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Underestimation of cardiac vagal control in regular exercisers by 24-hour heart rate variability recordings

René van Lien [a,*], Annebet Goedhart [a], Nina Kupper [a,b], Dorret Boomsma [a], Gonneke Willemsen [a], Eco J.C. de Geus [a]

[a] Department of Biological Psychology, VU University, EMGO+ institute, VU medical center, Amsterdam, The Netherlands
[b] CoRPS — Center of Research on Psychology in Somatic diseases, Department of Medical Psychology, Tilburg University, Tilburg, The Netherlands

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ABSTRACT

Objective: To examine whether ceiling effects at long inter beat intervals (IBIs)cause an underestimation of cardiac vagal control in regular exercisers by time and frequency-domain measures of respiratory sinus arrhythmia (RSA).
Methods: 24-hour ECG and respiration recordings were performed in 26 regularly exercising subjects, actively engaged in aerobic training for the past year, and enrolled in supervised training in the six weeks pre-study, and in 26 age- and sex-matched non-exercisers. Sleep and waking levels of cardiac vagal control were estimated by RSA obtained through the peak–valley method, by the standard deviation of the IBIs, the root mean square of successive IBIs, and the high frequency IBI spectral power.
Results: In 11 of the exercisers the IBI–RSA relationship was characterized by a quadratic relationship. This reflected a ceiling effect at very long IBI values attained by regular exercisers, particularly during the nighttime recording. Irrespective of this ceiling effect, RSA as well as other heart rate variability (HRV) measures was still significantly larger in the exercisers with a quadratic IBI–RSA relationship than in non-exercisers or exercisers with a linear IBI–RSA relationship.
Conclusions: We conclude that a subgroup of regular exercisers is characterized by a low heart rate paired to high levels of cardiac vagal control. In these exercisers, vagal control is underestimated from HRV measures in ambulatory recordings. Inspection of the IBI–RSA relationship should be routinely added when HRV measures are used to index cardiac vagal control.

1. Introduction

Regular vigorous exercise is associated with many favorable physiological adaptations, including a lower resting heart rate. Dual blockade studies point to a lower intrinsic heart rate as the most replicated source of this resting bradycardia in exercisers (Lewis et al., 1980; Bouchard et al., 1988; Bouchard et al., 1999; Katona et al., 1982; Smith et al., 1989; Goldberger et al., 2001; Kingwell et al., 1992; Uusitalo et al., 1996) and this is supported by findings in animals (Lin and Horvath, 1972; Negrao et al., 1992). In addition to lowered intrinsic heart rate, exercisers have long been hypothesized to possess a stronger vagal control over the heart rhythm (Ekblom et al., 1973). Animal studies and studies in cardiac patients generally support an effect of exercise on cardiac vagal control (Gutin et al., 2005; Goldsmith et al., 2000; Billman, 2002; Mueller, 2007; Goldsmith et al., 2000) but in healthy human subjects, the evidence for an exercise-induced shift in vagal control is more controversial (Goedhart et al., 2008).

Cardiac vagal control is most often quantified by time- or frequency domain indices of heart rate variability (HRV) in the respiratory frequency range, also called respiratory sinus arrhythmia (RSA) (Berntson et al., 1997; Martinmaki et al., 2006; Nunan et al., 2010; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996). A number of cross-sectional studies reported higher RSA in regular exercisers (Buchheit et al., 2005; Martinmaki et al., 2006; Dixon et al., 1992; Goldsmith et al., 1992; Shin et al., 1997) but not all studies support this difference (Hatfield et al., 1998; Goedhart et al., 2008) and some even report the opposite finding of lower RSA in exercisers compared to non-exercisers (Sacknoff et al., 1994). Notably, various randomized controlled training studies that assigned untrained subjects to a non-exercise control manipulation or a standardized exercise training program have failed to find a specific training-induced increase in RSA (Loimaala et al., 2000; Uusitalo et al., 2004; Boutcher and Stein, 1995; de Geus et al., 1996).

Here we hypothesize that a specific methodological problem in assessing RSA in well-trained exercisers may have led to an underestimation of the beneficial effects of regular exercise on cardiac

* Corresponding author at: Department of Biological Psychology, VU University, Van der Boechorststraat 1, 1081 BT Amsterdam, The Netherlands. Tel.: +31 20 59884508; fax: +31 20 5988832.
E-mail address: r.van.lien@psy.vu.nl (R. van Lien).

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vagal control in previous studies, both cross-sectional and longitudinal. We note that in a meta-analysis of training studies by Sandercock et al. (2005) a significant training-induced increase in RSA was seen during short-term laboratory recordings at rest, but *not* in 24-hour ambulatory recordings. Furthermore, some of the ambulatory studies suggested that training effects on HRV may be confined to the daytime but absent in the whole recording or nighttime levels (Schuit et al., 1999; de Geus et al., 1990). An obvious difference between (laboratory) daytime and ambulatory nighttime recordings is the absolute level of heart rate attained at night. As indicated as early as 1993 by Malik and Camm (1993) RSA may not be a reliable index of cardiac vagal control in subjects with a low heart rate.

