Time-frequency analysis of heart rate variability reveals cardiolocomotor coupling during dynamic cycling exercise in humans

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Blain G, Meste O, Blain A, Bermon S. Time-frequency analysis of heart rate variability reveals cardiolocomotor coupling during dynamic cycling exercise in humans. Am J Physiol Heart Circ Physiol 296: H1651–H1659, 2009. First published February 27, 2009; doi:10.1152/ajpheart.00881.2008.—To test the hypothesis that cycling exercise modulates heart rate variability (HRV), we applied a short-time Fourier transform on the electrocardiogram of subjects performing a maximal graded cycling test. A pedaling frequency component (PFC) in HRV was continuously observed over the time course of the exercise test and extracted from R-R interval series obtained from 15 healthy subjects with a heterogeneous physical fitness, exercising at three different pedaling frequency (n = 5): 70, 80, and 90 rpm. From 30 to 50% of the maximal power output (Pmax), in the 90 rpm group, spectral aliasing caused PFC to overlap with the respiratory sinus arrhythmia (RSA) band, significantly overestimating the PFC amplitude (APFC). In the meantime, APFC did not increase significantly from its minimal values in the 70 rpm (~1.26 ms) and 80 rpm (~1.20 ms) groups. Then, from 60 to 100% maximal power output (Pmax), workload increase caused a significant ~2.8-, ~3.3-, and ~3.4-fold increase in APFC in the 70, 80, and 90 rpm groups, respectively, with no significant difference between groups. At peak exercise, APFC accounted for ~43, ~39, and ~49% of the total HRV in the 70, 80, and 90 rpm groups, respectively. Our findings indicate that cycling continuously modulates the cardiac chronotropic response to exercise, inducing a new component in HRV, and that workload increase during intense exercise further accentuates this cardiolocomotor coupling. Moreover, because PFC and RSA overlapped at low workloads, methodological care should be taken in future studies aiming to quantify RSA as an index of parasympathetic activity.

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Miyamoto (27) showed that limb muscle contraction not only affects R-wave occurrence relative to the step cycle, but also modulates the duration of R-R intervals. These authors observed a positive chronotropic response when muscle contractions occur during systole, whereas muscle contractions occurring during diastole result in a negative chronotropic response.

To date, despite the physiological relevance of cardiolocomotor coupling, no study has examined the modulatory effect of locomotion on the variability of successive R-R intervals during exercise.

R-R interval variability, conventionally termed HR variability (HRV), is defined as variations in series of successive R-R intervals and is mainly mediated at rest by input from the autonomic nervous system (1, 2, 34, 36). During exercise, a marked decrease of HRV with workload increase has been demonstrated following vagal withdrawal (2, 4, 9, 31, 38), but no studies reported existence of a modulatory effect of locomotion.

The conventional methods used to quantify HRV are the spectral analysis techniques. These methods, which decompose HRV into its frequency components and evaluate the relative power of each component as a function of frequency, require stationarity of the signal studied (3, 36). Because this condition is difficult to achieve during exercise, studies aiming to examine HRV during exercise are scarce, and their conclusions are limited (3, 7, 12). To overcome the limitations encountered with spectral techniques, time-varying approaches have been developed to depict HRV spectrum during nonstationary conditions, such as dynamic exercise (7, 23). These methods estimate power as a function of time and frequency and produce instantaneous or evolutionary spectrum (7). For example, using the time-varying technique “short time Fourier transform” (STFT) on R-R interval series recorded during a maximal graded exercise test, our group successfully extracted and quantified the dynamic pattern of the HRV high-frequency band, related to the respiratory sinus arrhythmia (RSA) (4, 5, 23).

