Influence of Running Stride Frequency in Heart Rate Variability Analysis During Treadmill Exercise Testing

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***Abstract*—The analysis and interpretation of heart rate variability (HRV) during exercise is challenging not only because of the nonstationary nature of exercise, the time-varying mean heart rate, and the fact that respiratory frequency exceeds 0.4 Hz, but there are also other factors, such as the component centered at the pedaling frequency observed in maximal cycling tests, which may confuse the interpretation of HRV analysis. The objectives of this study are to test the hypothesis that a component centered at the running stride frequency (SF) appears in the HRV of subjects during maximal treadmill exercise testing, and to study its influence in the interpretation of the low-frequency (LF) and high-frequency (HF) components of HRV during exercise. The HRV of 23 subjects during maximal treadmill exercise testing is analyzed. The instantaneous power of different HRV components is computed from the smoothed pseudo-Wigner–Ville distribution of the modulating signal assumed to carry information from the autonomic nervous system, which is estimated based on the time-varying integral pulse frequency modulation model. Besides the LF and HF components, the appearance is revealed of a component centered at the running SF as well as its aliases. The power associated with the SF component and its aliases represents 22 $\pm$ 7% (median $\pm$ median absolute deviation) of the total HRV power in all the subjects. Normalized LF power decreases as the exercise intensity increases, while normalized HF power increases. The power associated with the SF does not change significantly with exercise intensity. Consideration of the running SF component and its aliases is very important in HRV analysis since stride frequency aliases may overlap with LF and HF components.**

***Index Terms*—Cardiolocomotor coupling, heart rate variability (HRV), running stride frequency (SF) component, smoothed pseudo-Wigner–Ville distribution (SPWVD), time-varying integral pulse frequency modulation model (TVIPFM), treadmill exercise test.**

NOMENCLATURE

ANS	Autonomic nervous system.
HF.	High frequency.
HR	Heart rate.
HRV	Heart rate variability.
LF	Low frequency.
MAD	Median absolute deviation.
MHR	Maximum heart rate.
$PETCO_2$	Partial end-tidal CO_2 pressure.
$PETO_2$	Partial end-tidal O_2 pressure.
RSA	Respiratory sinus arrhythmia.
RER	Respiratory exchange ratio.
SA	Stride frequency aliases.
SF	Stride frequency.
SPWVD	Smoothed pseudo-Wigner–Ville distribution.
TVIPFM	Time-varying integral pulse frequency modulation.
$\dot{V}CO_2$	Volume of consumed CO_2.
$\dot{V}O_2$	Volume of consumed O_2.
$\dot{V}E$	Ventilation.
VT1	First ventilatory threshold.
VT2	Second ventilatory threshold.
$a_{\mathfrak{m}}(n)$	Analytic signal of $\mathfrak{m}(n)$ (dimensionless).
$d_{\mathrm{HR}}(n)$	Instantaneous HR signal (in Hz).
$d_{\mathrm{HRM}}(n)$	Time-varying mean HR signal (in Hz).
$d_{\mathrm{HRV}}(n)$	HRV signal (in Hz).
$F_{\mathrm{R}}(n)$	Respiratory frequency signal (in Hz).
$F_{\mathrm{ST}}(n)$	Running stride frequency signal (in Hz).
F_s	Sampling frequency (in Hz).
$\mathfrak{m}(n)$	Modulating signal of the TVIPFM model (dimensionless).
$P_{\mathrm{J}}(n)$	Instantaneous power of the J HRV component (dimensionless).
$P_{\mathrm{HF}}(n)$	Instantaneous power of the HF component (dimensionless).
$P_{\mathrm{LF}}(n)$	Instantaneous power of the LF component (dimensionless).
$P_{\mathrm{S}}(n)$	Instantaneous power of the stride frequency coupling component (dimensionless).

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$P_{\mathrm{T}}(n)$ Total HRV instantaneous power (dimensionless).
$\overline{P_{\mathrm{LF}}^{\mathrm{I}}}$ Normalized $P_{\mathrm{LF}}(n)$ averaged in interval I.
$\overline{P_{\mathrm{HF}}^{\mathrm{I}}}$ Normalized $P_{\mathrm{HF}}(n)$ averaged in interval I.
$\overline{P_{\mathrm{S}}^{\mathrm{I}}}$ Normalized $P_{\mathrm{S}}(n)$ averaged in interval I.
$S_{\mathfrak{m}}(n,m)$ SPWVD of $a_{\mathfrak{m}}(n)$.

I. Introduction

SPECTRAL analysis of heart rate variability at rest has been widely used as a noninvasive technique for the assessment of the autonomic nervous system activity on the heart. Standards of measurement, physiological interpretation, and clinical use have been published [1]. However, during exercise the analysis and interpretation of HRV as a measure of ANS activity is still a matter of debate. Cardiovascular responses to exercise depend on the type, intensity, and volume of exercise. During dynamic exercise, the increase in sympathetic activity and the withdrawal of parasympathetic activity increase the HR [2], [3]. The relative role of the two drives depends on the exercise intensity [2], [4].