RSA is formally defined as the difference between the shortest inter beat interval (IBI) during inspiration and the longest IBI during expiration (Katona and Jih, 1975; Grossman, 1983) and the main physiological rationale to use it as an index of cardiac vagal control is that neural vagal activity is selectively inhibited during inspiration but not during expiration. However, at very high vagal activity, a ceiling effect may prevent lengthening of the IBI during expiration more than during inspiration (Malik and Camm, 1993). High vagal activity causes a large occupancy of the available muscarinergic receptors on the sinoatrial (SA) node, and at this level of saturation any further increases in acetylcholine may no longer linearly increase the IBI as it would at low to moderate levels of cardiac vagal activity (illustrated in Fig. 1).

This ceiling effect is expected to cause a quadratic relationship between IBI and RSA. A quadratic shape of the IBI–RSA relationship has indeed been found in studies manipulating vagal activity by phenylephrine and nitroprusside infusion (Goldberger et al., 2001). The quadratic shape of the IBI–RSA relationship will cause an underestimation of cardiac vagal control by RSA in subjects with low resting heart rates.

Because regular exercisers often have lower resting heart rates than non-exercisers we hypothesize 1) that exercisers more often have a quadratic IBI–RSA curve, 2) that RSA underestimates cardiac vagal control in these exercisers, and 3) that this underestimation is aggravated during 24-hour ambulatory monitoring that includes evening and night time recordings when vagal activity is high.

2. Methods

2.1. Subjects

The 52 subjects came from two studies described in detail elsewhere (Goedhart et al., 2008; Kupper et al., 2005). Briefly, 26 regularly exercising subjects (15 males, 11 females) with a mean age of 38.0 years (SD = 12.2 years) were recruited from several ministries in The Hague and a police station in Amsterdam. During study sign-up it was made explicit that subjects had to be actively engaged in leisure time aerobic training for at least 30 consecutive minutes a day, three days a week for the past year. During actual recruitment this was again confirmed by personal interview. To further ensure that they had been exposed to comparable high levels of vigorous exercise prior to ambulatory recording, they underwent a 6 week program of supervised training at the same fitness center. During this six week period they trained at least 3 times a week, for at least 1 h at a minimal intensity of 70% of the maximal heart rate (measured with a Polar A5 heart rate monitor), which had been established during an all-out test on a bicycle ergo meter (10 min warm-up at 130 bpm followed by two bouts of 60 s bicycling at an increasing resistance until exhaustion).

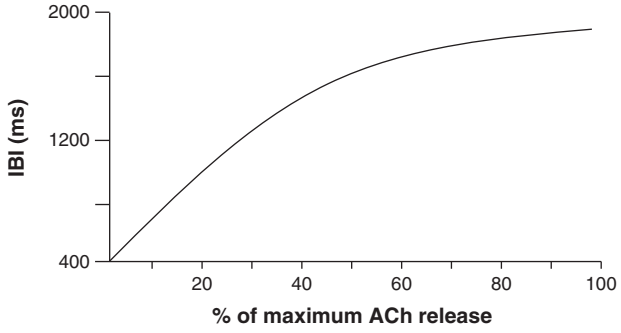

Fig. 1. Predicted relationship between the mean IBI and the release of Acetylcholine [ACh] in the sinoatrial (SA) node. This assumes a 1) near-linear relationship between fractional saturation of the cardiac muscarinergic receptors and the slowing of SA pacemaker cell depolarization, and 2) an asymptotic relationship between [ACh] and the fractional saturation of the cardiac muscarinergic receptors, known as the binding isotherm (Baudiere et al., 1987). Formal models that take into account phasic and tonic aspects cardiac vagal activity arrive at similar predictions (Pyetan et al., 2003).

Twenty-six sex- and age matched sedentary subjects with a mean age of 39.8 (SD = 9.8) were recruited from a family study in which 780 subjects, who had participated in a longitudinal study with biennial surveys on health and health behaviors and underwent 24-h ambulatory recording of RSA (Kupper et al., 2005). To ensure that we selected persistent sedentary subjects, they had to have indicated not to engage in any work-related or leisure time based regular exercise both in the months before ambulatory recording and in at least two past surveys.

All subjects were without a history of hypertension or cardiovascular disease and used no cardioactive medication (e.g. beta-blockers or antidepressants). Ambulatory recording protocols were approved by the Medical Ethics Committee of the VU University and the detraining study was approved by the Ethics Committee of the Faculty of Human Movement Sciences. All subjects gave written consent before entering the study.

2.2. Protocol

Subjects from both studies underwent an identical protocol of ambulatory monitoring using the VU University Ambulatory Monitoring System (VU-AMS). The VU-AMS continuously records the electrocardiogram (ECG) and the impedance cardiogram (ICG) (Goedhart et al., 2007) and produced an audible alarm approximately every 30 min (±10 min randomized) to prompt the subject to fill out an activity diary. Using the activity diary entries in combination with a visual display of the output of an inbuilt vertical accelerometer (measuring movement), the entire 24-h recording was divided into fixed periods. These periods were coded for posture (supine, sitting, standing, walking, bicycling), activity (e.g. desk work, dinner, meetings, watching TV), and physical load (no load, light, intermediate and heavy).

