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Investigating cardiolocomotor synchronization during running in trained and untrained males

Wollner Materko*, Jurandir Nadal, Antonio Mauricio Ferreira Leite Miranda de Sá

Abstract **Introduction:** This study aims at investigating the coupling of the cardiac and locomotor systems during running in trained and untrained males. **Methods:** Sixteen healthy young males subjects were submitted to an anthropometric evaluation, followed by a treadmill test at 70% to 75% of heart rate reserve. Based on the average velocity, they were divided into two groups of eight, trained group (TG) and untrained group (UT). The electrocardiogram and the electromyogram of the vastus lateralis muscle of the right thigh were digitized at a sampling rate of 1000 Hz, and processed off-line. Each cardiac and electromyographic cycle was detected to further investigate the coupling between cardiac and running rates in time domain, using the cross-correlation, and in the frequency domain, using a phase synchronization measure based on the Hilbert transform. A Shannon entropy index and magnitude squared coherence were also applied to improve analysis. **Results:** Both groups presented low cross-correlation (0.18 ± 0.07 TG and 0.15 ± 0.08 UG) values between these signals, and only four from 16 subjects presented short epochs of phase synchronization (4.1 ± 8.6% TG and 3.2 ± 7.3% UG) between signals, occurring at a low frequency band and random phase differences. The low to moderate coherence (0.67 ± 0.16 TG and 0.64 ± 0.16 UG) observed at 0.1 Hz appears to be an effect of the simultaneous action of sympathetic system over both cardiac and muscular rhythms. **Conclusion:** The combined results suggest that the chosen exercise protocol was not able to cause cardiolocomotor synchronization.

Keywords: Coupling, Cardiolocomotor, Coherence, Running, Synchronization.

Introduction

It is well known that the steady-state heart rate during exercise is closely related to metabolic demands with increasing oxygen supply, blood and energy substrates to sustain the energy demand of muscular action (Wasserman and Whipp, 1975).

For this reason, changes in autonomic modulation between the parasympathetic and sympathetic nervous systems for keeping a steady-state heart rate at various levels have physiological importance. Thus, it has been shown that during light exercise the heart rate response is predominantly mediated by a decrease in parasympathetic activity, whereas at higher levels of work sympathetic stimulation becomes increasingly important (Patel and Zheng, 2012).The heartbeat is influenced by a number of physiological mechanisms, including central interaction and afferentinputs from various receptors, such as arterial baroreceptors,chemoreceptors, and pulmonary stretch receptors (Patel and Zheng, 2012).

The synchronization is the interaction of various physiological rhythms that are generated by nonlinear dynamic system (Glass, 2001). Former studies have investigated the synchronization between different biological rhythms, such as cardiac and locomotor (Blain et al., 2009; Kirby et al., 1989; Niizeki, 2005; Nomura et al., 2003; Nomura et al., 2006), locomotor and respiratory (McDermott et al., 2003; O'halloran et al., 2012) and cardiac and respiratory (Busha, 2010; Santos et al., 2010). Some studies focused on phase synchronization, which is characterized as the phase coupling of some spectral components, with the amplitudes of signals remaining irregular and uncorrelated (Pikovsky et al., 2000; Tass et al., 1998).

In this sense, cardiac and locomotor synchronization have been associated with the presence of a neuronal circuit that modulates the cardiac pacemaker activity during the period of muscle contraction that exhibit nonlinear behavior (Nomura et al., 2003; Nomura et al., 2006).

Previous studies have shown that cardiac and locomotor synchronization occurs during walking (Kirby et al., 1989), running (Nomura et al., 2003; Nomura et al., 2006) and cycling (Blain et al., 2009; Nomura et al., 2003), hypothetically to optimize the blood flow supply to contracting muscle (Niizeki, 2005), therefore, favoring the oxygen transport to active muscles (Whipp and Ward, 1982) and, consequently,

*e-mail: wollner.materko@gmail.com
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minimizing the energy cost of cardiac muscle contraction (Funk et al., 1989).

The physiological adaptations associated with training are characterized, among other changes, by marked alterations in the cardiorespiratory system and the metabolic patterns during exercise. In this sense, maximal exercise performance is characterized by increases in maximal oxygen uptake (VO_2max), and submaximal exercise endurance presents increased cardiovascular function, with increases in heart rate and stroke volume, to adapt the cardiac output to the physiological needs. As a consequence of training, a cardiorespiratory conditioning occurs, with increases in cardiac dimensions and ventilatory efficiency (Bernasconi and Kohl, 1993).

As most of the research indicates that experience results in an increase in energetic economy and that stronger coordination may increase running performance (McDermott et al., 2003), it was hypothesized that a higher level of running experience would lead to stronger coordination between physiological rhythms during running, therefore, improving the cardiolocomotor coupling. Thus, when comparing trained and untrained subjects, it is expected a better synchronization in the group of trained subjects (Bračič Lotrič and Stefanovska, 2000), which is evidenced during the epochs of cardiorespiratory (Schäfer et al., 1998) and locomotor-respiratory (McDermott et al., 2003) synchronization. The purpose of the present study was to investigate the coupling of the cardiac and locomotor systems during running in trained and untrained males.

