfortable as possible, and that all auditory and visual stimuli were kept to a minimum. Subjects were asked to remain awake and relaxed.
throughout the testing procedures. As shown in figure 1, a total of nine recordings were obtained in all subjects: 2 supine rest, 1 standing, 2 seated rest, 2 exercise, and 2 recovery. Subjects rested in a supine position on a bed for 20 minutes prior to the recordings. Continuous ECG, blood pressure, and respiratory recordings were taken simultaneously. R-R acquisition time varied depending on the subject’s heart rate but a total of 532 beats were recorded for all subjects. Following the supine resting or baseline recording, subjects were asked to control their respiration rate by breathing in synchrony with a metronome set at 12 breaths/min (0.2 Hz). To test the effect of orthostatic stress, subjects were asked to stand and maintain a free standing position. Recordings were obtained following a 10 minute period in the upright position. Subjects were then seated and recordings were taken following a 10 minute adaptation period in the seated position. Recordings were again repeated in the seated position with subjects breathing at the imposed rate of 0.2 Hz. Following adjustment of the seat height, subjects were asked to exercise on a cycle ergometer at two different submaximal intensities. With a pedal rate of 70 rpm, the resistance was first increased until a steady state heart rate of 100 beats/min was maintained and recordings were obtained during steady state exercise. Steady state was achieved when the subject’s heart rate varied less than 5 beats/min. Recordings were again obtained at the second exercise level selected to produce a steady state heart
Figure 1. Timeline of experimental testing procedure. SR = spontaneous respiration; CR = controlled respiration at 12 breaths/min; S-S = steady state exercise at a heart rate of either 100 or 130 beats/min; • = duration of adaptation period; • = approximate length of recording.
rate of 130 beats/min. Recovery recordings were taken after 5 and 15 minutes, respectively, of passive recovery in the seated position.

*Instrumentation*

Five ECG electrodes were firmly attached to the anterior chest. Care was taken to ensure that a prominent R wave was obtained for data processing, while avoiding any movement artifact. Continuous ECG (modified lead III) signals were obtained with a physiograph (model 974429, Electronics for Medicine). Respiratory frequencies were recorded from a thermister probe placed subjacent to the nares. Continuous beat-to-beat arterial blood pressure was recorded (by photoplethysmographic transducer, Ohmeda Finapress, model 2300) using a small cuff placed around the index finger of the left hand. Blood pressure was also taken by conventional sphygmonomanometry after each condition.

Data acquisition was performed at a sampling rate of 250 samples/second for each experimental condition. This sampling rate was adequate to maintain the accuracy of the R wave, systolic and diastolic blood pressure detection algorithms and time interval measurements. ECG, respiratory, and diastolic and systolic blood pressure signals were recorded and stored using a signal acquisition program on a personal computer-based system equipped with an eight-channel analog-to-digital acquisition card (Data Translation model DT 2801). Time domain analysis of successive R-R intervals was performed to determine the mean and standard deviation of the R-R intervals. A record length
of 512 sample points were selected for power spectrum analysis. Signals were later analyzed off-line using spectral analysis software. R-R intervals, respiration, and blood pressure values were linearly interpolated at 0.8s intervals, since the equidistant data sampling of all time series is further required for a spectral emission. A moving fourth order polynomial was used to remove the baseline trend from all signals, which includes the very low frequency non-stationary below 0.005 Hz (Lepicovska et al., 1992). A Fast Fourier transform [with use of the Fourier algorithm (FFT) (Oppenheim & Schafer, 1985)] was performed on the R-R interval segment to obtain a frequency domain analysis. Spectral powers were averaged across the low frequency (0.05-0.15Hz) and a high frequency (0.16-0.5 Hz) ranges.

**Determination of VO$_2$max.** On a separate day, but not separated by more than three weeks from the heart rate variability recording, subjects were submitted to a direct graded maximal treadmill exercise test for determination of VO$_2$max. The subjects started at a treadmill speed of 6 mph (5 mph for females). The speed was increased by 2 mph every 2 minutes until 10 mph was reached for male subjects and 9 mph for female subjects. The grade of the treadmill was then increased by 2% every minute until the following criteria were met: 1) a lack of O$_2$ increase with an increase in work rate, 2) attainment of maximal heart rate predicted for each subjects age, and 3) a respiratory exchange ratio (O$_2$ uptake/CO$_2$ production)> 1.10. The Astrand-Ryhming (multiple load) bicycle test
was used to predict the VO2\textsubscript{max} of control subjects who did not want to perform a maximal oxygen consumption test.