Using this advanced method, the principal aim of this study was to determine the influence of locomotion on HRV during exercise. We hypothesized that 1) cycling exercise modulates the duration of successive R-R intervals, which results in a new frequency component in HRV centered at the pedaling frequency, and attests to cardiolocomotor coupling. 2) This coupling, named the pedaling frequency component (PFC), is accentuated by increases in workload, as shown by PFC amplitude increase. To identify this hypothesized PFC, we depicted the time-varying pattern of HRV spectrum through different pedaling frequencies and strengths, during a maximal graded cycling test.
METHODS

Subjects. Fifteen healthy male subjects, whose characteristics are shown in Table 1, participated in the present study. All subjects were nonsmokers, and none was taking any medication. The physical fitness in our group varied greatly, ranging from sedentary subjects to elite cyclists. Physical activity and alcohol and caffeinated beverage consumption were prohibited 24 h before the exercise testing session. Written, informed consent was obtained before participation, and ethical approval was granted by the local ethics committee. All experimental procedures were performed in accordance with the Declaration of Helsinki.

Experimental design. To test our hypotheses over different pedaling frequencies, the 15 subjects were divided into three different pedaling frequency groups: 70 rpm (n = 5), 80 rpm (n = 5), and 90 rpm (n = 5). Subjects were asked to select one of the pedal frequency groups according to what frequency at which they felt most comfortable pedaling.

Following a 5-min resting data collection, all subjects performed a continuous maximal graded exercise test on a cycle ergometer (ErgoMedic 824 E, Monark Exercise AB, Vansbro, Sweden), in a quiet temperature-controlled room (21°C). Subjects were instructed to pedal at a constant pedaling frequency and stay seated for the duration of the test. A maximal graded exercise test was chosen because it allowed us to test our hypotheses from a wide range of workloads and HRs. Initial workload and subsequent step (2 min long) workload increments were set to ensure a 12- to 15-min maximal exercise test interval.

Ventilatory indexes and gas exchanges were measured by an automatic ergospirometer (Metasys TR-M, Brainware, Toulon, France). Subjects breathed through a silicon facemask connected to a two-way non-rebreathing valve (Hans Rudolph, Kansas City, MO). Inspired and expired O2 and CO2 concentrations were measured by paramagnetic and infrared sensors, respectively. Before each test, the gas analyzers were calibrated with gases of known composition, and an accurate controlled volume syringe adjusted the pneumotachograph. During the test and the preceding 5 min (rest), a three-lead ECG (Cardiopac II, Datex Engstrom, Helsinki, Finland) was recorded. Both the respiratory and the ECG signals were digitized in phase online by a 12-bit analog-to-digital converter (DAS 1600, Keithley Instruments, Taunton, MA) at a sampling rate of 1,000 Hz.

ECG processing. During exercise, body motion could distort the ECG signal, both in amplitude and shape, and bias any method of R-R interval estimation (16). To limit the potential pedaling artifacts resulting from body motion, care was taken to use a robust R-peak occurrence calculation using a double threshold-crossing technique added to an amplitude demodulation stage. Moreover, the maximum value of each R-wave peak was extrapolated and used to test the potential influence of body motion on the ECG. Using time-varying frequency analysis on this extrapolated signal, we failed to quantify any significant power in the frequency band corresponding to the pedaling frequency. Accordingly, we concluded that an artifact resulting from body motion could not explain any pedaling effects on R-R interval.

Then, the successive R-R intervals, defined as RR(k), were calculated as the difference:

$$RR(k) = t_k - t_{k-1}$$

where \( t_k \) is defined as the \( k \)th R-wave peak occurrence. Resampling of the ECG was not used to avoid interpolation artifacts, such as low-pass effects. Moreover, because the HR is a nonlinear transformation of the basic R-R interval measure, the analysis of R-R interval series (index of heart period) was preferred to the analysis of HR series (3). R-R interval series were visually inspected to ensure absence of artifacts. In case of artifacts arising from a spurious R-wave detection, the R-R interval was restored by summing the two or more spuriously short periods. In cases of undetected R-wave, the erroneous R-R interval was replaced by the mean of the two surrounding R-R interval values. Those artifacts did not exceed 0.1% of the data.