Minimum duration of periods was 5 min and maximum duration was 1 h. If periods with similar activity and posture lasted more than 1 h (e.g., during sleep), they were divided into multiple periods of maximally 1 h. All periods were classified into three main ambulatory conditions: 1) lying asleep, 2) sitting during the day, or 3) mild physical activity (standing/walking) based on the dominant posture/activity reported in that period; the exact timing of changes in posture/activity was verified using the accelerometer signal from the ambulatory device. For the exercisers the 24-hour recordings took place at least one day after a training session; both exercisers and non-exercisers were instructed to keep physical activity at a minimal level during the ambulatory recording day.

2.3. IBI and HRV measures

The ECG and changes in the thorax impedance (dZ) were recorded continuously using six disposable, pregelled Ag/AgCl electrodes as described in detail elsewhere (Goedhart et al., 2008; Kupper et al., 2005).

The IBI time series was obtained from the ECG by an online automated R-wave peak detector, where IBI is the interval in milliseconds between two adjacent R waves of the ECG. Artifact pre-processing was performed on the IBI data. When the IBI deviated more than 3 SD from the moving mean of a particular period it was automatically identified as an artifact and accepted or overruled by visual inspection. Since artifacts cannot simply be deleted because the continuity of time would be lost for frequency analysis, spuriously short IBIs were summed and missing beats were 'created' by splitting spuriously long IBIs.

In the time domain we computed the mean heart rate across the ambulatory conditions as 60,000/IBI. To assess RSA we used the 'peak–valley' method (Grossman and Wientjes, 1986; Goldberger et al., 1994; de Geus et al., 1995; Goldberger et al., 2001; Grossman et al., 1990). In this method, RSA is scored from the combined respiration and IBI time series by detecting the shortest IBI during inspiration and the longest IBI during expiration on a breath-to-breath basis. From the dZ we obtained a continuous respiration signal in which we scored the onset of inspiration and expiration on a breath to breath basis according to the procedures detailed elsewhere (Houtveen et al., 2006; de Geus et al., 1995). Per breath, estimates of peak–valley RSA were obtained by subtracting the shortest IBI in the inspirational phase (which was made to include 1500 milliseconds from the following expiration to account for phase shifts) from the longest IBI in the expirational phase (including 1500 milliseconds from the following expiratory pause/inspirational phase). The shortest IBI in inspiration had to be part of an acceleration in heart rate and the longest IBI in expiration had to be part of a deceleration in heart rate. This means that the selected IBI's not only had to be within the correct part of the respiratory cycle, but also be part of a clear downward or upward slope in the IBI time series. Automatic scoring of RSA was checked by visual inspection of the respiratory signal from the entire recording. Breathing cycles that showed irregularities like gasps, breath holding, coughing etc., were not considered valid and were removed from further processing. In the remaining data the shortest and longest breaths that deviated more than 3 SD from the mean were automatically removed from the entire recording before averaging RSA across all remaining breaths to a single mean value for each of the labeled periods. We discarded on average 14.5% of all automatically scored breaths.

In a small percentage of the valid breaths no respiratory phase-related acceleration or deceleration was found or the shortest beat in inspiration was longer than the longest beat during expiration. It is common practice to set RSA to zero in such breaths, assuming that cardiac vagal control truly is low here. This variable is labeled "RSAzero" in our results. This procedure might, however, bias estimation of RSA to lower values in subjects with a quadratic IBI–RSA relationship, which we expect to be more prevalent in the group of exercisers, because the ceiling effect may cause a larger percentage of breaths with no valid shortest or longest IBI. We will therefore apply an additional strategy that averages RSA only across breaths that have a valid shortest and longest IBI, and a positive RSA value (variable is labeled "RSA" in the results). Finally, various other measures of HRV often used in the literature were computed from the corrected IBI time series. In the time domain we computed the standard deviation of the IBIs (SDNN) and the root mean square of successive IBI differences (RMSSD). The latter was defined as:

$$\mathrm{RMSSD} = \sqrt{\frac{1}{n}\sum(\mathrm{IBIi}-\mathrm{IBIi}-1)^2}.$$

In the frequency domain, total spectral power (TP), very low frequency (VLF), low frequency (LF) and high frequency (HF) power were extracted from the IBI time series by Wavelet decomposition (see Houtveen and Molenaar, 2001 for more information regarding this procedure). Total power was computed as the variance in the 0.0078125–0.5 Hz window, the VLF power as the variance in the 0.0078125–0.0625 Hz window, LF power as the variance in the 0.0625–0.125 Hz window, and HF as the variance in the 0.125–0.5 Hz window.

To be able to detect potentially confounding group differences in respiratory behavior mean respiration rate (RR) was derived for each subject across the three ambulatory conditions.