Methods

Subjects

This study was designed as a cross-sectional, community-based survey, in which participants were randomly drawn from gym academies in Rio de Janeiro city, Brazil. After anthropometric measurements, 16 healthy male volunteers were submitted to a treadmill test. All subjects were nonsmokers, with no history of cardiopulmonary disease, and none was taking any medication.

The tests were conducted in a quiet room with temperature maintained at 22 °C. All volunteers were instructed to avoid strenuous activity in the 24 hours prior to each testing session and to avoid alcohol, caffeine, smoking as well as the consumption of large meals for, at least, three hours prior testing, and to maintain well-hydrated during the exercise.

The study protocol was approved by a local Ethical Human Research Committee (protocol: 257.728), and conducted according to the instructions of the Helsinki Declaration of 2008. Each subject was oriented about testing procedures and time commitment required for participation in this study and signed a written informed consent prior to any procedure.

Anthropometric measurements

The volunteers were assessed for height, body mass, age and skinfold measurement (Table 1). The height was measured in centimeters, while the body mass was measured in kilograms with a mechanical scale (Filizola, Brazil). A skinfold caliper (Cescorf, Brazil) was used to take skinfold measurement. Body density (Jackson and Pollock, 1978) was estimated based on skinfolds and the percent body fat was determined based on body density using the Siri (1961) equation:

$$Body\ Fat = \left(495 / Body\ Density\right) - 450 \qquad (1)$$

Experimental protocol

The tests were conducted on a treadmill 1580Itv (Bayaction, Brazil) and the protocol was accomplished in four phases: (1) passive resting - 5 min; (2) warm-up - 5 min period at 5 km/h speed; (3) test -10 min 70% to 75% of heart rate reserve, speed ranged from 5 and 12 km/hat 0% grade; and (4) recovery - 5 min of active resting (5 km/h speed) followed by a 5 min of passive rest period.

The maximum heart rate was estimated as proposed by Inbar et al. (1994):

$$HR_{\max} = 205.8 - 0.68 \times age \qquad (2)$$

According to Robergs and Landwehr (2002), this is the estimate that presents the best correlation coefficient and the least standard error among a set of different methods.

Afterward, lower and upper limit of heart rate reserve for each volunteer were calculated, according to Equations 3 and 4, respectively (Karvonen et al., 1957):

$$HRR_{lower} = \left(HR_{\max} - HR_{rest}\right) \times 0.70 + HR_{rest} \qquad (3)$$

$$HRR_{upper} = \left(HR_{\max} - HR_{rest}\right) \times 0.75 + HR_{rest} \qquad (4)$$

where HR_{rest} is the resting heart rate, HR_{max} - HR_{rest} heart rate reserve.

The heart rate was thus monitored during the test with a FT1 monitor (Polar, Finland). For the first 2 min of the measurement period, the treadmill speed was gradually increased until a target of 70-75% heart rate reserve was achieved, and it was then maintained at that speed throughout the remainder of the test session,

Table 1. Physical and anthropometric characteristics of the sample.

Variable	Trained Group	Untrained Group
Age (years)	23.7 ± 5.1	24.1 ± 3.3
Height (cm)	173.7 ± 6.5	174.2 ± 4.6
BM (kg)	77.5 ± 8.5	82.4 ± 12.1
BFP (%)	16.4 ± 5.1	17.1 ± 4.8

BM=body mass; BFP=body fat percentage; values are mean ± standard deviation

for 8 min. Therefore, this period were extracted and used for analysis.

Instrumentation

The surface electromyogram (EMG) was measured by a system CNX_04 (EMG System, Brazil) using a differential amplifier with gain 1000, 120 dB common mode rejection and band limited between 20 Hz and 500 Hz, first order. A specific channel of this system was for the acquisition of the electrocardiogram (ECG) in the modified lead DII to improve R wave amplitudes.

All signals were acquired and simultaneously digitized using a data acquisition board model NI USB-6008 (National Instruments, USA), with 12-bits resolution analog-to-digital converter and dynamic range ±5 V, which provides a quantization error of 1.22 μV with the gain 1000, at a sampling rate of 1000 Hz and stored on a microcomputer for offline processing. All procedures for the acquisition and control data were performed in the software DAS (Pino et al., 2004), build in LabVIEW version 5.0 (National Instruments, USA) in a micromputador with Windows 7 operating system (Microsoft, USA).

Physical conditioning assessment

To investigate the hypothesis that cardiolocomotor coupling is influenced by physical conditioning, the 16 subjects were divided into two groups, eight subjects each, following the percentile of the average velocity measured during treadmill running: trained group (percentile > 50, 11.5 ± 0.6 km/h; mean ± standard deviation) and untrained group (percentile ≤ 50, 7.5 ± 0.3 km/h).