Table 1. Protocol and physical characteristics of the subjects

<table>
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<th>Subject No.</th>
<th>( F_p ), rpm</th>
<th>Initial Workload, W</th>
<th>Increment, W</th>
<th>( P_{max} ), W</th>
<th>( \dot{V}O_2peak ), ml min(^{-1}) kg(^{-1})</th>
<th>Age, yr</th>
<th>Height, cm</th>
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<td>181.0±4.2</td>
<td>76.1±5.5</td>
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Values are means ± SD. \( F_p \), pedaling frequency; \( P_{max} \), maximal power output; \( \dot{V}O_2peak \), peak O2 consumption. *P < 0.05 compared with group 70 rpm; †P < 0.05 compared with group 80 rpm.
The first processing applied to RR(k) was to remove the trend due to workload increase using a polynomial fitting \( po(k) \) (order equal to 20). The second processing step was a 100th-order high-pass filter (the cutoff frequency was 0.03, with 0.5 corresponding to one-half of the normalized sampling rate) applied to RR(k), where the trend \( po(k) \) has been removed. The resulting signal was termed \( m(k) \) (example in Fig. 1).

Time-varying amplitude estimate. Although subject's pedaling frequency was kept constant throughout the test, the PFC frequency from \( m(k) \), defined as \( F_{PFC} \), would not be constant, because HR (which represents the sampling rate in the case of HRV analysis) was time varying in response to successive increases in workload (see Fig. 3, A–C). The relation between pedaling frequency \( (F_p) \) and \( F_{PFC} \) was given by:

\[
F_{PFC}(k) = po(k) \cdot F_p
\]

In this study, \( F_{PFC} \) was used as the center frequency of the band-pass filter designed to quantify PFC. Because \( F_{PFC} \) was time varying (Eq. 1), the time-varying technique STFT was thus chosen to compute the amplitude of the PFC \( (A_{PFC}) \) rather than a conventional spectral method (see DISCUSSION). The filtering window, within which \( A_{PFC} \) was quantified, was \( F_{PFC} \pm 0.1 \text{ Hz} \) (corresponds to the pedaling frequency \( \pm 6 \text{ rpm} \)) to fully encompass the PFC modulation. Visual inspection of R-R interval series spectrogram was performed before each \( A_{PFC} \) estimate to confirm that the filtering window boundaries (white solid lines in Fig. 2) accurately tracked \( F_{PFC} \) time course throughout the test (Fig. 2).

Defining the STFT of \( m(k) \) as \( M(k, f) \), \( A_{PFC} \) was computed as followed.

\[
A_{PFC}(k) = \sqrt{\frac{1}{K} \sum_{f=F_{PFC}(k)-\delta}^{F_{PFC}(k)+\delta} |M(k, f)|^2}
\]

where \( K \) and \( \delta \) delineate, respectively, the length and the bandwidth of the STFT filtering window, and \( f \) corresponds to the time-varying frequencies contained in \( m(k) \). \( K \) was equal to 128 consecutive points, and \( \delta \) was adapted such that the signal was filtered in the range of \( F_{PFC} \pm 0.1 \text{ Hz} \).

\( A_{PFC} \) was computed both in absolute (ms) and relative (\( A_{PFC\%} \)) units. \( A_{PFC\%} \) determines the relative contribution of \( A_{PFC} \) to the total power of the HRV spectrum and was calculated as:

\[
A_{PFC\%}(k) = \frac{A_{PFC}(k) \times 100}{A_{Total\ Spectrum}(k)}
\]

where \( A_{Total\ Spectrum} \) represents the sum of all of the HRV spectral line amplitudes.

ECG preprocessing was performed using Matlab software 6.0 R12 (MathWorks, Natick, MA).