2.4. Data analysis

As outlined above, the entire 24-h recording was divided into fixed periods of lying asleep, sitting during the day, or mild physical activity. To determine the shape of the relationship between IBI and RSA/RSAzero we further subdivided these periods into bins no longer than 10 min. The mean IBI and RSA/RSAzero were determined per bin and the correlation across these mean IBI and RSA/RSAzero means were depicted in a separate scatter plot for each of the subjects in the study. Two examples for the IBI–RSA relationship are shown in Fig. 2 (full set of scatter plots available upon request from the first author). Significance of the regression weights ($\beta 1$ and $\beta 2$) in the linear and quadratic terms was tested by the SPSS CURVEFIT procedure. To be classified as quadratic, the $\beta 2$ parameters had to be significantly different from zero, the quadratic solution had to explain >20% of the variance in RSA, and the quadratic solution had to improve on the linear solution by at least 10% additional explained variance. Two raters, blinded to exercise status, then verified this algorithmic classification of the scatter plots by visual inspection. Virtual identical classification was obtained when RSAzero was used in the scatter plots rather than RSA. For brevity, we will only use the classification based on the IBI–RSA relationship throughout.

Based on the shape of their IBI–RSA scatter plots, the exercisers and non-exercisers were divided into subgroups with a linear shape of the IBI–RSA relationship and a quadratic shape of the IBI–RSA relationship. To test whether exercisers more often had a quadratic shape than non-exercisers we used a χ^2 test. To test the hypothesis that the different shape of the IBI–RSA relationship in exercisers would underestimate RSA we used a mixed model ANOVA with sex, exercise and shape status as between-subject factors. To test the hypothesis that the potential underestimation of RSA would be larger at night, ambulatory condition (sleep, sitting, mild physical activity) was added as a within-subject factor. Because the distribution of the HRV measures was skewed a logarithmic transform was used for the analyses. Tables and figures present original values for the time domain measures and log-transformed values for the frequency domain measures.

3. Results

In the group of exercisers 11 subjects (7 females) showed a quadratic relationship between IBI and RSA and 15 subjects (8 females) showed a predominantly linear IBI–RSA relationship. In the group of non-exercisers only 2 out of the 26 subjects showed a quadratic IBI–RSA relationship (both males). This group difference is significant ($\chi^2 = 9.57$, $p<0.002$). Typical examples from both groups are shown in Fig. 3.

Based on the observed IBI–RSA scatter plots, the exercisers were subdivided into a group with a linear shape of the IBI–RSA relationship and a quadratic shape of the IBI–RSA relationship. A third group was formed by all non-exercisers (including two non-exercisers with a quadratic shape). No main effects of sex were found and no interactions involving group by sex. Table 1, therefore, depicts the HRV measures as a function of ambulatory condition (night-time sleep, awake sitting, and awake physical active) and exercise/shape status collapsed across males and females. A main effect of condition was found on IBI ($F(2, 49) = 259.55$, $p<0.0001$), SDNN ($F(2, 49) = 64.41$, $p<0.0001$), RMSSD ($F(2, 48) = 10.62$,

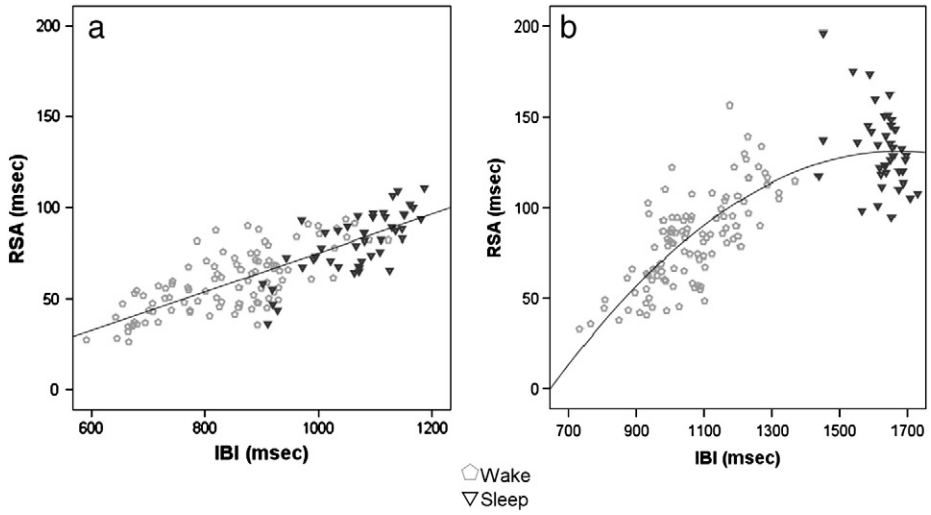

Fig. 2. Scatter plots of the mean IBI and RSA across 10 min bins of a 24 h ambulatory for recording two different individuals (a, b). The IBI–RSA relationship for individual a (N = 167 bins) was judged linear, the IBI–RSA relationship for individual b (N = 127 bins) was judged quadratic. Curves represent the best fitting linear or quadratic function. Data collected during sleep are triangle shaped; data collected during the awake period are pentagon shaped.