Power in the LF band (0.04–0.15 Hz) of HRV is considered to be a measure of sympathetic and parasympathetic activity, together with other regulatory mechanisms such as the rennin–angiotensin system and baroreflex [1], [2]. Its interpretation is controversial when, for example, the respiratory frequency lies in the LF band [5]. Several works have reported a reduction in the LF power with exercise [4], [6]–[8] but there are inconsistent results regarding normalized LF power [4].

Power in the HF band (0.15–0.4 Hz) of HRV is considered to be a measure of parasympathetic activity, mainly due to RSA, at least in resting conditions [1]. The fact that respiratory frequency is not restricted to the range (0.15–0.4 Hz) during exercise makes it necessary to redefine the HF band [5]. In some studies, the HF band is extended to include the whole range of physiological respiratory frequencies [4], [9], the upper limit being half the mean HR. In other cases, the HF band is centered on the respiratory frequency with constant or time-dependent bandwidth [5], [7], [8], [10]. While some consider HF power as a valid marker of parasympathetic activity during exercise [11], which is consistent with the decrease observed in HF power during moderate exercise [6], others support the idea that during heavy exercise, when cardiac vagal control is no longer effective, the HF power of HRV is due to the mechanical effect of breathing on the sinus node [4], [7], [12]. This is confirmed by the increase observed in the HF power of HRV around the VT1 reported in [4], [7], and [12]. Furthermore, previous results show the persistence of HRV during exercise after complete ganglionic autonomic blockade [13], [14] and the reappearance of HF power in the HRV of heart transplant recipients during exercise [15].

Besides the LF and HF components of HRV, a component centered at the pedaling frequency has been observed in maximal graded cycling tests. This is considered to reflect cardiolocomotor coupling and increases with workload [16]. Cardiolocomotor coupling is assumed to be responsible for the synchronization between locomotor rate or cadence and HR [17] but also to modulate HRV [18]. Moreover, HRV indices evaluated at different exercise workloads are reported to be influenced by cycling cadence [19].

TABLE I
STUDY POPULATION CHARACTERISTICS. MEAN ± STANDARD DEVIATION

Age (yr)	Height (cm)	Mass (kg)	Body mass index ($\mathrm{kg \cdot m^{-2}}$)	$\dot{V}O_2$ max ($\mathrm{ml\ O_2 \cdot kg^{-1} \cdot min^{-1}}$)
34.8±5.0	178.4±5.7	74.8±7.8	23.5±2.5	57.8±6.1

Not only is the interpretation of HRV during exercise challenging. Its analysis is also challenging due to its nonstationary nature. Several approaches to nonstationary analysis of HRV have been proposed in the literature [20], of which time–frequency (TF) analysis is the most common. This approach can be divided into three categories: 1) nonparametric methods based on linear filtering, including the short-time Fourier transform [16], [21], [22] and the wavelet transform [23]; 2) nonparametric quadratic TF representations, including the Wigner–Ville distribution and its filtered versions [4], [8], [24]; and 3) parametric methods based on autoregressive models with time-varying coefficients [21].

Furthermore, during exercise the mean HR is not constant but increases with exercise intensity. It has been demonstrated that if the ANS evolution is derived from HRV analysis, a correction of HRV measurements with the time-varying mean HR should be considered in order to avoid overestimation or underestimation of ANS activity due to changes in mean HR [21], [24].

II. Methods

In this paper, the HRV of subjects during maximal treadmill exercise test is analyzed. Our hypothesis is that a component centered at the running SF[1] will appear due to cardiolocomotor coupling, similar to the pedaling frequency component described in [16]. The objectives of this study are as follows: 1) to characterize the running SF component of HRV and its evolution during the exercise test and 2) to study the influence of the SF component in the interpretation of the LF and HF components of HRV during the exercise test.

A. Subjects

Twenty-three males volunteered to participate in the study. Some study population characteristics are reported in Table I. All of them were physically active, doing at least 3 days/week of regular aerobic training. The subjects were all familiar with exercise testing. They were not taking prescribed medications and they had normal blood pressure levels and electrocardiographic patterns. None of the subjects were underperforming or complaining about any type of fatigue at the time of the study. The subjects were fully informed of the purpose and possible risks and benefits of their participation in the study before giving their written informed consent. The study was approved by the University's Human Ethics Committee in accordance with the Declaration of Helsinki.

[1]Running stride is the cycle of movement from the time one foot touches the ground to the time it touches the ground again. SF is the number of the steps taken in a given amount of time or over a given distance.