Statistical analysis. The means and 95% confidence interval of individual pattern of \( A_{PFC} \), \( A_{PFC\%} \), HR, ventilation (\( V_t \)), respiratory frequency (\( F_R \)), and tidal volume (\( V_t \)) were constructed as a function of the percentage of the \( P_{max} \). For statistical analysis purpose, \( A_{PFC} \), \( A_{PFC\%} \), HR, \( V_t \), \( F_R \), and \( V_t \) were filtered with an eighth-order Chebyshev type I low-pass filter, and the resulting signal was then resampled at a 10% \( P_{max} \) rate. Before statistical analysis, normality of the data and homogeneity of the variances of the distributions (equal variance) were confirmed using the Shapiro-Wilk test and the Levene test, respectively. The difference between groups for \( P_{max} \), peak \( O_2 \) consumption (\( V_{O2peak} \)), age, height, and weight were tested using a one-way ANOVA. The effects of workload increase, groups, and their possible interactions on \( A_{PFC} \), \( A_{PFC\%} \), HR, \( V_t \), \( F_R \), and \( V_t \) were tested using a two-way repeated-measures ANOVA. When appropriate, a multiple-comparisons analysis was performed.
using the Holm-Sidak test. To characterize the relationship between $A_{1FRC}$ and $\%P_{max}$, a linear regression analysis was also performed for each subject. Statistical analysis was performed using SigmaPlot software 11.0 (Systat Software, Tulsa, OK).

RESULTS

Maximal graded exercise test. All subjects completed the maximal graded exercise test without any clinical abnormalities or discomfort. $P_{max}$ and $V_{O2peak}$ achieved during the test are summarized in Table 1. A significant ($P < 0.05$) group difference was found when $P_{max}$ and $V_{O2peak}$ data from the three groups were pooled, with subjects with the highest $P_{max}$ and $V_{O2peak}$ distributed in the highest frequency groups. Figure 3 shows the pattern of HR, $V_{i}$, $F_{R}$, and $V_{t}$ throughout the test. A statistically significant increase with workload was found in HR and $V_{i}$ from 30 to 100% $P_{max}$, in $F_{R}$ from 60 to 100% $P_{max}$, and in $V_{t}$ from 30 to 80% $P_{max}$ ($P < 0.05$). The increase in $V_{i}$ was significantly greater in the 90 rpm compared with the 70 rpm group ($P < 0.05$). No significant group differences were found in HR, $F_{R}$, and $V_{t}$.

Fig. 3. Representations of HR and ventilatory parameters during exercise. Mean (black solid lines) and 95% confidence interval (gray dotted lines) values of HR (A–C), minute ventilation ($V_{i}$, D–F), respiratory frequency ($F_{R}$, G–I), and tidal volume ($V_{t}$, K–M) in the 70 rpm (left), 80 rpm (middle), and 90 rpm (right) groups are shown. bpm, Beats/min.
Consistent with our previous studies (4, 5), R-R interval significantly and continuously shortened with workload increase, whereas its variability demonstrated a biphasic evolution: it decreased from the beginning of exercise to ~60% $P_{\text{max}}$ and then increased during the highest intensities (example in Fig. 1).

The dynamic pattern of the PFC. In all subjects, visual inspection of R-R interval series spectrogram showed 1) a conspicuous PFC in HRV, clearly indicating the existence of a modulatory effect of cycling exercise on cardiac activity at the pedaling frequency (Fig. 2), and 2) that the filtering window boundaries, defined as the theoretical pedaling frequency ± 0.1 Hz (white solid lines in Fig. 2), completely encompassed and tracked PFC time course throughout the test. The dynamic pattern of $A_{\text{PFC}}$ was then accurately extracted.

Spectral aliasing can cause overestimation of the PFC from mild to moderate workloads. Figure 4 shows one example for each group of the dynamic pattern of the estimated $F_{\text{PFC}}$ and the extracted $A_{\text{PFC}}$ throughout the entire exercise test.