*Treatment of Data*

Time domain and frequency domain variables were obtained for every subject under each of the experimental conditions. The time domain variables included the mean (absolute units), standard deviation, and variance (standard deviation squared) of the R-R interval and beat-to-beat systolic and diastolic blood pressures. The frequency domain variables included HF component, LF component, and LF/HF ratio of R-R interval variability, and beat-to-beat systolic and diastolic blood pressure variability. For each subject the mean and standard deviation were determined for all the time and frequency domain variables. The group mean and standard deviation for each dependent variable were then obtained by taking the average of all the subjects in the group.

The LF and HF components are presented both in absolute (ms\textsuperscript{2}) and in normalized (nu) units. The normalized units are obtained by dividing the power of each component in ms\textsuperscript{2} by the total variance minus the VLF and multiplying the result by 100 (Malliani et al., 1991a; Pagani et al., 1986).
Statistical Analysis

The data is expressed as mean (SEM). Independent t tests were performed on resting heart rate, diastolic and systolic resting blood pressure, submaximal workloads, and VO\textsubscript{2}max to compare athletes with non-athletes. A 2 X 9 analysis of variance (ANCVA) was performed to compare heart rate and blood pressure variability responses between athletes and non-athletes during various conditions. For significant treatment effects, planned comparisons were performed using Systat statistics package. Differences were considered significant at a value of p<0.05.
RESULTS

Circulatory parameters and responses to orthostatic challenges

Subjects of both groups were of similar height (C: 173.9(2.6); A: 171.7(1.8) cm), weight (C: 76.7(5.3); A: 65.3(2.5) kg), and age (C: 25.3(1.1); A: 25.7(1.2) yrs). Resting circulatory parameters are shown in Table 1. As can be seen from this table, there were significant differences between the groups for resting heart rates, R-R interval, systolic blood pressure and VO_{2}max. As expected, during steady state submaximal cycling at a set heart rate of 100 beats/min, the athletes were at significantly higher workloads, with workloads and VO_{2}max equivalent to 93.8(7.1) W and 4.47(0.24) L/min for athletes compared with 49.39(3.2) W and 3.19(0.21) L/min in control subjects. Similarly during exercise at 130 beats/min, workload values were 157.5(9.6) W for the athletes compared with 109.8(5.0) W for the control subjects. In both control and athletic subjects these work loads corresponded to relative exercise intensities of 28(1.2) and 29(2.9)% VO_{2}max and 52(2.6) and 48(1.1)% VO_{2}max during exercise at 100 and 130 beats/min, respectively.

As seen in Table 2, bradycardia was observed at rest and also under every experimental condition, except for exercise at 100 and 130 beats/min where the heart rate was controlled. Interestingly, systolic blood pressure (SBP) was significantly higher in the athletes at rest, in the standing position, and during the first and second exercise levels. The diastolic blood pressure (DBP) values were similar between the athletes and non-athletes.
Table 1. Subject's cardiorespiratory characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=10)</th>
<th>Athletic group (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting heart rate (bpm)</td>
<td>65.1 (2.1)</td>
<td>53.1 (1.8) t</td>
</tr>
<tr>
<td>R-R Interval (ms)</td>
<td>929.7 (28.4)</td>
<td>1143.5 (38.1) t</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>107.4 (4.3)</td>
<td>115.6 (2.8) t</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>57.9 (3.1)</td>
<td>64.7 (2.1)</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>41.38 (2.0)</td>
<td>67.24 (2.1) t</td>
</tr>
</tbody>
</table>

Mean (SEM)