B. Experimental Protocol

All the subjects completed one test session. Prior to the test, they were asked to adhere to the following pretest instructions: 1) wear comfortable, loose-fitting clothing; 2) drink plenty of fluids over the 24-h period preceding the test; 3) avoid food, tobacco, alcohol, and caffeine for 3 h prior to taking the test; 4) avoid exercise or strenuous physical activity on the day of the test; and 5) get an adequate amount of sleep (6–8 h) the night before the test. All the subjects were tested in an environmentally controlled exercise laboratory (22–23 °C) between 9:00 and 13:00 h. They were first monitored for 5 min at rest (seated, without any movement or talking) to measure resting cardiorespiratory variables. The subjects then performed an incremental maximal test to exhaustion on a motorized treadmill (Quasar Med 4.0, h/p/cosmos, Nussdorf–Traunstein, Germany). The incremental running test began with an initial speed of 8 km·h^{-1} and increased 1 km·h^{-1} every minute until the subjects stopped due to volitional exhaustion. After the end, subjects were required to remain running at 8 km·h^{-1} for another 5 min. However, subjects behavior just after the stress peak was not consistent. Some subjects continued running at a slower SF but others jumped out of the treadmill and took some seconds until running was reestablished. Respiratory and HR responses were continuously recorded during the maximal test and the recovery period.

Ventilatory and exchange gases were analyzed breath-by-breath by an open-circuit sampling system (Oxycon Pro, Jaeger-Viasys Healthcare, Hoechberg, Germany). The metabolic cart was calibrated with a known gas mixture (16% oxygen, O_2, and 5% carbon dioxide, CO_2) and volume prior to the first test each day as recommended by the company. The highest $\dot{V}O_2$ attained during the maximal exercise test was denoted $\dot{V}O_2$max. $\dot{V}O_2$max was considered to have been achieved if the subject met at least two of the following criteria: 1) a RER equal to or greater than 1.15; 2) plateau of the $\dot{V}O_2$ during the last stage of exercise; and 3) maximal HR within ± 10 beats/min of predicted values. Due to their importance in the definition of exercise intensity, ventilatory thresholds were determined as described in [25]. The VT1 or aerobic threshold[2] was established as the speed at which the ratio between $\dot{V}E$ and $\dot{V}O_2$, i.e., $\dot{V}E/\dot{V}O_2$ and $PETO_2$ began to increase without a simultaneous increase in $PETCO_2$. The VT2 or anaerobic threshold[3] was determined as the speed corresponding to the first decrease in $PETCO_2$, with a corresponding increase in the ratio between $\dot{V}E$ and $\dot{V}CO_2$, i.e., $\dot{V}E/\dot{V}CO_2$, after the steady-state phase following the VT1. All threshold measurements were made by visual inspection of each relevant respiratory variable measured in the test plotted against time. The visual inspections were made by two experienced exercise physiologists, and the results were compared and then averaged. Respiratory frequency and HR were also recorded by the open-circuit system breath-by-breath.

[2]The aerobic threshold could be defined as the exercise intensity at which anaerobic energy pathways start to operate. Below this point, the muscles derive their energy from oxygen-dependent pathways.

[3]The anaerobic threshold could be defined as the exercise intensity above which aerobic energy production is supplemented by anaerobic mechanisms, causing a sustained increase in lactate and metabolic acidosis [26].

RR intervals were recorded beat-to-beat using an HR monitor (RS800, Polar Electro Oy, Kempele, Finland) which uses a sampling frequency of 1000 Hz for the ECG signal, providing an accuracy of 1 ms for each RR period. Moreover, the equipment provided running SF, using a stride sensor (S3, Polar Electro Oy, Kempele, Finland).

The synchronization between the open-circuit sampling system and the HR monitor measurements was assessed using the HR recorded by both devices.

C. TF Analysis of HRV

The modulating signal assumed to carry information from the ANS is estimated based on the TVIPFM model, described in [24]. It is estimated from the beat occurrence time series, derived from the recorded RR intervals. Briefly, the instantaneous HR signal is estimated with the procedure described in [24], sampled at a sampling frequency $F_s = 4$ Hz and denoted $d_{\mathrm{HR}}(n)$. Then, the time-varying mean HR signal is estimated by low-pass filtering $d_{\mathrm{HR}}(n)$ with a cutoff frequency of 0.03 Hz and denoted $d_{\mathrm{HRM}}(n)$. The HRV signal is obtained as $d_{\mathrm{HRV}}(n) = d_{\mathrm{HR}}(n) - d_{\mathrm{HRM}}(n)$. Finally, the modulating signal is estimated as $\mathfrak{m}(n) = d_{\mathrm{HRV}}(n)/d_{\mathrm{HRM}}(n)$.

The discrete SPWVD is applied to $\mathfrak{m}(n)$

$$S_{\mathfrak{m}}(n,m) = \sum_{k=-K+1}^{K-1} |h(k)|^2 \times \left[\sum_{n'=-N+1}^{N-1} g(n') a_{\mathfrak{m}}(n+n'+k) a_{\mathfrak{m}}^*(n+n'-k) \right] \times e^{-j\pi \frac{m}{M} k}; \quad m = -M+1, \ldots, M \tag{1}$$

where n and m are the time and frequency indices, respectively. The analytic signal $a_{\mathfrak{m}}(n)$ is defined as $a_{\mathfrak{m}}(n) = \mathfrak{m}(n) + j\hat{\mathfrak{m}}(n)$, where $\hat{\mathfrak{m}}(n)$ represents the Hilbert Transform of $\mathfrak{m}(n)$ [27]. The terms $g(n)$ and $|h(k)|^2$ represent the time and frequency smoothing windows, chosen to be Hamming windows of length $2N-1$ and $2K-1$, normalized so that $\sum_{n'=-N+1}^{N-1} g(n') = 1$ and $|h(0)|^2 = 1$, respectively. In this study, $2M = 1024$, $2N-1 = 203$ and $2K-1 = 513$ [8], [24].