Accurate examination of the HRV’s spectral decomposition showed that, because the HR (i.e., the sampling rate) was not consistently at least twice the pedaling frequency (i.e., did not comply with Shannon theorem), spectral aliasing occurred in all groups. Consequently, the original $F_{\text{PFC}}$ was “undersampled” and appeared folded to lower frequencies (Fig. 4, A–C).

Because spectral aliasing was more accentuated in the 90 rpm group than in the other groups, PFC overlapped with RSA (Fig. 4C). $A_{\text{PFC}}$ was thus overestimated (Fig. 4, F and I).

Fig. 4. Representative examples of the time-frequency analysis of the PFC during exercise. Normalized PFC frequency (black dotted and solid line, A–C) and PFC amplitude are shown in absolute ($A_{\text{PFC}}$; D–F) and normalized ($A_{\text{PFC}}\%$; G–I) units, from subjects 1 (left, group 70 rpm), 8 (middle, group 80 rpm), and 12 (right, group 90 rpm). The y-axis in F has been expanded for the purpose of illustration ($A_{\text{PFC}}$ was 18 ms at 30% $P_{\text{max}}$). Although subjects’ pedaling frequency was kept constant throughout the test, PFC frequency was time-varying because HR (i.e., the sampling rate) increased with workload (see METHODS). Moreover, because HR (i.e., the sampling rate) was not at least twice the pedaling frequency during part of the exercise test, the original PFC frequency was “undersampled” and appeared folded to lower frequencies (black dotted line, A–C). Note that this aliasing phenomenon was more pronounced in the 90 rpm subject (C), leading PFC frequency to overlap with the respiratory sinus arrhythmia (RSA) frequency (gray line). This caused $A_{\text{PFC}}$ overestimation from 30 to 50% $P_{\text{max}}$ in subjects from the 90 rpm group (F and I), which reflected the sum of PFC and RSA amplitudes. RSA frequency time course was determined using a previously described method (4, 23).
reflecting the sum of APFC and RSA amplitudes. This phenomenon was more pronounced at the beginning of exercise, and APFC was maximal at 30% \( P_{\text{max}} \) (mean \( 90 \text{ rpm} = 23.0 \text{ ms} \), range \( 90 \text{ rpm} = 17.2–30.0 \text{ ms} \)). With exercise-induced tachycardia, PFC and RSA progressively separated, leading to APFC decrease until 50% \( P_{\text{max}} \). APFC then remained at its minimal values up to 60% \( P_{\text{max}} \) (mean \( 90 \text{ rpm} = 1.12 \text{ ms} \), range \( 90 \text{ rpm} = 1.0–1.5 \text{ ms} \)).

In contrast, in both the 70 and 80 rpm groups, PFC did not overlap with RSA (Fig. 4, A and B), and no significant change was found in APFC with workload increase. APFC remained at its baseline values up to 60% \( P_{\text{max}} \) (mean \( 70 \text{ rpm} = 1.26 \text{ ms} \), range \( 70 \text{ rpm} = 1.0–1.4 \text{ ms} \); mean \( 80 \text{ rpm} = 1.20 \text{ ms} \), range \( 80 \text{ rpm} = 1.0–1.3 \text{ ms} \)).

When expressed as a percentage of the total spectrum, APFC% demonstrated similar patterns to APFC: from the beginning of exercise to 60% \( P_{\text{max}} \), no change was observed in the 70 and 80 rpm groups, whereas APFC% significantly decreased with increases in workload in the 90 rpm group. At 50% \( P_{\text{max}} \), the contribution of PFC to the total spectrum was minimal in all groups.

The PFC increases during intense exercise. Figure 5 shows the mean APFC evolution for each group with an expanded abscissa.