* t significantly different from control values (p < 0.05)
Table 2. Results of circulatory responses to orthostatic challenges and recovery from exercise.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>CR</th>
<th>Sit CR</th>
<th>CR</th>
<th>Stand</th>
<th>Ex 100</th>
<th>Ex 130</th>
<th>Rec 5</th>
<th>Rec 15</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (bpm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C</td>
<td>65.1(2.1)</td>
<td>65.8(1.7)</td>
<td>68.8(1.8)*</td>
<td>72.0(1.9)</td>
<td>82.0(2.4)*</td>
<td>102.4(0.4)*</td>
<td>131.2(0.2)*</td>
<td>81.9(1.6)*</td>
<td>76.9(1.6)*</td>
</tr>
<tr>
<td>A</td>
<td>53.1(1.8)</td>
<td>54.3(1.7)l</td>
<td>56.0(1.9)l*</td>
<td>56.8(2.4)l</td>
<td>68.1(2.6)l*</td>
<td>102.6(0.9)*</td>
<td>131.5(0.3)*</td>
<td>65.0(1.7)l*</td>
<td>62.4(1.6)l*</td>
</tr>
<tr>
<td><strong>R-R (ms)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>929.7(28.4)</td>
<td>917.3(23.2)</td>
<td>877.9(23.3)*</td>
<td>838.4(22.1)</td>
<td>729.3(20.7)*</td>
<td>586.2(2.0)*</td>
<td>457.4(0.7)*</td>
<td>735.5(14.8)*</td>
<td>783.2(15.9)*</td>
</tr>
<tr>
<td>A</td>
<td>1143.6(38.1)l</td>
<td>1116.2(34.1)l</td>
<td>1083.8(35.2)l*</td>
<td>1076.3(42.8)l</td>
<td>895.2(34.6)l*</td>
<td>585.0(4.8)*</td>
<td>456.4(1.2)*</td>
<td>930.4(23.3)l*</td>
<td>968.1(25.0)l*</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>C</td>
<td>106.6(2.6)</td>
<td>106.5(2.5)</td>
<td>98.4(3.9)</td>
<td>99.3(3.9)</td>
<td>98.9(3.4)</td>
<td>121.4(4.3)*</td>
<td>135.6(3.9)*</td>
<td>100.1(4.4)</td>
<td>101.2(4.0)</td>
</tr>
<tr>
<td>A</td>
<td>115.9(2.8)l</td>
<td>113.6(3.5)</td>
<td>108.2(3.3)</td>
<td>108.2(3.3)</td>
<td>112.8(4.0)l</td>
<td>151.3(4.5)l*</td>
<td>160.2(4.9)l*</td>
<td>106.2(2.6)</td>
<td>108.0(2.9)</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>60.8(3.3)</td>
<td>60.2(3.5)</td>
<td>61.1(2.9)</td>
<td>60.7(2.4)</td>
<td>63.0(3.6)</td>
<td>64.7(4.1)</td>
<td>62.8(4.9)</td>
<td>60.5(4.5)</td>
<td>61.8(3.2)</td>
</tr>
<tr>
<td>A</td>
<td>64.9(2.2)</td>
<td>64.6(2.5)</td>
<td>61.5(2.5)</td>
<td>63.8(3.0)</td>
<td>67.3(1.9)</td>
<td>69.8(4.1)</td>
<td>66.8(4.1)</td>
<td>62.1(3.8)</td>
<td>65.8(3.3)</td>
</tr>
</tbody>
</table>

HR= heart rate; R-R= R-R interval; SBP= systolic blood pressure; DBP= diastolic blood pressure; C= control subjects; A= athletic subjects; CR= controlled respiration at 12 breaths/min; Ex 100 and Ex 130= submaximal exercise at a heart rate of 100 and 130 beats/min, respectively; Rec 5 and Rec 15= post-exercise recovery at 5 and 15 minutes, respectively.

Mean (SEM); * significantly different from baseline values (p< 0.05); † significantly different from control subjects (p< 0.05)
Heart rate variability: At rest, the total R-R interval and variance were larger in the athletes (1143.5(38.1) ms and 7762(2052.3) ms²) than in the non-athletes (929.7(28.4) ms and 5151.5(1781.8) ms²), with the total R-R interval variability showing statistical significance.

Figure 1 illustrates the HF and LF components of R-R interval variability under basal state, standing, seated, controlled respiration in the supine and seated position, and two levels of exercise. The upper panel shows absolute units and the lower panel shows normalized units. As expected from the longer R-R intervals observed in athletes in the resting condition, the HF component of the athletes is somewhat higher than that of the control subjects in all conditions except exercise, although the difference is not statistically significant. When the R-R interval is standardized, such as during exercise at 100 and 130 beats/min, there is no longer any difference between the two groups. Similarly when values are expressed in normalized units (lower panel), differences between the groups are minimized or abolished. The HF component decreased upon standing and during both levels of exercise, while controlled respiration increased the HF component in both the standing and sitting positions as compared to supine baseline values. As expected a mirror image was found for the LF component of R-R interval variability. However, examination of the kinetics of response to the experimental conditions reveals similar patterns whether expressed in absolute or normalized units (figure 2).
Figure 1. Comparison of absolute (ms²) and normalized (nu) units for high frequency (HF) and low frequency (LF) spectral components of R-R interval variability at rest (baseline), during controlled respiration at 12 breaths/min (CR), and during orthostatic challenges in athletes (A) and control (C) subjects. Ex 100 and Ex 130 = submaximal exercise at heart rates of 100 and 130 beats/min. Data are expressed as means and SEM.
Figure 2. Comparison of absolute (ms²) and normalized (nu) units for the LF/HF spectral ratio of R-R interval variability at rest (baseline), during controlled respiration at 12 breaths/min (CR), and during orthostatic challenges in athletes (closed bars) and control (open bars) subjects. Ex 100 and Ex 130 = submaximal exercise at heart rates of 100 and 130 beats/min. Data are expressed as means and SEM.