Data collection

The skin was prepared by shaving the hair, abrasion with sandpaper and alcohol cleaning. The EMG signal was measured from the right vastus lateralis muscle using bipolar leads. Surface electrodes of silver-silver chloride (Ag/AgCl) disposable Kendall Meditrace 200 (The Ludlow, Chicopee, USA) were placed along the longitudinal axis of the muscle, approximately 10 cm superior to the head of the fibula to avoid EMG baseline wandering by leg motion; such that the inter-electrode distance was set 20 mm, according the procedure of SENIAM (Hermens et al., 1999). The ECG signal was measured following the procedure of Misra (2010), with electrodes attached in the fourth intercostal space on the left side near the sternum (positive polarity) and on the midline between the first electrode and the right shoulder (negative polarity). The reference electrode was placed on the styloid process of the right ulna.

Signal processing

All signal processing was developed using programs written in Matlab version 6.5 (The MathWorks, USA). Besides, all measurement units for acquiring of the amplified signals at the input of the AD converterhave been considered in Volts.

Firstly, each cardiac and electromyographic cycle was detected. Subsequently, the synchronization was analyzed in time domain, using the cross-correlation function, and in the frequency domain, using a phase synchronization test and spectral coherence function.

Heartbeat detection

To measure the beat-to-beat RR intervals (RRI), the ECG signal was pre-processed by a 2th order Butterworth band-pass filter with cutoff frequencies 5 and 20 Hz, applied in both direct and reverse directions of the signals to avoid phase distortions. The instants of the peaks of R waves were detected through zero crossing of the derivative of the filtered signal. Thus, the algorithm selected all signal peaks above a threshold of 0.5 V and removed the peaks whose refractory period were less than 250 ms. In addition, the algorithm allowed manual editing with visual inspection, in order to exclude ectopic heartbeats.

Muscle activity detection

The myoelectric activity of right vastus lateralis muscle is maximal approximately at the initial contact of the right foot with the treadmill surface during running, and the rhythm of the muscle contraction reflects the locomotor rhythm. The muscle contraction interval (MCI) was defined as the interval between the onsets of two consecutive muscle contractions. The EMG signal was firstly submitted to a digital filter proposed by Mello et al., (2007), which included a 2th order Butterworth high-pass filter with cutoff frequency 10 Hz and a 4th order Butterworth low-pass filter with cutoff at 400 Hz. In addition, six 1st order stop-band filters, 2 Hz bandwidth, centered at 60 Hz and its harmonics until 360 Hz, to remove mains noise. The resulting filter was applied in both direct and reverse directions of the signals to avoid phase distortions.

The Teager–Kaiser energy operator (TKEO) (Kaiser, 1990) was thus applied to the filtered EMG to improve MCI measurements, according to equation:

$$TKEO(n) = emgfilt^2(n) - emgfilt(n+1)emgfilt(n-1) \quad (5)$$

where *emgfilt* is the filtered EMG signal and *n* is the sample number.

Subsequently, the *TKEO* signal was filtered by a 2nd order Butterworth low-pass filter, with cutoff at 3 Hz, also applied bidirectionally, and used to calculate the root mean square (RMS), to obtain the envelope of the muscle activity.

Finally, the algorithm for detecting cycles of muscle contraction was applied. It consists in detecting zero crossings in the derivative envelope signal, and selecting all signal peaks above a threshold of 0.04 V^2. The signal is then transformed into a binary signal with muscle contraction and no muscle contraction represented by 1 and 0, respectively. A manual editing was performed by visual inspection to avoid detection errors.

The sequences of RRI and MCI were interpolated by cubic splines and resampled with a frequency of 5 Hz to obtain equally sampled signals *RRI*(*t*) and *MCI*(*t*).

Synchronization analysis

The cross-correlation analysis between the gait and the cardiac signals was adapted to evaluate the strength of coupling between heart rate and locomotion in time domain, according to equation:

$$C_{RRI,MCI}(k) = \sum_{k=-\infty}^{\infty} RRI(t)MCI(t+k) \quad (6)$$

where *k* is the time lag.

The interaction between the rhythms in the frequency domain was studied using a phase synchronization measure based on the Hilbert transform (Rosenblum et al., 2004).This method is based on fitting a linear function to the instantaneous phase difference of analyzed signals in a moving window. Synchronization is accepted when this regression is constant along the window.