From 60 to 100% \( P_{\text{max}} \), increase in workload caused a significant \( (P < 0.05) \) \( \approx 2.8\text{-}, \approx 3.3\text{-}, \text{ and } \approx 3.4\text{-fold increase} \) in APFC in the 70, 80, and 90 rpm groups, respectively. No significant difference between groups or interaction between groups and \%\( P_{\text{max}} \) were found in APFC increase. This increase in APFC with workload increase was confirmed in all subjects by the significant \( (P < 0.001) \) positive slope in the relationship between APFC and \%\( P_{\text{max}} \). The regression equation was \( \text{APFC} = 4.9 \times \%\( P_{\text{max}} \) - 1.8 \ (r = 0.80, \ P < 0.001), \text{APFC} = 6.3 \times \%\( P_{\text{max}} \) - 3.1 \ (r = 0.83, \ P < 0.001), \text{and APFC} = 5.9 \times \%\( P_{\text{max}} \) - 2.7 \ (r = 0.89, \ P < 0.001) \) in the 70, 80, and 90 rpm groups, respectively.

When expressed as a percentage of the total spectrum, APFC% demonstrated a significant \( (P < 0.05) \) \( \approx 5.3\text{-}, \approx 4.0\text{-}, \text{ and } \approx 4.5\text{-fold increase} \) in the 70, 80, and 90 rpm groups, respectively. No significant difference between groups or interaction between groups and \%\( P_{\text{max}} \) were found in APFC% increase.

Together, the significant increase in APFC and APFC% with increase in workload demonstrates a greater contribution of PFC to HRV spectrum and a greater degree of cardiolocomotor coupling during intense exercise.

**DISCUSSION**

In the present study, existence of a new HRV component related to the pedaling frequency was demonstrated using time-varying analysis of R-R interval series recorded during a maximal graded cycling test. This PFC was identified for different ranges of pedaling frequencies (70, 80, and 90 rpm) and fitness levels and attests to a consistent cardiolocomotor coupling during dynamic cycling exercise. Our findings also showed that the APFC significantly increased with workload from mild to maximal exercise, demonstrating a higher degree of cardiolocomotor coupling during intense exercise.

**Methodological considerations.** Spectral analysis techniques, such as Fourier transform or autoregressive modeling, have been used to analyze HRV. However, these methods can suffer from spectral aliasing, especially at high pedaling frequencies. In our study, we observed that spectral aliasing was less pronounced in the 70 and 80 rpm groups, where PFC did not overlap with RSA. This may be due to the lower pedaling frequency and workload in these groups. In contrast, in the 90 rpm group, where PFC and RSA were more separated, spectral aliasing was more pronounced. In future studies, it would be beneficial to use techniques that are less susceptible to spectral aliasing, such as wavelet analysis or empirical mode decomposition, to accurately characterize the PFC and RSA components.
the most extensively used methods to quantify HRV (36). However, these methods have two main limitations (3, 7, 12). First, spectral techniques require stationarity of R-R interval series along the considered analysis window. Indeed, presence of slow or irregular trends in the data series, such as R-R interval shortening with workload increase during exercise, distorts analysis and can lead to misinterpretation. Second, spectral techniques do not provide temporal information about variations in frequency components over the analyzed time period.

In recent years, time-varying approaches have been developed to overcome spectral limitations and investigate the dynamic properties of HRV spectral parameters during transient physiological or pathological episodes, such as dynamic exercise, rest-tilt maneuvers, vasovagal syncope, or acute ischemic periods (4, 5, 7, 12, 17, 23). Like previously proposed in similar works (4, 5, 21), the STFT was chosen among the available time-frequency techniques because it allows a clear time-frequency decomposition and direct and simple implementation of time-varying filters (7, 23). Although the time frequency resolution is reduced with STFT compared with more advanced methods, such as the Wigner-Ville distribution, time-varying autoregressive model, and wavelets, this resolution is sufficient to depict the dynamic characteristics of HRV time-varying spectral lines during nonstationary conditions, such as exercise (12, 14, 23). Moreover, in contrast to Wigner-Ville distribution, the STFT is not significantly affected by cross-term interference in the time-frequency phase decomposition, which not only requires a heavy computation burden, but can distort analysis and lead to misinterpretation (12, 14).