The instantaneous power of each HRV component is estimated as

$$P_{\mathrm{J}}(n) = \frac{1}{2}\frac{1}{2M} \sum_{m=m_l}^{m_u} S_{\mathfrak{m}}(n,m) \tag{2}$$

where subscript J denotes a specific HRV component and m_l and m_u are the discrete indices corresponding to the lower and upper frequency limits, F_l and F_u, of the band associated with the J component, i.e., $m_l = \lceil \frac{F_l}{F_s} M \rceil$ and $m_u = \lfloor \frac{F_u}{F_s} M \rfloor$.

The following frequency bands are considered:

1) *LF band:* It is defined as in classical HRV analysis from 0.04 to 0.15 Hz [1].
2) *HF band:* It is defined as centered on the respiratory frequency $F_{\mathrm{R}}(n)$ with a bandwidth $\Delta F = 4\left(\frac{4F_s}{2K-1}\right)$, dependent on the frequency resolution, which, for a Hamming window of $2K-1$ samples is $\frac{4F_s}{2K-1}$ Hz. In this

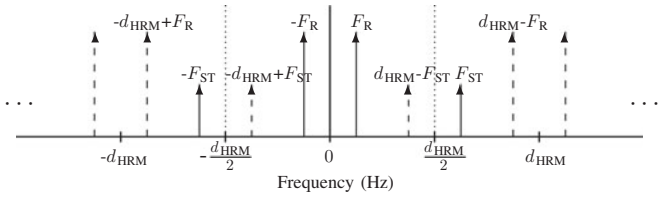

Fig. 1. Superposition of replicas of the spectrum of $\mathfrak{m}(t)$ shifted at multiples of the mean HR, d_{HRM}. The theoretical $\mathfrak{m}(t)$ is assumed to be composed of two pure sinusoids at frequencies F_{R} and F_{ST}. Dotted lines represent the limits of the frequency range with physiological meaning in HRV analysis.

study, $\Delta F = 4\left(\frac{4\cdot 4}{513}\right) = 0.125$ Hz. The respiratory frequency $F_{\mathrm{R}}(n)$ is derived from that provided by the Oxycon Pro system, described in Section II-B, by interpolation of the respiratory frequency series at F_s and low-pass filtering with a cutoff frequency of 0.03 Hz to avoid artificial jumps of the frequency series. The upper limit of the HF band is limited by $\frac{d_{\mathrm{HRM}}(n)}{2}$, while its lower limit is limited by 0.15 Hz. The rationale for limiting the lower limit of the HF band to 0.15 Hz is that when the LF and HF components overlap, an entrainment between the two rhythms occurs and the separation of sympathetic and parasympathetic activities is not trivial since nonlinear mechanisms and interactions are involved [28]. In this study, respiratory frequency is always above 0.15 Hz during exercise and recovery, but in some subjects it is below during the 5-min rest.

3) *Running SF band:* It is defined as centered at the running SF $F_{\mathrm{ST}}(n)$ with a bandwidth ΔF. The SF $F_{\mathrm{ST}}(n)$ is obtained from the S3 stride sensor, described in Section II-B, again after interpolation at F_s and low-pass filtering with a cutoff frequency of 0.03 Hz. The upper and lower limits of the SF band are limited by $\frac{d_{\mathrm{HRM}}(n)}{2}$ and 0.15 Hz, respectively, as in the HF band.

The intrinsic sampling frequency of the HRV is given by the HR. As a result, the maximum frequency with physiological meaning that can be analyzed is defined as half the mean HR in the analyzed interval [29]; this is why, the upper limit of the HF and SF bands is limited to $\frac{d_{\mathrm{HRM}}(n)}{2}$. Moreover, if the modulating signal with information on the ANS activity contains components whose frequencies exceed this limit, aliasing will occur at the natural sampling of the heart and appear at frequencies below half the mean HR [5]. To exemplify this phenomenon, consider an artificial ANS modulating signal $\mathfrak{m}(t)$ composed of two pure sinusoids at frequencies F_{R} and F_{ST} representing the HF and SF components. Due to the natural sampling of the heart, the spectrum of the modulating signal $\mathfrak{m}(n)$, estimated from the beat occurrence time series, will consist of a superposition of scaled replicas of the spectrum of $\mathfrak{m}(t)$ shifted to the multiples of the sampling frequency, i.e., the HR. In Fig. 1, a schematic representation of the spectrum of $\mathfrak{m}(n)$ is shown, where F_{ST} is above half the mean HR, denoted d_{HRM}. It can be appreciated that the alias at frequency d_{HRM}–F_{ST} appears below the limit $\frac{d_{\mathrm{HRM}}}{2}$. If the running SF component is not a pure sinusoid, not only will harmonics of the fundamental frequency F_{ST} appear in the spectrum of $\mathfrak{m}(n)$, but also their aliases, which may lie in the frequency range of interest (see the Appendix for further justification of SF harmonics). To take into account all the HRV power contributed by the running SF coupling, the power in the following band is also computed:

1) *Running SA band:* This is defined as the union of all bands centered at each of the SA which eventually intercept the frequency range with physiological meaning, namely, considering two harmonics $d_{\mathrm{HRM}}(n)-F_{\mathrm{ST}}(n)$ and $-d_{\mathrm{HRM}}(n)+2F_{\mathrm{ST}}(n)$. Each band has a bandwidth of ΔF and its lower and upper limits are bounded by $\frac{d_{\mathrm{HRM}}(n)}{2}$ and 0.15 Hz, respectively.

Finally, the power associated with the running SF is computed summing the power in the SF and SA bands, and denoted $P_{\mathrm{S}}(n)$. The powers associated with the LF and HF components are denoted $P_{\mathrm{LF}}(n)$ and $P_{\mathrm{HF}}(n)$, respectively. Total HRV power is computed in the frequency band from 0.04 Hz to $\frac{d_{\mathrm{HRM}}(n)}{2}$, and denoted $P_{\mathrm{T}}(n)$.

D. Physiological Intervals

The evolution of $P_{\mathrm{LF}}(n)$, $P_{\mathrm{HF}}(n)$, and $P_{\mathrm{S}}(n)$ during the exercise is studied using different time intervals with physiological meaning.

The following intervals are defined according to:

1) *Physiological standardized exercise intensity:* Three 1-min intervals are defined—centered at VT1, at VT2, and preceding the instant of MHR.
2) *Percentage of $\dot{\mathrm{V}}O_2$:* Three intervals are defined where $\dot{\mathrm{V}}\mathrm{O}_2$ lies within 40–60% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$, 60–80% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$, and 80–100% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$, respectively, where $\Delta\dot{\mathrm{V}}\mathrm{O}_2 = \dot{\mathrm{V}}\mathrm{O}_2\mathrm{max} - \dot{\mathrm{V}}\mathrm{O}_2\mathrm{bas}$, and $\dot{\mathrm{V}}\mathrm{O}_2\mathrm{bas}$ is the basal $\dot{\mathrm{V}}\mathrm{O}_2$ computed as the mean value of $\dot{\mathrm{V}}\mathrm{O}_2$ during the 5-min rest.
3) *Percentage of speed excursion:* Five intervals are defined where the speed lies within 0–20% Δs, 20–40% Δs, 40–60% Δs, 60–80% Δs, and 80–100% Δs, where Δs is the difference between the maximum speed achieved in the test and the initial speed.

E. Statistical Analysis

To test whether the contribution of each HRV component to the total HRV power is significantly different between the different intervals, a statistical analysis is conducted. First, the instantaneous powers $P_{\mathrm{LF}}(n)$, $P_{\mathrm{HF}}(n)$, and $P_{\mathrm{S}}(n)$ are normalized by the total power $P_{\mathrm{T}}(n)$. Then, the normalized powers are averaged in each of the defined intervals, and denoted $\overline{P^{\mathrm{I}}_{\mathrm{LF}}}$, $\overline{P^{\mathrm{I}}_{\mathrm{HF}}}$, and $\overline{P^{\mathrm{I}}_{\mathrm{S}}}$, respectively, where I represents the corresponding interval. Due to the non-normality of the data as revealed by a Kolmogorov–Smirnov test, a Wilcoxon signed rank test is applied to all possible pairwise comparison of the different intervals defined according to physiological standardize exercise intensity, percentage of $\dot{\mathrm{V}}\mathrm{O}_2$ and percentage of speed excursion. Statistical significance is considered for p-values ≤ 0.02.

III. Results

Ventilatory thresholds could not be properly identified in one of the 23 subjects. This subject was excluded from the

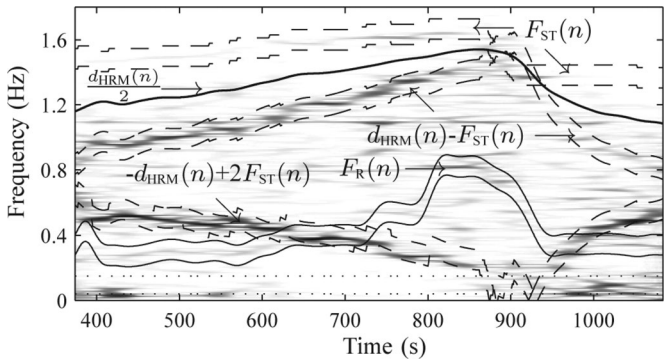

Fig. 2. Decimal logarithm of the SPWVD of $\mathfrak{m}(n)$ for one subject of the database from the first minute after the beginning of the exercise until the fourth minute of the recovery period. In the grayscale, lighter grays stand for lower values and darker grays for higher values. The thicker solid line represents the limit $\frac{d_{\mathrm{HRM}}(n)}{2}$. The LF band is limited by dotted lines, the HF band by solid lines, and the SF and SA bands by dashed lines.

analysis relating to the physiological standardized exercise intensity intervals. SF was not recorded for three subjects. These were excluded from the analysis relating to the SF coupling.