In order to emphasize the low frequency (LF) band associated with the heart rate regulation process, the signal *RRI*(*t*) was band pass filtered by a bidirectional 4th order Butterworth filter, cutoff frequencies 0.05 Hz and 0.15 Hz, removing very LF oscillations and high frequency (HF) oscillations predominantly associated with respiration, in order to emphasize the frequency band related to sympathetic activity during running. The same filter was also applied to *MCI*(*t*)

The first step in quantifying phase synchronization between two signals is to determine their phases. To calculate the phase of heart rate and muscle contraction the analytic signal $\varsigma(t)$ was estimated for the signal *X(t)* obtained as a result of bandpass filtering of *RRI*(*t*) or *MCI*(*t*). The signal $\varsigma(t)$ is a complex function of time defined as:

$$\varsigma(t) = X(t) + jX_H(t) = A(t)e^{j\varphi H(t)} \quad (7)$$

where $j = \sqrt{-1}$, *A(t)* and $\varphi(t)$ are, respectively, the amplitude and the phase of the analytic signal, and $X_H(t)$ is the Hilbert transform of *X(t)*, given by:

$$X_H(t) = \pi^j P.V. \int_{-\infty}^{\infty} \frac{X(\tau)}{t-\tau} dt \quad (8)$$

where *P.V.* means that the integral is taken in the sense of the Cauchy principal value. The phase $\varphi(t)$ from equation 7 is defined as:

$$\varphi_X(t) = \arctan \frac{X_H(t)}{X(t)} \quad (9)$$

Afterward, the phases of both signals were submitted to an unwrap function, which corrects the phase angles to produce a continuous phase vector of each signal by adding multiples of ±2 π radians.

For various subjects, it was observed epochs of signals where the ratio of two heartbeats per cycle of muscle contraction occurs (Figure 1). Thus, to detect synchronization between the LF heart rate and muscle contraction oscillations the instantaneous phase difference was calculated according to:

$$\varphi_{RRI,MCI} = \phi_{RRI}(t) - 2\phi_{MCI}(t) \quad (10)$$

where: $\phi_{RRI}(t)$ is the phase of LF oscillations of heart rate and $\phi_{MCI}(t)$ is the phase of LF oscillations of muscle contractionand $\varphi_{RRI,MCI}$ is the generalized phase difference, or relative phase, considering in this case the relationship 2:1 – two RRI for each MCI.

For the detection of epochs of phase synchronization, it was developed an algorithm based on a linear

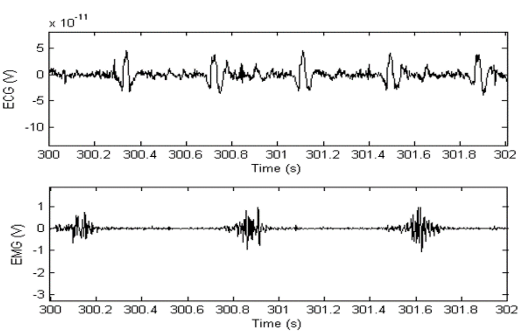

Figure 1. Segments of an ECG signal (above) and EMG signal (below) in one subject studied here.

approximation of the instantaneous phase difference in moving windows with 10 s width.To each window, a straight line is fitted and the respective angular coefficient is taken as the phase difference estimate α_i related to the time t_i corresponding to the middle of the window. By moving the window one point forward along the time series of instantaneous phase difference, the estimate α_{i+1} is obtained for t_{i+1} and so on. In the regions of phase synchronization the relative phase exhibits plateaus resulting in small values of $|\alpha|$. A given region will be candidate for synchronization episodes if $|\alpha|$ is below the threshold based in Student's *t* probability density function. A second necessary condition for the detection of synchronization is a sufficiently large duration of the region of small $|\alpha|$values. The duration of this region should exceed 16 s to exclude short regions with a high probability of accidental coincidence of instantaneous phases of oscillations (Karavaev et al., 2009).

All epochs of synchronization were detected in the phase differences plot, and the respective time duration were summed and thus expressed in percentage of the duration *T* of the entire record. The percentage of phase synchronization was obtained for each subject by:

$$S = \frac{\sum_{K=1}^{N} d_k}{T} \times 100 \tag{11}$$

where *dk* is the duration of the *k*th epochs of synchronization, *N* is the number of epochs and S is the percentage of phase synchronization for the subject.

Estimation of statistical significance of phase synchronization

As soon as the phases of two signals are estimated by appropriate methods, the relationship between phases can be quantified. Phase synchronization in noisy systems can be understood in a statistical sense as the appearance of a peak in the distribution of the cyclic relative phase:

$$\psi_{RRI,MCI} = \phi_{RRI,MCI} \bmod 2\pi \tag{12}$$

An index can be based on the Shannon entropy of the distribution of the cyclic relative phase. The entropy describes irregularity, complexity or level uncertainty of a signal (Johnson and Shore, 1984). It is defined as:

$$\tilde{\rho}_{n,m} = \frac{S_{\max} - S}{S_{\max}} \tag{13}$$

where $S = -\sum_{k=0}^{N} P_k \ln P_k$ is the entropy of the distribution of the cyclic relative phase, Smax = 1n N, and N is the number of bins used for the distribution estimation.