Because of technical constraints, no direct measurement of the subjects’ pedaling frequency was obtained in this study. One may state that, if the real pedaling frequency varied from the assigned experimental pedaling frequency during the test, this could bias PFC measurement and contribute to the observed dynamic pattern of A_PFC. At least five different aspects of our study suggest that the dynamic pattern of A_PFC is not explained by variations in the real pedaling frequency or weakness inherent to the analysis technique. First, a simulation study was performed to test the validity and robustness of the technique we used; this study rejected the notion that noise or biased estimation contributed to our findings (22). Second, the instantaneous real pedaling frequency was closely monitored throughout the test and strictly maintained within the range of ±3 rpm to prevent substantial variations in pedaling frequency. Third, the filtering window used to quantify A_PFC was set to the pedaling frequency ± 6 rpm (twice the range of the authorized pedaling frequency variation) to fully encompass PFC. Fourth, visual inspection of R-R interval series spectrogram was performed before each A_PFC estimate to confirm both the existence of PFC and that the filtering window boundaries accurately encompassed PFC (Fig. 2). Finally, PFC was identified throughout the entire test, and a progressive increase in PFC with workload increase was always found in distinct frequency groups and populations. This PFC pattern is unlikely to be caused by either variation in the pedaling frequency or bias in the analysis technique.

Pedaling modulates HRV. Most protocols aiming to study HRV under exercise conditions have been limited to constant load or very slow trend ramp load to reduce the nonstationarity of R-R interval series (2, 6, 9, 24, 31, 32). From low to moderate workloads (<60% P_max), these studies consistently reported a marked decrease in the overall HRV spectral power following vagal withdrawal (2, 4, 9, 31, 38). Using glycopyrrolate as a muscarinic blocker, Warren et al. (38) stated that the reduction of RSA amplitude from the beginning of exercise to ~60% of VO_2peak could provide a reliable index of cardiac vagal withdrawal during exercise. In this study, we demonstrated that, because of spectral aliasing, RSA and PFC can overlap from mild to moderate workloads. This overlapping leads to an overestimation of the RSA amplitude and thus the vagal contribution to HRV, even though the PFC contribution to HRV is probably limited at low workloads. Indeed, when PFC and RSA were completely separated because of the tachycardia-induced sampling rate increase, A_PFC was largely reduced (~1.12 ms, range = 1.0–1.5 ms), suggesting that the high values of A_PFC measured at the beginning of exercise were almost entirely determined by RSA. This is consistent with previous findings (4) that showed that RSA amplitude was ~21 ms at 30% of VO_2peak, which is very close from the A_PFC values measured in this study at 30% of P_max (~23 ms). Thus, to accurately examine the dynamic pattern of RSA amplitude during exercise and limit the influence of PFC on this index, it is recommended to 1) design specific experimental conditions where the pedaling frequency is adapted to HR (since the spectral aliasing is accentuated at high pedaling frequency, lower HRs should be accompanied by a lower pedaling frequency); and 2) use time-frequency analysis with a narrow band-pass time-varying filter, instead of the conventional large band-pass filter, which substantially delays RSA and PFC splitting and tends to accentuate RSA overestimation.

From moderate-to-heavy exercise (>60% P_max), despite nearly complete vagal withdrawal, RSA was shown to be the main mechanism regulating HRV (2, 4, 9, 31). Our group previously demonstrated that, in healthy humans who performed a maximal graded exercise test, RSA contributes ~60% to the overall HRV at peak exercise (4). Our present findings show that, at the same exercise intensity, the A_PFC contribution to HRV reached a mean value of ~43% across groups (range = 39–49%). Taken together, these results suggest that, during intense exercise, HRV is mainly mediated by two components: PFC, which accounts for ~40%, and RSA, which accounts for the remaining 60%.