* significantly different from baseline value (p<0.05).
Blood pressure variability: At rest, the total SBP variance was higher in the athletes (43.7(3.3) mmHg²) compared with the control subjects (26.6(4.3) mmHg²). The total DBP variance was similar between the two groups (C:6.9(2.1) vs A:9.0(1.3) mmHg²).

The HF component of the beat-to-beat SBP variability was higher in the control group than in the athletic group (33.6(5.3) vs 18.8(6.3) nμ) at rest, while the LF component was higher in the athletic group than in the control group (16.7(4.9) vs 9.3(3.2)nμ). Neither the HF nor the LF components were significantly different between the two groups. The LF/HF ratio however, was significantly higher in the athletes than in the control group (11.4(3.6) vs 2.6(0.5)). The HF, LF, and LF/HF ratio of DBP variability were all similar between athletes and non-athletes.

Response to Controlled Breathing

As expected, controlled respiration increased the HF component of R-R interval variability from baseline by approximately 1.3 and 1.4 fold, and decreased the LF/HF ratio from baseline by 0.5 and 0.4 fold in both the control and athletic groups, respectfully (figure 3). The influence of controlled respiration only reached statistical significance in the control group in the seated position although changes were similar (1.4 vs 1.9) in the two groups. There were no differences in the HF component or the LF/HF ratio of R-R interval variability between the athletic and control groups.
Figure 3. High frequency (HF) spectral component and LF/HF ratio during spontaneous and controlled respiration in supine and seated conditions in athletes (closed bars) and control (open bars) subjects. CR = controlled respiration at 12 breaths/min; nu = normalized units. Data are expressed as means and SEM.

* significantly different from baseline value (p<0.05); t = significantly different from seated value (p<0.05).
Similar observations were found for beat-to-beat SBP variability in response to controlled respiration in the athletes while in a supine position, whereas this change was observed in the seated position for the non-athletes. Again, no differences in HF component and LF/HF ratio of SBP variability were found between the athletes and control subjects. In contrast, controlled respiration had no effect on the HF component or the LF/HF ratio of DBP variability in either the supine (C:18.2(4.0) nu or 5.2(1.6); A:16.1(5.2) nu or 10.7(4.2) nu) nor the seated (C:23.6(6.2) nu or 11.7(2.4); A:17.6(4.3) or 9.0(2.2)) position in either group.

Response to standing, exercise, and recovery

Figure 4 (upper panel) shows that the HF spectral components of R-R variability were significantly lower than baseline values (C:48.0(4.0); A:44.2(6.9) nu) in both groups during standing and seated conditions. There was no statistical differences in the HF components observed from seated to either exercise intensities, although a slight non-significant increase was observed at the second exercise level. Similarly the HF values found at either recovery times were not significantly different than those seen in the seated position, although significantly lower than baseline values in both groups and from exercise at 130 beats/min in the athletes. No significant differences between groups were found under any experimental condition. The LF component is shown in the lower panel. Findings are similar to those of the HF component although in the
Figure 4. Spectral power of the low frequency (LF) and high frequency (HF) components of R-R interval variability at rest (baseline), during orthostatic challenges, and recovery from exercise in athletes (closed bars) and control (open bars) subjects. Ex 100 and Ex 130 = submaximal exercise at heart rates of 100 and 130 beats/min; Rec 5 and Rec 15 = post-exercise recovery at 5 and 15 minutes; nu = normalized units. Data are expressed as means and SEM.