An estimator of the total bin number is given for *T* data points by:

$$Bin = \exp(0.626 + 0.4\ln(T-1)) \tag{14}$$

For uncoupled oscillators the distribution of the cyclic relative phase is uniform, whereas the interaction makes this distribution unimodal, or bimodal in the case of 2:1 relationship. As the $\rho_{n,m}$ index is normalized, $0 \le \rho_{n,m} \le 1$, $\rho_{n,m} = 0$ corresponds to a perfectly predictable unimodal distribution and $\rho_{n,m} = 1$ corresponds to the complete independence between signals that generated the cyclic relative phase (Tass et al., 1998).

Spectral coherence

The power density function of each signal was estimated in epochs of 25 s, by applying a Hamming window and using an autoregressive model (AR) in order 20, Burg's method, as proposed by Martinmäki et al. (2006).

The auto-spectra of *RRI*(*t*) and *MCI*(*t*) and the cross-spectrum of these signals were thus estimated, respectively, by:

$$\hat{S}_{RRI,RRI}(f) = \frac{1}{M.T_N} \sum_{i=1}^{N} \left| RRI_i(f) \right|^2 \tag{15}$$

$$\hat{S}_{MCI,MCI}(f) = \frac{1}{M.T_N} \sum_{i=1}^{N} \left| MCI_i(f) \right|^2 \tag{16}$$

$$\hat{S}_{RRI,MCI}(f) = \frac{1}{M.T_N} \sum_{i=1}^{N} \left| RRI_i(f)^* MCI_i(f) \right|^2 \tag{17}$$

where M, is the number of windows, T_N is the duration of each window (25 s); * is the complex conjugate, $RRI_i(f)$ and $MCI_i(f)$ are power spectral density of the i^{th} epoch of *RRI*(*t*) and *MCI*(*t*), respectively.

To estimate the frequency interaction between the RRI and MCI, the magnitude-squared coherence function $\hat{\gamma}^2(f)$ was calculated by:

$$\hat{\gamma}^2(f) = \frac{\left| \hat{S}_{RRI,MCI}(f) \right|^2}{\hat{S}_{RRI,RRI}(f)\hat{S}_{MCI,MCI}(f)} \tag{18}$$

where $\hat{\gamma}^2(f)$ have values ranging from zero (uncorrelated signals) to unity (linearly dependent signals at frequency *f*).

Subsequently, Fisher's Z transform has been used to approximate the probability density function of the coherence estimates to a Gaussian distribution.

The magnitude-squared coherence differs from phase synchronization, since the coherence does not distinguish the effects of amplitude and phase in the interrelations between signals.

Statistical analysis

Descriptive statistical analysis of the data was expressed as mean ± standard deviation. The Lilliefors test confirmed the normality of sampling distribution. The difference in anthropometric and physical characteristics between trained group and untrained group was tested through Student's paired t test for independent variables. The results of the cross-correlation, coherence and percentage of phase synchronizationof the trained group and untrained group were comparedby analysis ofvariance (ANOVA) one way, and the post-hoc Tukey's test applied whensignificant differences occur. Finally, the number of heartbeats per cycle of muscle contraction for trained group and untrained group was tested through Student's t test one sample.All procedures assumed $p \leq 0.05$ for statistical significance. All data were processed in the Matlab v 6.5 (Mathworks, EUA).

Results

No significant differences were found between trained group and untrained group about the anthropometric and physical characteristics, representing a homogeneous group of volunteers.

Both groups presented the same variation of RRI during the tests, 0.39 ± 0.05 s, with the heart rate ranging from 152 to 158 bpm in trained subjects and from 154 to 160 bpm in untrained ones. Also the variation of the MCI presented no significant difference between groups, 0.73 ± 0.03 s for untrained group and 0.70 ± 0.02 s for trained subjects.

The results for the cross-correlation between *RRI*(*t*) and *MCI*(*t*) were lower for both groups, 0.18 ± 0.07 in trained group (Figure 2a) and 0.15 ± 0.08 in untrained group (Figure 2b), showing no significant difference ($p > 0.05$). Furthermore, the correlation between these signals presented peaks at different lags, showing variable delays between signals.

Figure 3 illustrates the phase from signals *RRI*(*t*) and *MCI*(*t*) obtained by the Hilbert transform approach after the unwrap procedure for the two subjects, one from each group, which presented RRI:MCI ratios nearest to 2:1. Both trained (Figure 3a) and untrained (Figure 3b) subjects presented smaller values of relative phase than the average of the respective group. In addition, the untrained subject presented increased variability of *RRI*(*t*), which also appears in the respective relative phase (Figure 3d). Just two of the eight subjects for each group presented epochs of synchronization (as examples in Figures 3c and 3d).

The total time of synchronization corresponded to 4.1 ± 8.6%, equivalent to 19.6 ± 41.2 s for trained group and 3.2 ± 7.3%, equivalent to 15.3 ± 35 s for untrained group, showing no significant difference ($p > 0.05$).

The Shannon entropy index resulted in 0.80 ± 0.02 for subjects in trained group and 0.78 ± 0.01 for untrained subjects, with no significant difference between groups.