$p<0.0001$), RSA ($F(2, 49)=16.95$, $p<0.0001$), RSAzero ($F(2, 49)=22.47$, $p<0.0001$), TP ($F(2, 48)=59.93$, $p<0.0001$), HF power ($F(2, 48)=4.97$, $p=0.011$), LF power ($F(2, 48)=5.02$, $p=0.010$), VLF power ($F(2, 48)=55.87$, $p<0.0001$), and RR ($F(2, 49)=18.62$, <0.0001). IBI, RMSSD, RSA, RSAzero, and HF power were highest during sleep, intermediate during sitting, and lowest during mild physical activity. TP, SDNN, and VLF decreased from sleep to awake sitting but higher levels were again found during physical activity. The increase in TP and SDNN during physically active conditions is likely caused by the increase in VLF that, in turn, is due to the heterogenetic nature of this condition, which included periods of standing alternated with periods of walking or sitting. Respiration

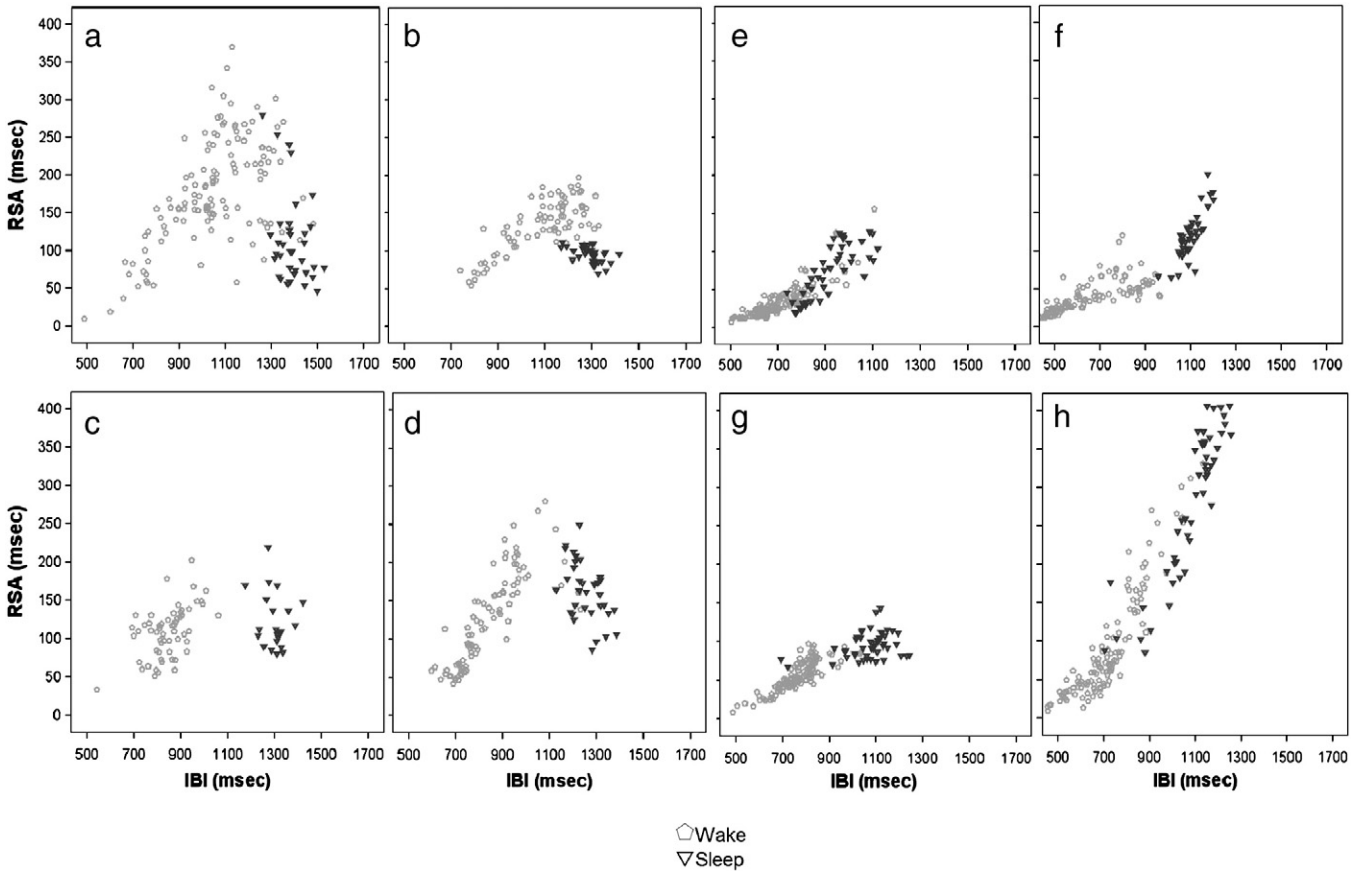

Fig. 3. Representative scatter plots of IBI and RSA for exercisers (a, b, c and d) and non-exercisers (e, f, g and h). The IBI and RSA values were averaged across 10 min bins throughout the 24-hour recording period. Data collected during sleep are triangle shaped, data collected during the awake period are pentagon shaped.