* significantly different from baseline value (p<0.05); horizontal line connect conditions that are significantly different (p<0.05).
Figure 5. Ratio of LF and HF spectral powers of R-R interval variability at rest (baseline), during orthostatic challenges, and recovery from exercise in athletes (closed bars) and control (open bars) subjects. Ex 100 and Ex 130 = submaximal exercise at heart rates of 100 and 130 beats/min; Rec 5 and Rec 15 = post-exercise recovery at 5 and 15 minutes. Data are expressed as means and SEM. * significantly different from baseline value (p<0.05).
opposite direction, thus standing and sitting both induced significant increases in the LF component from baseline levels. During exercise LF values remained unchanged or only slightly decreased from the seated condition. The LF component during recovery was not different from values seen during exercise and the seated position but remained significantly higher than those seen under supine baseline conditions. Again no differences were observed between the athletes and non-athletes. As expected from the previous discussion of HF and LF spectral power components, the LF/HF ratio (figure 5) increased from baseline under all the conditions and returned towards baseline during recovery, although values remained higher. Once more, no differences between the two groups were observed.

In order to examine the sensitivity of response of athletes and controls, changes induced with standing or sitting were calculated from baseline values and are shown in Table 3. Results indicate similar magnitudes of relative changes in R-R interval were found in both groups associated with similar increases in LF, decreases in HF, and increases in LF/HF ratio. As expected the greatest magnitudes of change were seen from baseline to standing and from baseline to sitting.

**Blood pressure variability:** Figures 6 and 7 illustrate the spectral power components of beat-to-beat SBP and DBP variability, respectively. Overall as can be seen from figure 6, standing and sitting had no effect on the HF or LF/HF
Table 3. Relative changes in R-R interval, low and high frequency spectral components of heart rate variability in response to orthostatic challenges.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>( \Delta \text{RR} ) (%)</th>
<th>( \Delta \text{LF} ) (%)</th>
<th>( \Delta \text{HF} ) (%)</th>
<th>( \Delta \text{LF/HF Ratio} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline-Sit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-5.23(2.3)</td>
<td>48.31(17.0)</td>
<td>-38.04(11.2)</td>
<td>265.5(85.1)</td>
</tr>
<tr>
<td>A</td>
<td>-5.02(1.7)</td>
<td>27.13(12.4)</td>
<td>-24.6(10.8)</td>
<td>218.1(105.6)</td>
</tr>
<tr>
<td>Baseline-StanC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-21.06(2.7)</td>
<td>74.93(19.3)</td>
<td>-65.15(5.2)</td>
<td>610.9(190.9)</td>
</tr>
<tr>
<td>A</td>
<td>-21.57(2.1)</td>
<td>71.51(27.16)</td>
<td>-50.15(13.2)</td>
<td>508.7(158.0)</td>
</tr>
</tbody>
</table>

Contrasts are relative changes from baseline (supine) or seated conditions. C= control subjects; A= athletic subjects; RR= R-R interval; LF and HF= low and high frequency spectral components of HRV; Mean (SEM).
Figure 6. High frequency (HF) spectral component and LF/HF ratio of systolic blood pressure variability at rest (baseline), during orthostatic challenges, and recovery from exercise in athletes (closed bars) and control (open bars) subjects. Ex 100 and Ex 130 = submaximal exercise at heart rates of 100 and 130 beats/min; Rec 5 and Rec 15 = post-exercise recovery at 5 and 15 minutes; nu = normalized units. Data are expressed as means and SEM.

* significantly different from baseline value (p<0.05); horizontal lines connect conditions that are significantly different (p<0.05).
Figure 7. High frequency (HF) spectral component and LF/HF ratio of diastolic blood pressure variability at rest (baseline), during orthostatic challenges, and recovery from exercise in athletes (closed bars) and control (open bars) subjects. Ex 100 and Ex 130 = submaximal exercise at heart rates of 100 and 130 beats/min; Rec 5 and Rec 15 = post-exercise recovery at 5 and 15 minutes; nu = normalized units. Data are expressed as means and SEM.

* significantly different from baseline value (p<0.05); horizontal lines connect conditions that are significantly different (p<0.05).
ratio of SBP variability. Exercise increased the HF component from the seated condition, with statistical significance being reached in both groups at the higher exercise intensity. A return to baseline values was already observed during the earliest recovery period. Similarly the LF/HF ratio was generally unchanged from baseline during standing and sitting but was decreased during exercise, with the decrease reaching statistical significance in the athletic group. Again a return to baseline values was observed at 15 minutes of recovery. There were no significant differences observed between the athletes and non-athletes. As shown in figure 7, the HF component of DBP variability was not statistically significant between the two groups and was not significantly changed from baseline in any of the experimental conditions. The LF/HF ratio was however decreased in both groups from baseline to exercise at 130 beats/min and from the seated condition to both levels of exercise. Once more, the recovery values had returned to baseline levels within 5 minutes of recovery.
DISCUSSION