The untrained group showed peaks of magnitude squared coherence ($\gamma^2 = 0.57 \pm 0.05$) located at the frequency 0.10 ± 0.04 Hz, while the trained group showed similar values ($\gamma^2 = 0.62 \pm 0.08$), but in a wider frequency range of 0.17 ± 0.11 Hz. After applying the Fisher z-transformation, the γ^2 values 0.67 ± 0.16 and 0.64 ± 0.16 for the trained and untrained groups, respectively, showed no significant differences ($p > 0.05$).

The number of heartbeats per cycle of muscle contraction varies for each subject in both groups (Table 2), resulting in average 1.84 ± 0.11 heartbeats per gait cycle in the untrained group and 1.85 ± 0.08 in the trained group, with no significant difference ($p < 0.05$).

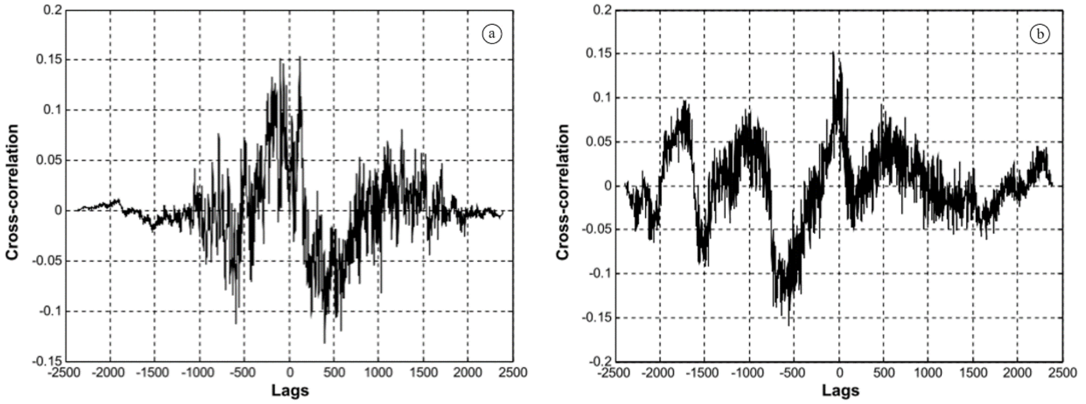

Figure 2. Cross-correlation between RRI and MCI signals in one subject of the trained group (a) and untrained group (b). Lags is N–1:N+1 and N is total sample.