Table 1
Means and standard deviations for inter beat interval (IBI), standard deviation of normal-to-normal RR interval (SDNN), root mean square of successive differences (RMSSD), respiratory sinus arrhythmia (RSA), RSA with missing breaths set to zero (RSAzero), total IBI power (TP), high frequency IBI power (HF), low frequency IBI power (LF), very low frequency IBI power (VLF) and respiration rate (RR) by exercise/shape status and ambulatory condition.

		Sleep		Sitting		Mild activity			
		Mean	SD	Mean	SD	Mean	SD	N	Effects
IBI (ms)	Non-exercisers	927.4	94.9	756.5	73.1	667.8	74.4	26	
	Linear exercisers	989.6	145.8	842.9	105.7	746.1	85.5	15	
	Quadratic exercisers	1235.9	247.2	945.8	165.6	818.9	125.9	11	a,b,c
SDNN (ms)	Non-exercisers	93.3	32.8	66.0	14.6	76.9	15.1	26	
	Linear exercisers	91.3	36.8	70.3	30.7	86.9	29.7	15	
	Quadratic exercisers	137.1	50.2	111.5	44.2	136.9	50.9	11	a,b,c
RMSSD(ms)	Non-exercisers	57.7	41.3	36.9	17.1	33.2	12.2	26	
	Linear exercisers	64.9	47.1	50.5	35.4	46.4	32.6	15	
	Quadratic exercisers	94.8	52.8	79.4	52.0	71.5	43.0	11	a,b
RSA (ms)	Non-exercisers	75.1	48.7	53.6	18.2	45.1	13.2	26	
	Linear exercisers	73.4	51.6	56.1	32.5	47.4	25.7	15	
	Quadratic exercisers	96.7	41.9	107.0	57.4	92.7	51.0	11	a,b,c
RSAzero(ms)	Non-exercisers	66.9	41.5	43.7	17.1	34.6	11.5	26	
	Linear exercisers	63.7	48.6	45.8	28.2	36.8	20.6	15	
	Quadratic exercisers	77.5	33.9	83.4	41.6	70.8	37.6	11	a,b,c
logTP	Non-exercisers	8.7	0.6	8.2	0.4	8.6	0.4	26	
	Linear exercisers	8.7	0.8	8.2	0.9	8.7	0.7	15	
	Quadratic exercisers	9.4	0.7	9.1	0.7	9.7	0.7	11	a,b
logHF	Non-exercisers	6.3	0.9	5.8	0.8	5.7	0.7	26	
	Linear exercisers	6.1	1.3	5.8	1.2	5.7	1.1	15	
	Quadratic exercisers	6.7	1.1	6.9	1.1	6.9	1.0	11	a,b,c
logLF	Non-exercisers	6.4	0.9	6.3	0.7	6.2	0.64	26	
	Linear exercisers	6.2	1.3	6.2	1.1	6.1	1.1	15	
	Quadratic exercisers	7.2	0.9	7.4	0.7	7.3	0.6	11	a,b
logVLF	Non-exercisers	7.2	0.6	7.1	0.5	7.3	0.5	26	
	Linear exercisers	7.8	0.8	7.1	0.8	7.3	0.9	15	
	Quadratic exercisers	8.5	0.7	8.0	0.7	8.3	0.7	11	a,b
RR (br/min)	Non-exercisers	15.6	1.6	17.6	1.3	17.7	0.8	26	
	Linear exercisers	16.2	2.0	17.6	1.0	17.6	0.7	15	
	Quadratic exercisers	16.8	2.2	17.8	1.2	17.7	0.9	11	a

a = Significant main effect of ambulatory condition ($p<0.05$).
b = Significant effect of exercise/shape status ($p<0.05$).
c = Significant interaction between ambulatory condition and exercise/shape status ($p<0.05$).

rate was lower during sleep than during the awake time. Mean 24-hour RSA did not correlate significantly with mean RR ($r=0.06$).

A significant main effect was found of exercise/shape status on IBI ($F(2, 49)=14.75$, $p<0.0001$), SDNN ($F(2, 49)=11.44$, $p<0.0001$), RMSSD ($F(2, 48)=5.36$, $p=0.008$), RSA ($F(2, 49)=6.41$, $p=0.003$), RSAzero ($F(2, 49)=4.71$, $p=0.013$), TP ($F(2, 48)=8.89$, $p=0.001$), HF power ($F(2, 48)=3.68$, $p=0.033$), LF power ($F(2, 48)=6.46$, $p=0.003$), and VLF power ($F(2, 48)=8.50$, $p=0.001$) but not on RR. Post-hoc testing of the effect of exercise/shape revealed that it derived mainly from the difference between the exercisers with a quadratic IBI–RSA relationship and the other two groups. Exercisers with a quadratic IBI–RSA relationship had longer IBIs and significant higher levels of SDNN, RMSSD, RSA, RSAzero, TP, HF, LF, and VLF compared to non-exercisers (p's<0.0001) and also significant higher levels of SDNN, RSA, RSAzero, TP, HF, LF, and VLF compared to exercisers with a linear IBI–RSA relationship. The exercisers with a linear IBI–RSA relationship had a longer IBI but showed otherwise comparable levels of RSA, RSAzero and the other HRV measures to those of the non-exercisers.