Results from the present study indicate that the sympathoadrenal influence on the sinus node at rest is not different between highly trained endurance athletes and healthy but untrained age-matched control subjects. In addition, responses to sitting, standing and exercise were all similar in both groups. These observations are in contrast to the popular belief that training-induced bradycardia results from an increase in resting parasympathetic influence on the sinus node. The controversy surrounding this issue is also apparent from a close review of the literature into the mechanism responsible for training-induced bradycardia using pharmacological blockade in animals and humans. For example, in animals, the response to a parasympathetic blockade using atropine was found to be unchanged (Barnard et al., 1976) or attenuated following endurance-training (Lin & Horvath 1972; Negrao et al., 1992; Tipton & Taylor, 1965). In humans, the comparison of response to the administration of atropine between endurance-trained athletes and non-athletes revealed either no difference (Katona et al., 1982; Raab et al., 1960) or a greater increase in the heart rate of the athletes (Smith et al., 1989). Meanwhile, no change (Maciel et al., 1985) or an increase in the heart rate response (Ekblom et al., 1973) was reported following 10 and 5 weeks of training, respectively. Reasons for the discrepancy in findings may be related to the fact that the administration of atropine may trigger a non-predictable compensatory reflex response.
Heart rate variability analysis is a technique that is non-invasive and non-selective for identifying the vagal influence on the sinus node (Malik et al., 1996). There is to date only a limited number of studies that have used heart rate variability to examine the mechanism responsible for training-induced bradycardia (Bernardi et al., 1990; Dixon et al., 1992; Furlan et al., 1993; Goldsmith et al., 1992). Results indicate either an increase (Dixon et al., 1992; Goldsmith et al., 1992), a decrease (Furlan et al., 1993) or no change (Bernardi et al., 1990) in the high frequency or vagal component of the spectral analysis of heart rate variability. However, because responses were not always reported in normalized units and because confounding factors such as caffeine ingestion or previous exercise training were not controlled for it is difficult to appropriately compare findings. In addition, in the study by Bernardi et al. (1990) the trained subjects did not exhibit a lower heart rate than the untrained subjects, making it difficult to interpret the findings in terms of training-induced bradycardia.

In the present study, athletes were selected for their high aerobic fitness state, as reflected by their VO_{2\text{max}} values which is comparable to that of competitive endurance athletes (McArdle et al., 1991). In addition, testing was always done at the same time of day and subjects were instructed to avoid exercise in the 24-hours previous to testing and to refrain from taking caffeine or medication prior to testing. The present observation of a similar sympathovagal balance in athletes and controls despite a 12-beat lower heart rate in athletes, may be taken to reflect an exercise-induced adaptation in the intrinsic heart rate.
A decrease in the firing rate of isolated atrial tissue has previously been reported in rats following 12 weeks of treadmill running (Schaefer et al., 1992) or 15 weeks of swimming (Bolter et al., 1973). A decrease in the resting heart rate was also found after 12 weeks of treadmill running in rats after sympathectomy and vagotomy (Sigvardsson et al., 1977), suggesting an effect on the intrinsic heart rate. Similarly, using double pharmacological blockade to eliminate both sympathetic and parasympathetic influences on the sinus node, a decrease in the intrinsic heart rate was reported in trained animals (Bamard et al., 1976; Hughson et al., 1976; Lin & Horvath, 1972; Negrao et al., 1992) while endurance athletes were found to have a lower heart rate than controls following atropine and propranolol administration (Frick et al., 1967; Katona et al., 1982; Lewis et al., 1980; Smith et al., 1989). The present observations of no difference in any of the components of the spectral analysis of heart rate variability under any experimental conditions despite the persistence of the lower heart rate in the athletes suggest that training-induced bradycardia is not explained by an increase in the resting vagal influence but could rather be the result of a decrease in the intrinsic heart rate. Alternately, a post-synaptic adaptation such as altered β-receptor density or sensitivity. Reductions in β-adrenergic receptor density have been observed in both miniswine and rats following chronic treadmill training or swimming (Hammond et al., 1987, 1988; Plourde et al., 1991; Werle et al., 1990) which was associated with a marked training
bradycardia (Hammond et al., 1987, 1988). The decrease in receptor density was also associated with a decrease in β-adrenergic sensitivity as reflected by an attenuation in G-protein coupling (Plourde et al., 1991). However, other investigators have failed to demonstrate any change in β-adrenergic receptor density with exercise training in rats (Moore et al., 1982; Takeda et al., 1985; Williams et al., 1984). In the present study, no differences were observed between the spectral components of heart rate variability in the athletes and non-athletes. Considering that the electrocardiographic evidence inherent to heart rate variability determination is a reflection of a post-synaptic event, any ensuing decrease in post-synaptic β-adrenergic receptor density would have had to be compensated for by an increase in their sensitivity.