The SPWVD $S_{\mathfrak{m}}(n,m)$ for one subject of the database is displayed in Fig. 2. It is displayed from the first minute after the beginning of the exercise until the end of the fourth minute of the recovery period (for visualization purposes the decimal logarithm of the SPWVD is displayed). The $\frac{d_{\mathrm{HRM}}(n)}{2}$ limit is shown as well as limits of the LF band, the HF band, centered at $F_{\mathrm{R}}(n)$, and the SF and SA bands, centered at $d_{\mathrm{HRM}}(n)-F_{\mathrm{ST}}(n)$ and $-d_{\mathrm{HRM}}(n)+2F_{\mathrm{ST}}(n)$. In this example, the SF band only appears in the frequency range of interest during a brief interval just after the MHR is achieved, since the SF $F_{\mathrm{ST}}(n)$ is above the limit $\frac{d_{\mathrm{HRM}}(n)}{2}$ elsewhere during the displayed interval. Note that SF coupling appears not only during exercise but also during recovery.

Fig. 3 shows the power associated with the LF and HF components, and the SF coupling, $P_{\mathrm{LF}}(n)$, $P_{\mathrm{HF}}(n)$, and $P_{\mathrm{S}}(n)$, respectively. It also shows the $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ for the same subject and time interval as Fig. 2, including the 5-min resting period. The times when VT1, VT2, and MHR are achieved are indicated. It can be appreciated that $P_{\mathrm{S}}(n)$ is higher than $P_{\mathrm{LF}}(n)$ and $P_{\mathrm{HF}}(n)$ during the exercise phase. Power $P_{\mathrm{LF}}(n)$ decreases notably with the increase in exercise intensity, almost disappearing after VT2 is achieved. It increases rapidly during recovery. The trend of $P_{\mathrm{HF}}(n)$ deserves further attention. The increase in $P_{\mathrm{HF}}(n)$ around MHR may be due to the mechanical stretching of the sinus node at high exercise intensity. However, the peaks around 670 and 980 s cannot be explained by the RSA. Note that from 620 to 720 s, and from 960 to 1000 s, approximately, the HF and SA bands centered at $-d_{\mathrm{HRM}}(n)+2F_{\mathrm{ST}}(n)$ overlap, and the power in this overlapped band contributes both to $P_{\mathrm{HF}}(n)$ and $P_{\mathrm{S}}(n)$. It is impossible to distinguish if its cause is the respiratory or stride modulation. If the evolution of the RSA was to be assessed from $P_{\mathrm{HF}}(n)$, an increase at around 670 and 980 s would be erroneously interpreted due to the overlapping between the HF and SA bands. Regarding $P_{\mathrm{S}}(n)$, a decrease is observed around MHR which may be partially explained by the overlapping between the SA band centered at $-d_{\mathrm{HRM}}(n)+2F_{\mathrm{ST}}(n)$ and the LF band. In this case, power in the overlapped band contributes only to $P_{\mathrm{LF}}(n)$. In this particular example, the transitory period at the beginning of exercise lasts approximately until 400 s, as suggested by the running