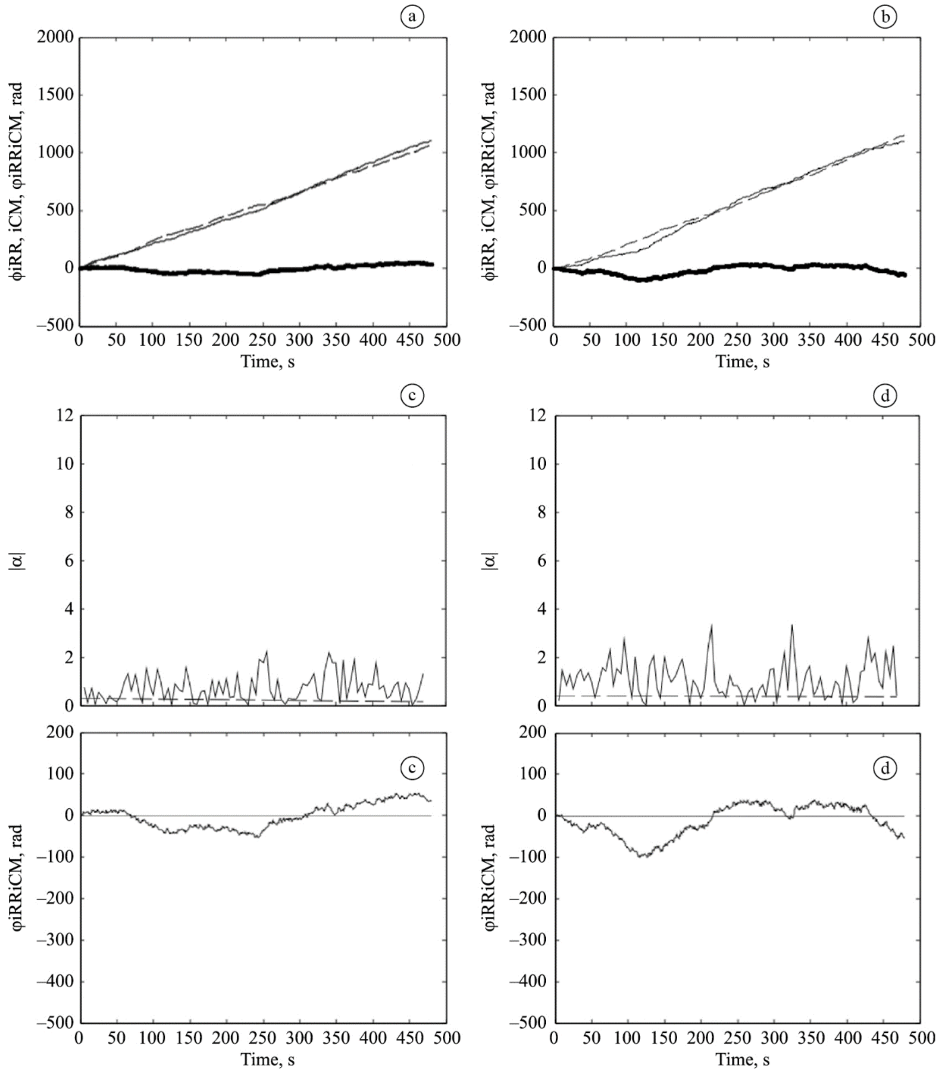

Figure 3. The phase RRI and MCI signals and the phase difference are show by continuous line, dotted line and dark black line, respectively, in one subject (number four for both group) of the trained group (a) and untrained group (b).The linear approximation of the instantaneous phase difference of in a moving window, in the subject four number of the trained group (c) and untrained group (d), respectively. |α| is slope of the approximating line, continuous line is instantaneous phase difference in zero and dotted line is the threshold based in Student's *t* probability density function that demonstrate the presence of the phase synchronization.

Table 2. The number of heartbeats per cycle of muscle contraction for each subject of the trained and untrained group.

Subjects	Number of heartbeats per cycle	of muscle contraction
	TrainedGroup	UntrainedGroup
1	1.81	1.75
2	1.78	1.88
3	1.80	1.92
4	2.00	1.97
5	1.92	1.76
6	1.77	1.65
7	1.80	1.97
8	1.93	1.88
mean ± sd	1.85 ± 0.08	1.84 ± 0.11

Discussion

This study aimed at identifying the phase synchronization of the cardiac and locomotor systems during running in groups of trained and untrained males with similar anthropometric and physical characteristics. The results did not confirm the investigated interaction between the oscillating processes, excepting the higher coherence at frequencies around 0.1 Hz, which sounds a weak evidence of coupling between human heart rate and muscle contraction.

Various studies suggest the presence of cardiolocomotor synchronization in physical exercise

(Blain et al., 2009; Kirby et al., 1989; Niizeki, 2005; Nomura et al., 2003; Nomura et al., 2006). Nevertheless, differently from the present work, none of these studies investigated the coupling at different fitness levels or the instantaneous phase difference between signals. The latter has being employed to analyze synchronization cardiorespiratory (Bartsch et al., 2007) and between cardiovascular variables (Karavaev et al., 2009).

Exercise acts as a perturbation in the system that alters the mean cardiac and locomotor frequencies and changes the amount of their modulation by low-frequency oscillations, mainly related to sympathetic regulation, which occurs principally when the effort is anaerobic. Considering that a cardiolocomotor coupling occurs by some physiological reason, the hypothesis is that trained subjects will better explore this phenomenon. Additionally, the untrained ones are expected to be most affected by fatigue, which could become the most important factor affecting the synchronization between these systems (Kenwright et al., 2008).

The time domain analysis based on cross-correlation between $RRI(t)$ and $MCI(t)$ shows the absence of correlation between rhythms, since it resulted small correlation peak values at variable delays between signals, therefore justifying the need of use other approaches, as phase coupling (Pikovsky et al., 2000; Tass et al., 1998).

The analysis of phase was firstly addressed by the approach used by various authors (Tass et al., 1998; Pikovsky et al., 2000): the difference of the unwrapped phases determined by the Hilbert transform of $RRI(t)$ and $MCI(t)$. The method is based on testing the linear approximation of the instantaneous phase difference between signals in a moving window. In the present study, an objective assessment was introduced, based on the Student t distribution density function.

The results suggested the occurrence of short epochs of phase locking between cardiac and locomotor rhythms, without significant difference between groups. In accordance, Bartsch et al. (2007) studied cardiorespiratory phase synchronization during different sleep stages for a large database of healthy subjects and also observed short epochs of phase locking between cardiac and respiratory rhythms: 0.6% during REM sleep and 3.8% during non-REM sleep. Conversely, Karavaev et al., (2009) reported high percentage of phase synchronization between resting electrocardiogram and blood pressure for healthy individuals ($34.4 \pm 16.1\%$) and patients after acute myocardial infarction ($16.0 \pm 9.5\%$).

Considering the occurrence of synchronization, it is reasonable expecting a same phase difference between signals. Thus, the Shannon entropy was applied over the cyclic signal obtained by wrapping the relative phase to investigate the possible occurrence of distribution peaks. This index quantifies the complexity or irregularty of a distribution (Johnson and Shome, 1984). The obtained results, with entropies around 0.8 for all subjects, describe almost homogeneous distributions of the cyclic relative phase, contrarily to the expected with synchronization.