Another factor impacting on heart rate variability analysis is the unit of measurement. In the present study, when results were reported in absolute and not normalized units, slightly higher although non-significant, total spectral power and HF spectral power were observed (Figure 1). This is similar to what Goldsmith et al. (1992) reported in athletes and can be explained by the fact that the standard deviation of an R-R interval which is larger as a result of a lower heart rate, translates into greater total variance (SD^2) and thus, in a greater total power and consequently, greater HF and LF powers. For this reason, it has been suggested that reporting the frequency spectral power components in normalized units would be more appropriate since they minimize the effect of
change in the total power on the values of the HF and LF components, thereby emphasizing the vagal and sympathetic behavior to the sinus node (Malik et al., 1996). However, as can be seen in the present study from Figure 2, identical LF/HF ratios are obtained either when computed using spectral power expressed in normalized units or in absolute units. Thus, the use of normalized units may not be crucial if comparisons between groups are made on the basis of the ratio of spectral power components.

Variations in the spectral components of heart rate variability observed in the present investigation in response to the various experimental stimuli (controlled respiration, standing, exercise) are in accordance with the general understanding related to heart rate variability responses (Kamath et al., 1993; Malliani et al., 1991a). As previously reported, controlled respiration induced an increase in the HF or vagal component of the heart rate variability (Pagani et al., 1986; Pomeranz et al., 1985) which in the present study has been equally observed with subjects in the supine or in the sitting position. The fact that the observed increase in the HF component was of a similar magnitude in athletes and non-athletes indicates a similar vagal sensitivity to the stimulus. As expected, standing and dynamic exercise both induced a decrease in the HF and an increase in the LF component as compared to the supine resting state. A 34 - 69% decrease in the HF and inversely a 43 - 55% increase in the LF component is generally reported in healthy control subjects aged 16 to 32 years (Dixon et al., 1992; Furlan et al., 1993; Pagani et al., 1986). This is in agreement
with the 41.5 - 65.5% decrease in the HF and 52.5 - 67.8% increase in the LF component found in both our athletic and non-athletic populations, respectfully.

Although there exists limited data on the heart rate variability responses to dynamic exercise, the changes in HF and LF found in the present study at submaximal exercise intensities equivalent to heart rates of 100 and 130 beats/min respectively, are also in agreement with the general direction and magnitude of change reported under similar circumstances (Bernardi et al., 1990; Casadei et al., 1995). The HF and LF spectral power responses to both levels of submaximal exercise were similar in both the athletes and control subjects and may therefore reflect similar patterns of sympathetic and vagal influence on the sinus node. The fact that the LF/HF ratio was similar in both groups at the same heart rate while the workload was higher in the athletes confirms previous findings that the level of sympathetic stimulation is related to the relative rather than the absolute work intensity since the VO\textsubscript{2} during these submaximal exercise bouts were similar in both groups and equivalent to approximately 28 and 50% VO\textsubscript{2max} or heart rate max, respectively. As, expected results from recordings obtained during recovery indicate an decrease in the LF/HF ratio, values having returned to the resting seated baseline in both groups after 15 minutes of recovery although they remained elevated compared to supine values. Again, no differences in the kinetics of return towards baseline were found between groups, suggesting similar rates of vagal reappearance in athletes and controls.
Finally, it is interesting that when reporting spectral components in normalized units, a slight although non-significant increase in the vagal or HF component and conversely a slight decrease in the LF component are seen in both groups as exercise intensity increases. This is contrary to what would normally be expected from our current understanding of the interaction of dynamic exercise and sympathetic and parasympathetic behaviors. Similar findings however, have also been reported in response to dynamic exercise in healthy subjects; the greater the intensity of work the greater the increase in the HF power component (Bernardi et al., 1990; Casadei et al., 1995; Rimoldi et al., 1992). A clear explanation for this observation remains to be provided. The fact that similar findings were reported in response to submaximal exercise in heart transplant patients may be taken to suggest the involvement of a non-neural factor. As can be seen from the reproduction of absolute spectral power of heart rate variability (Figure 1), the latter is significantly reduced upon the first exercise level and is almost completely eliminated at an exercise intensity of 130 beats/min. A similar observation is reported by Casadei et al. (1995) and Yamamoto et al. (1991). The increase in the HF component in normalized unit can therefore not be accounted for by true physiological R-R interval variation. Considering that data acquisition sampling rates in these studies vary between 250 and 600 msec, a sampling error rate between ± 8 msec and 3.2 msec may be calculated which may result in an artifactual variation of the R-R interval induced by cursor identification of the junctional point of the QRS complex. It
follows therefore that when the standard deviation of successive R-R intervals is drastically reduced to values ranging between 4.6 (Bernardi et al., 1990) and 13 msec (Casadei et al., 1995) such as occurs with intensive exercise, the sampling rate error may be equal or very close to the R-R variability itself, introducing a methodological artifact. Considering the high frequency range of these sampling rates, the influence of the sampling rate error is predominantly observed on the HF component. In turn, because expression of the variance using normalized units reflects the proportional contribution of the variability component, an increase in the HF component conversely results in a reduction in the LF component.