Our findings of an increase in A_PFC with workload increase are consistent with a mechanically induced PFC, originating from a dynamic modulation of venous return by rhythmic limb muscles contractions. Indeed, the alternation of concentric and relaxing phases during a sequence of dynamic contractions makes the venous outflow pulsatile, increasing during contraction and being reduced during relaxation (10, 15, 20). The increase in venous outflow on a contraction-by-contraction basis may, in turn, accentuate right heart preload and activate a stretch-induced positive chronotropic response of the sinus node at the locomotor frequency. This hypothesis agrees with previous findings in anesthetized animals (19, 33), which demonstrated that an increase in the diastolic volumetric load enhances atrial wall stretching and directly shortens R-R interval. It also agrees with findings from heart transplant patients (2) or from healthy subjects during ganglion blockade (6), which showed that RSA persistence during intense exercise is explained by the enhancement of a nonneural mecha-
nism at the heart level, in response to the breath-by-breath modulation of venous return by ventilation.

Moreover, although HRV is mainly mediated by input from the autonomic nervous system at rest (1, 2, 34, 36), a neural contribution to $A_{PFC}$ increase with increases in workload is unlikely because, not only is vagal withdrawal nearly complete at mild workloads (11, 13, 37), but both the sympathetic and parasympathetic nervous systems respond too slowly to modulate HRV in the pedaling frequency range (1, 3, 35).

The PFC contributes to cardiolocomotor coupling. Numerous studies have associated cardiolocomotor coupling with the synchronization between a step cycle and R-wave occurrence (18, 26, 28, 29). For example, Kirby et al. (18) observed a 1:1 HR and locomotion rate synchronization (one heartbeat for one step) in walking and running, at treadmill speed ranging from 4 to 17 km/h. However, all of these studies reported that cardiolocomotor synchronization can only be observed when the heart and locomotion rates are close to each other, is not modulated by workload increase, and occurs intermittently over time (periods of synchronization alternating on a minute-by-minute basis with periods of absence of coupling) (18, 26, 28–30). Consequently, it can be argued that this apparent coupling can be transiently found by chance alone, when heart and locomotor rates intersect. In contrast to this cardiolocomo-
tor synchronization, our findings demonstrated that PFC does not require the heart and the pedaling rates to intercept, is consistently identified over a broad range of HRs, and is increased with increase in workload. It is thus obvious that PFC and synchronization are two different aspects of the cardiolocomotor coupling.

Numerous studies (18, 25, 26, 29, 30) have proposed that coupling between the locomotor and cardiac systems optimizes blood flow to the contracting muscles and minimizes the energy cost of cardiac muscle contraction. For example, Niizeki (25), using computer-assisted bilateral thigh cuff occlusion to simulate rhythmic intramuscular pressure changes during bipedal locomotion in the human, showed that an HR-induced increase in cardiac output occurs during absence of cuff pressure, so that the peak arterial flow in the thigh was not overlapped by an elevated intramuscular pressure. Results from our study, showing low values of $A_{PFC}$, probably limit the physiological contribution of PFC to cardiovascular optimization during exercise. However, although it is possible that the observed PFC only reflects homeostatic response of the heart, it can also be postulated that a PFC from mechanical origin, increasing the HR when ventricles are full of blood rather than empty, could optimize, at least to a small extent, heart con-
traction efficiency.

In summary, our findings demonstrate that the cardiac chro-
notropic activity is significantly modulated by cycling exercise. This new element of the cardiolocomotor coupling does not require the heart and the pedaling rates to be close to each other and is consistently identified throughout a broad range of workloads and HRs. Because this coupling is increased with increase in workload, it contributes to the HRV increase observed from moderate to maximal exercise.

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