To a deeper frequency analysis, the magnitude squared coherence was employed. Higher coherence peaks were observed around 0.10 and 0.17 Hz for untrained and trained subjects, respectively, without significant difference. This result is compatible with the frequency band of sympathetic activity, thus justifying the use of a preprocessing band pass filter adopted in the phase analysis. The coherence values reduces with the increase of frequency, as observed by Niizeki et al. (1993), who obtained $\gamma^2 = 0.2$ at a frequency of 0.5 Hz. However, why the phase analysis did not confirm the possible synchronization suggested by the coherence at low frequencies?

Two hypotheses of physiological mechanisms emerge as responsible for the synchronization: (1) A peripheral neural circuit in which afferent signals from mechanoreceptors within active skeletal muscle may modulate the heartbeat interval (Legramante et al., 2000), by inhibiting the cardiac vagal component of the baroreceptor reflex and playing a role in shortening the heartbeat interval (McWilliam and Yang, 1991); and (2) A non-neural mechanism, such as an intrinsic property of the heartbeat interval to shorten via mechanoelectric feedback, within the myocardium is stimulated by stretching of the atrial wall associated with the diastolic volumetric load (Kohl et al., 1999).

On the other hand, it is known that sympathetic system is used by the central nervous system to simultaneously activate various body organs during exercise. Thus, both cardiac and muscle activity are increased with low frequency stimulus, mainly when the vagal modulation is strongly reduced (Malliani et al., 1991; Victor et al., 1995). By considering this, high low frequency coherence should be expected, independently of the occurrence of synchronization. In this sense, the present study is not conflicting former studies that have observed interactions between cardiac (Malliani et al., 1991) and locomotor (Victor et al., 1995) rhythms at low frequency, which were also related to sympathetic activity. Karavaev et al., (2009) investigated the low frequency synchronization between the blood pressure and heart rate signals, governing the cardiovascular dynamics in humans with a fundamental frequency close to 0.1 Hz, was considered to be a Mayer wave, which is a physiological marker of sympathetic activity.

The adopted protocol consisting of 10 min of running at 70-75% of the heart rate reserve (152-160 beats.min^{-1}) is similar to the study by Nomura et al., (2003) who studied ten healthy subjects performed during running at 150 beats.min^{-1}, while electrocardiograms and electromyograms were monitored continuously and these results suggested the occurrence of periods of coupling between cardiac and running rhythms. However, at this load the studied subjects presented a mean ratio of 1.85 heartbeats per running cycle (ranging from 1.65 to 2.00), which does not represent the most favorable condition for the eventual occurrence of coupling in a 2:1 proportion pointed out by Kirby et al. (1989) and Nomura et al. (2003). The different results could be attributed to differences among studies groups, and the exercise protocols.

We can hypothesize that an increase in stress intensity of the exercise should reduce the chance of cardiac and locomotor systems synchronization during running. However, if we force the 2:1 ratio, we also increase the need of discharging the interpretation of the forced coincidence as a physiological coupling. Additional studies at different stages of exercise, as well as improved analytical methods are necessary to test the hypothesis of cardiolocomotor synchronization.

It is worth to mention that the use of an EMG amplifier to record the ECG causes distortion in the acquired signal. This distortion arises due distinct setups in both the amplifier gain and cutoff frequencies. Thus, the use of a band-pass filter within 20 and 500 Hz (first order) clearly attenuates the QRS complexes and hence reduces the signal to noise ratio. An example of such distortion is shown in Figure 1 (above signal), where the signal presents high frequency noise, probably due to respiratory muscles EMG. Although such a signal may not be suitable for diagnostic purposes, the described algorithm for R-wave detection reemphasizes de R-waves with a Butterworth band-pass filter with cutoff frequencies 5 and 20 Hz, second order, allowing the detection of all R-waves without loss, as proven by visual inspection. Indeed, this filter has a central frequency slightly lower than those recommended by Thakor et al. (1984) and Schlindwein et al. (2006). Thakor et al. (1984) recommended a filter centered in 17 Hz, with a quality factor equal to 5, which corresponds to a bandwidth of 3.4 Hz, while Schlindwein et al. (2006) found the optimal performance for their QRS complex detector by using a bandpass filter centered at 19 Hz and with 9 Hz bandwidth. The filter in the present study was chosen for both compensating the above-mentioned attenuation of lower frequencies of the QRS waves, as well as for cutting-off the high frequency noise caused by the EMG filter used during data acquisition.

In conclusion, the occurrence of phase synchronization in just short periods of four from 16 subjects, conjugated to an almost uniform distribution of phase difference and coherence values ranging from low to moderate between heart rate and muscle contraction suggest the absence of cardiolocomotor synchronization during the running at 70 to 75% of the heart rate reserve, both in trained and untrained males.

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Authors

Wollner Materko[1]*, Jurandir Nadal[1], Antonio Mauricio Ferreira Leite Miranda de Sá[1]

[1] Biomedical Engineering Program – PEB, COPPE Institute, Universidade Federal do Rio de Janeiro, P.O. Box 68510, CEP 21941-972, Rio de Janeiro, RJ, Brazil.