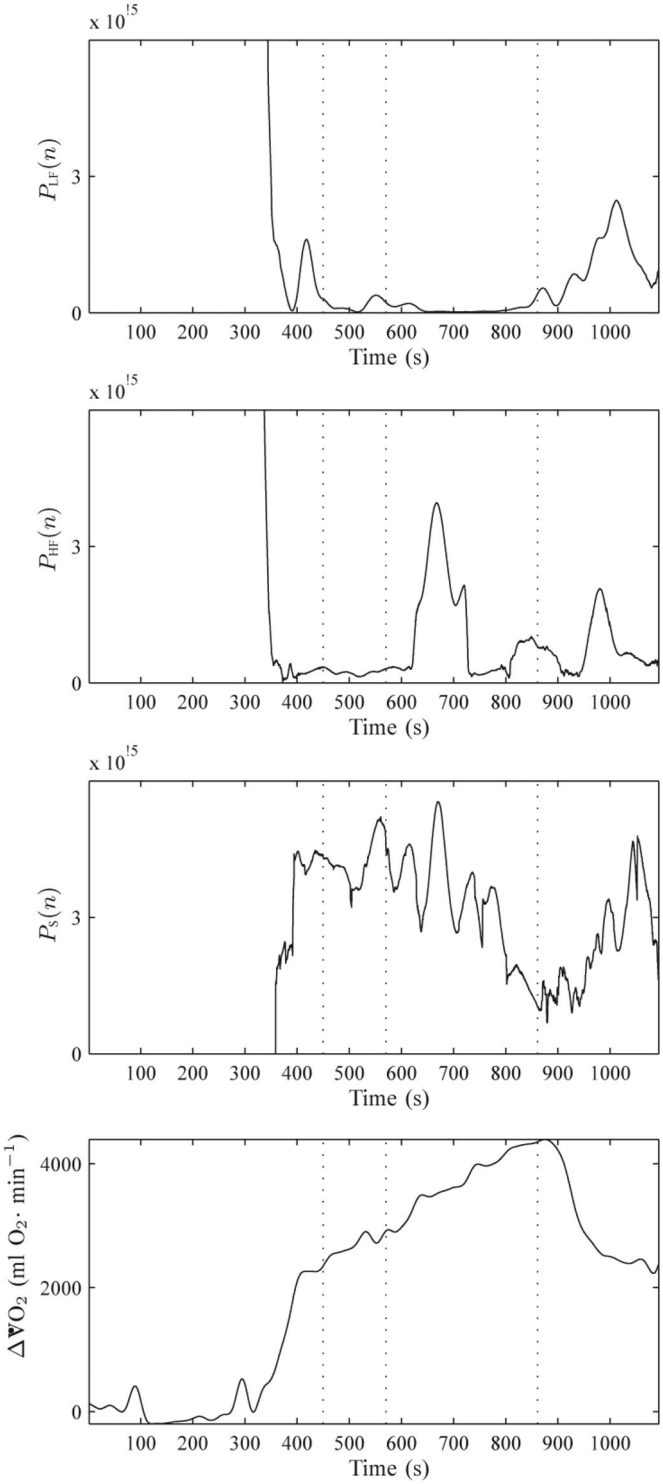

Fig. 3. From top to bottom: $P_{\mathrm{LF}}(n)$, $P_{\mathrm{HF}}(n)$, $P_{\mathrm{S}}(n)$, and $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ for the same subject of Fig. 2 including the 5-min resting period. Dotted lines represent from left to right the time when VT1, VT2, and MHR are achieved.

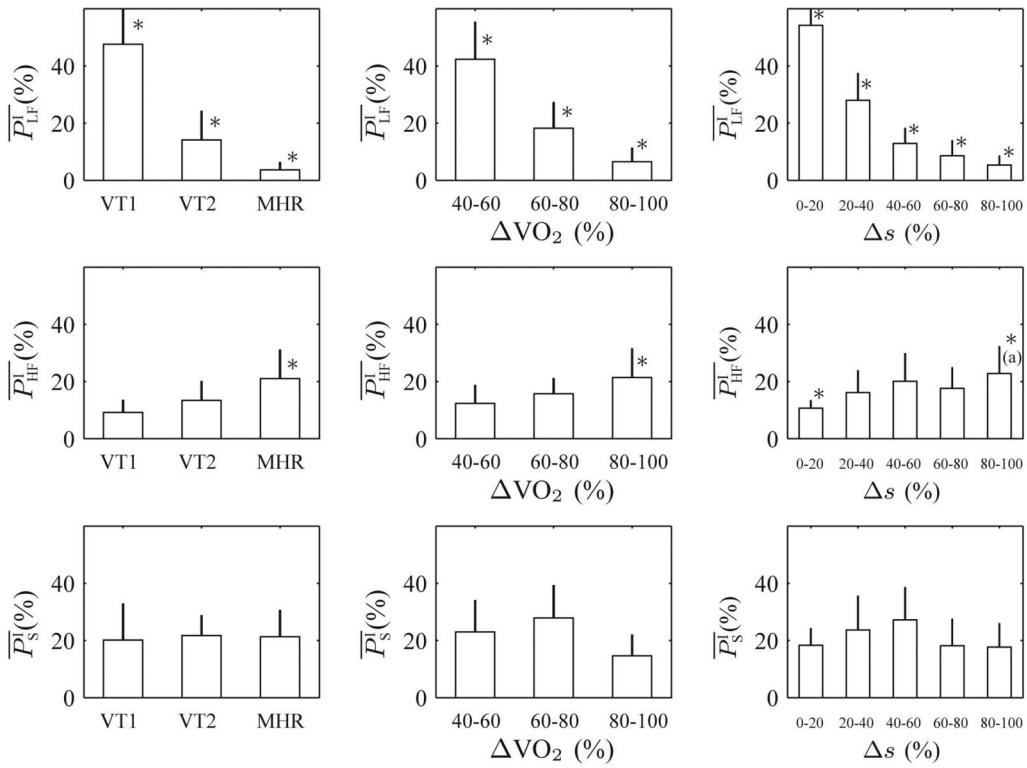

Fig. 4. Median ± MAD of $\overline{P^{\mathrm{I}}_{\mathrm{LF}}}$, $\overline{P^{\mathrm{I}}_{\mathrm{HF}}}$, and $\overline{P^{\mathrm{I}}_{\mathrm{S}}}$ during the different intervals I defined according to (left) physiological standardized exercise intensity, (center) percentage of $\dot{\mathrm{V}}\mathrm{O}_2$, and (right) percentage of speed excursion. $*$ significantly different (p-value < 0.02) from all the other intervals considered, (a) except for intervals 40–60 and 60–80.

stride and respiratory frequency trends. During this period subjects may still be walking rapidly, which would explain the peculiar trends of $P_{\mathrm{LF}}(n)$, $P_{\mathrm{HF}}(n)$, and $P_{\mathrm{S}}(n)$.