The effect of exercise/shape status was different during the awake and nighttime recordings. A significant interaction between exercise/shape and ambulatory condition was found on IBI ($F(4, 49)=6.46$, $p<0.001$), SDNN ($F(4, 49)=2.56$, $p=0.047$), RSA ($F(4, 49)=2.49$, $p=0.05$), RSAzero ($F(4, 49)=2.64$, $p=0.045$), and HF power ($F(4, 48)=3.01$, $p=0.027$). Post hoc analysis revealed a significant difference between non-exercisers, exercisers with a quadratic IBI–RSA relation and exercisers with a linear IBI–RSA relation during the sitting or physical active conditions for all variables but this failed to reach significance during sleep for RMSSD, RSA, RSAzero, HF power and LF power. The interactive effect is illustrated for RSA in Fig. 4.

3.1. Analyses that do not distinguish between a quadratic and linear IBI–RSA relationship

The above analyses suggested that in cross-sectional comparisons between exercisers and non-exercisers, higher RSA levels in the exercisers derive entirely from exercisers with a quadratic IBI–RSA relationship, and that grouping the exercisers with quadratic and

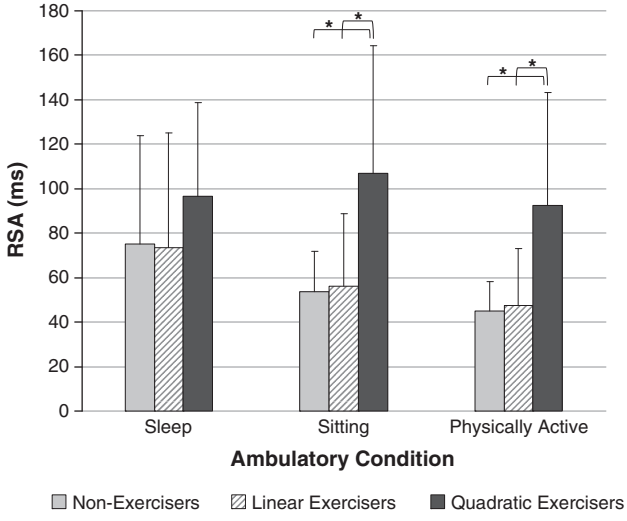

Fig. 4. RSA as a function of ambulatory condition in the non-exercisers and exercisers with a linear or quadratic shape of the IBI–RSA relationship. * = significant group differences at $p=0.05$.

linear shapes might lead to different conclusions. To test this, we repeated the analyses without the distinction between the exercisers with a linear and a quadratic IBI–RSA relationship. In this analysis IBI ($F(1, 50) = 16.3$, $p < 0.0001$), SDNN($F(1, 50) = 6.28$, $p = 0.016$), RMSSD ($F(1, 49) = 5.78$, $p = 0.020$), were still significantly higher in the total group of exercisers when compared to the non-exercisers, but no significant difference was found for the four measures that are most commonly used in the field: RSA ($F(1, 50) = 3.22$, $p = 0.078$) RSAzero ($F(1, 50) = 2.36$, $p = 0.13$), LF power ($F(1, 50) = 1.74$, $p = 0.192$) and HF power ($F(1, 50) = 1.47$, $p = 0.230$).

4. Discussion

Prospective studies have shown that regular vigorous exercise in leisure time (e.g. sports, jogging, aerobics) is associated with a reduced risk for myocardial infarction and sudden death (Powell et al., 1987; Williams, 2001). An exercise-induced increase in cardiac vagal control is one of the mechanisms put forward to explain this reduced risk in regular exercisers (Frick et al., 1967; Lewis et al., 1980; Billman, 2002; Goldsmith et al., 2000; Scheuer and Tipton, 1977; Goldsmith et al., 2000) but the empirical evidence to support this idea has been mixed. Specifically, a large number of studies using time or frequency domain measures of RSA have not found higher RSA in highly trained regular exercisers (Hatfield et al., 1998; Buchheit et al., 2006; Scott et al., 2004; Sacknoff et al., 1994). Here we show that these previous null findings may reflect a systematic underestimation of cardiac vagal control by RSA measures in a substantial (40%) subset of exercisers who are characterized by a quadratic relationship between IBI and RSA. During the day, RSA was significantly higher in these quadratic exercisers compared to linear exercisers and non-exercisers, in keeping with a higher vagal control. At night, however, when the IBI–RSA relationship was in the downward arm of the quadratic curve for quadratic exercisers, the difference between the RSA of quadratic exercisers and the RSA of linear exercisers/non-exercisers was greatly attenuated. The quadratic IBI–RSA relationship likely reflects a high level of fractional saturation of sino-atrial muscarinergic receptors, characteristic of high vagal tone. This is in keeping with the much lower resting heart rate in these exercisers. Comparison of the total group of exercisers with the non-exercisers without taking the IBI–RSA relationship into account, would have lead to the conclusion that exercise is not associated with an increase in cardiac vagal control.