**Blood pressure variability**

The present observations indicate a significantly higher SBP blood pressure in athletes than in non-athletes both at rest as well as during standing and exercise. A clear explanation for this remains to be provided. Spectral analysis of beat-to-beat SBP variability show a slightly higher \( p \leq 0.10 \) SBP HF power in athletes than in control subjects. The fact that there was no difference between groups for the HF component of the beat-to-beat variability in R-R suggests that the SBP HF power may not be affected by vagally mediated changes in heart rate. This has also been substantiated on the basis of observations in patients with denervated donor hearts (Bernardi et al., 1989; Peters et al., 1988) or in 24-hour monitoring of blood pressure variability in
hypertensive patients (Malliani et al., 1991b). In fact, it is generally recognized that the HF SBP power is mainly caused by the mechanical effects of respiration (Parati et al., 1995).

The SBP variance observed in the present study for control subjects under supine resting conditions is in agreement with values previously reported in healthy subjects 20-60 years of age (Pagani et al, 1986). Athletes demonstrated a significantly higher SBP variance which however could be related to their higher resting blood pressure. In accordance with the observation of similar DBP mean values in athletes and controls DBP variance was not different between groups.

Standing did not induce any change in either the HF or LF component of the beat-to-beat SBP variability in either athletes or control subjects nor did it affect either the HF or the LF components of the DBP. There exists very few studies that have examined blood pressure variability during standing or tilting in healthy control subjects (Pagani et al., 1986; Veglio et al., 1995) and to my knowledge, none during exercise. When examining the effects of passive head-up tilt, Pagani et al. (1986) and Veglio et al. (1995) both observed significant increases in the LF components and conversely decreases in the HF components of the SBP beat-to-beat variability in their healthy untrained population. Similar results were found for the DBP beat-to-beat variability, except that Veglio et al. found no change in the DBP HF component. The discrepancy in findings may be related to the hemodynamic differences.
associated with passive tilt and active standing or to a difference in the age of
the subjects; Pagani et al's and Veglio et al's subjects ranged in age from 20 and
60 years. Exercise at 130 beats/min resulted in a significant increase in the HF
SBP power from seated rest and conversely a significant decrease in LF while
values for the LF and HF DBP powers remained unchanged in both groups. A
clear explanation for the response in SBP variability remains to be determined. It
should be noted however that the breathing rates recorded during exercise in the
present study increased to values ranging between 15 and 30 breaths/minute
(0.25 to ≤ 0.50 Hz) at the second exercise level. Similarly, the mean HF SBP
power during this exercise was centrally located at a frequency of 0.35 Hz. This
observation may thus be taken to reinforce the contention that the HF SBP
power is mainly caused by the mechanical effects of respiration.

In summary, the present observations indicate that training-induced
bradycardia cannot be attributed to differences in resting sympathovagal balance
or in differences in the kinetics of vagal withdrawal with standing or exercise as
determined through heart rate variability. Similarly, the significantly higher
systolic blood pressure found in athletes can not be attributed to any difference
in the control of blood pressure as reflected by blood pressure variability. These
results may thus be taken to confirm previous observations of a training-induced
adaptation in the intrinsic heart rate of endurance-trained athletes, which cannot
be determined using the heart rate variability technique. Future investigations
may thus be warranted in animals to examine the effects of endurance training
on sinoatrial node membrane electrophysiological properties and β-adrenergic receptor characteristics.
REFERENCES