The present study shows that regular exercisers constitute a heterogeneous group with regard to cardiac autonomic regulation. There is a group of exercise with a very low heart rate and a quadratic IBI–RSA relationship and a second group with less dramatic bradycardia and a linear IBI–RSA relationship. A first likely explanation for this dichotomy is difference in the amount and duration of regular exercise between our quadratic and linear exercisers, such that the exercisers with a quadratic IBI–RSA relationship are the more vigorous exercisers. This would be in keeping with previous studies showing that moderate exercisers had higher RSA levels than sedentary subjects but that vigorous exercisers did *not* have higher levels than moderate exercisers (Melanson, 2000; Sandercock et al., 2005) or even RSA that had returned to the sedentary level (Buchheit et al., 2004; Buchheit et al., 2006). In the present study large differences in the amounts of regular exercise are unlikely because we selected only individuals in the exercise group that had been engaged in high levels of regular exercise (3 times a week) for at least a year, the intensity and frequency of which had been under our own supervision in the last six weeks preceding testing.

A second explanation is that the dichotomy within the exercisers reflects innate differences in the responses to exercise. There are large individual differences in extent of cardiorespiratory fitness adaptation to comparable levels of regular exercise exposure (de Geus et al., 1993; de Geus et al., 1990) that appear substantially heritable (Bouchard and Rankinen, 2001). Importantly, these heritable differences in trainability include genetic effects on the bradycardiac response to a standardized training program (An et al., 2003; Rice et al., 2002). It is possible, therefore, that the quadratic exercisers simply have a more favorable genetic make-up that makes them respond to regular exercise behavior with a strong increase in cardiac vagal control. However, it is additionally possible that subjects with a quadratic IBI–RSA relationship posses an innate bradycardia and high vagal control that is partly independent of their exercise behavior. Previously, it has been shown that non-exercisers selected for an innate bradycardia also showed high resting HRV (Boutcher et al., 1998). In our study, high cardiac vagal tone was not limited to exercisers as demonstrated by the two non-exercisers that also showed a quadratic IBI–RSA relationship in spite of reporting not to have engaged in regular exercise in the past years. The highest mean RSA (287 ms) was in fact observed in one of these non-exercisers during sleep (in Fig. 3, subject in the lower right corner). Twin or family studies that explicitly test the heritability of the shape of the IBI–RSA relationship are needed to resolve this.

Three important limitations of this study must be noted. A first limitation is that we have not confirmed higher vagal activity in quadratic exercisers using pharmacological blockade of the muscarinergic receptors. However, in light of our findings, an unresolved question is whether this golden standard would not also suffer from the ceiling effects depicted in Fig. 1. A second limitation is that we cannot exclude that the subjects in the quadratic and linear groups were different on confounding variables that affect IBI, RSA or their relationship. These include, but are not limited to, the type of regular exercise they had been engaged in before the standardized training program, body composition, smoking behavior and alcohol use, oral contraceptives use, recent life stress, social support, and mental health (i.e. depressive symptomatology).

A third limitation is that this study used cross-sectional data only. It is unclear whether training studies that examined the effects of a vigorous training program on RSA have been similarly affected by ceiling effects. Based on our findings we would expect that the underestimation may not be as severe in training studies, because the heart rate differences between exercisers and non-exercisers in cross-sectional studies is often much larger (10–15 bpm) than the heart rate effects of training (3–5 bpm). Nonetheless, underestimation may still occur in the subset of subjects with the largest training-induced decrease in heart rate. In keeping, exercise training studies that did not find a significant training-induced increase in RSA tend to be characterized by significant reductions in heart rate and show low (<60 bpm) post-training absolute heart rate levels (Stein et al., 1999; Loimaala et al., 2000; de Geus et al., 1996; Boutcher and Stein, 1995; de Geus et al., 1990; Uusitalo et al., 2002) whereas training studies that do find an increase in RSA often do not find a significant decrease in resting heart rate, the absolute value of which is in the normal range (around 70 bpm) (Melanson and Freedson, 2001; Sandercock et al., 2005), although there are exceptions (Levy et al., 1998; Schuit et al., 1999). We tentatively conclude that the failure to find a significant increase in RSA in training studies may partly derive from trained individuals with a low post-training heart rate and a quadratic IBI–RSA relationship.

Previous studies using pharmacological manipulation of vagal activity in a laboratory setting had already voiced concerns about the use of RSA as an index of cardiac vagal control under these specific experimental conditions (Goldberger et al., 1994; Goldberger et al., 2001). The present study shows that these concerns extend to recordings made in normal naturalistic conditions, and suggest that the problem is aggravated in exercisers during nighttime sleep recordings. In about 40% of the regular exercisers cardiac vagal control was underestimated by RSA. This underestimation of vagal control by RSA was not specific to the peak–valley method, since other

time domain measures (SDNN, RMSSD) and spectral powers in the low and high frequency range showed an identical pattern. Future studies comparing RSA in exercisers and non-exercisers or studies comparing RSA pre- and post-exercise training should aim to stratify the sample by the shape of the IBI–RSA relationship. More generally, inspection of the IBI–RSA relationship should be routinely added when using HRV measures as an index of cardiac vagal control.

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