SF coupling appeared during exercise in all the subjects analyzed, although its contribution to the total HRV power varied substantially. In some subjects, the SF band was not shown since it was above the limit $\frac{d_{\mathrm{HRM}}(n)}{2}$. In other subjects, it was the SA band centered at $d_{\mathrm{HRM}}(n) - F_{\mathrm{ST}}(n)$ which exceeds the upper limit. In some cases, the SA band $-d_{\mathrm{HRM}}(n) + 2F_{\mathrm{ST}}(n)$ was not visible since it overlapped with the LF band during the whole exercise test. Moreover, the relative contribution of the SF and each of the SA bands to the total SF power varied greatly among subjects depending on the instantaneous HR, the SF and the particular way of running of each subject.

The median ± MAD of $\overline{P^{\mathrm{I}}_{\mathrm{LF}}}$, $\overline{P^{\mathrm{I}}_{\mathrm{HF}}}$, and $\overline{P^{\mathrm{I}}_{\mathrm{S}}}$ during the different intervals I defined according to physiological standardized exercise intensity, percentage of $\dot{\mathrm{V}}\mathrm{O}_2$, and percentage of speed excursion are displayed in Fig. 4. Parameter $\overline{P^{\mathrm{I}}_{\mathrm{LF}}}$ decreases significantly (p-value < 0.02) during VT2 with respect to VT1, and during MHR with respect to both VT1 and VT2, while $\overline{P^{\mathrm{I}}_{\mathrm{HF}}}$ increases significantly during MHR with respect to both VT1 and VT2. There were no significant differences in $\overline{P^{\mathrm{I}}_{\mathrm{S}}}$ during VT1, VT2, or MHR. The median ± MAD values were 22 ± 11%, 21 ± 7%, and 20 ± 10%, respectively. Parameter $\overline{P^{\mathrm{I}}_{\mathrm{LF}}}$ decreases significantly during interval 60–80% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ with respect to interval 40–60% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$, and during interval 80–100% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ with respect to intervals 40–60% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ and 60–80% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$. Parameter $\overline{P^{\mathrm{I}}_{\mathrm{HF}}}$ increases significantly during interval 80–100% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ with respect to intervals 40–60% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ and 60–80% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$. There were no significant differences in $\overline{P^{\mathrm{I}}_{\mathrm{S}}}$ during intervals 40–60% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$, 60–80% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$, and 80–100% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$. The median ± MAD values were 23 ± 11%, 28 ± 11%, and 15 ± 7%, respectively. Parameter $\overline{P^{\mathrm{I}}_{\mathrm{LF}}}$ decreases significantly during all the intervals considered as the speed increases, while $\overline{P^{\mathrm{I}}_{\mathrm{HF}}}$ increases from interval 0–20% Δs with respect to all the other intervals, and from interval 20–40% Δs with respect to interval 80–100% Δs. There were no significant differences in $\overline{P^{\mathrm{I}}_{\mathrm{S}}}$ during the analyzed intervals, with median ± MAD values of 18 ± 6% during 0–20% Δs, 24 ± 12% during 20–40% Δs, 27 ± 11% during 40–60% Δs, 18 ± 9% during 60–80% Δs, and 18 ± 8% during 80–100% Δs.

IV. Discussion

A. Methodological Aspects

In this study, HRV is estimated from the RR interval series recorded by an HR monitor. Although the HR monitor used (Polar RS800) is an improved version of the Polar S810i, which has been validated during exercise against different ECG systems [30], [31] and compared to other HR monitors [31], it has not been validated specifically for maximal treadmill exercise tests. Thus, the proprietary QRS detection algorithm used by the HR monitor and the unavailability of the simultaneous ECG

signal introduce a degree of uncertainty about the validity and origin of the HRV derived.

Various TF methods have been applied for the analysis of HRV during exercise testing. These include the wavelet transform [23], time-varying autoregressive methods [32], a method which combines a time-varying autoregressive model to estimate HRV component frequencies, and the short-time Fourier Transform to estimate their amplitudes [21], the SPWVD [4], [24], and a method which includes a parametric decomposition of the SPWVD [8], [33]. The advantages of SPWVD are that it provides better resolution than nonparametric linear methods, independent control of time and frequency filtering, and HRV power estimates with lower variance than parametric methods when rapid changes occur [34]. The main drawback of the SPWVD is the presence of cross terms, which can be attenuated by time and frequency filtering. In this study, the SPWVD was preferred to track the rapid changes in respiratory and stride frequencies. The Hamming windows used for time and frequency smoothing gave a time and frequency resolution, defined as the full width at half maximum of $g(n)$ and the Fourier transform of $|h(k)|^2$, respectively, of 27 s and 0.019 Hz. The study of HRV in specific time-varying frequency bands makes the problem of cross terms attenuation less serious.

Besides the presence of SF coupling during exercise in all the subjects analyzed, in the SF band and/or in one or more of the defined SA bands, it has been observed that sometimes the SA bands overlap with the LF and HF bands. Due to the frequency bands definition used in this study, when the overlapping occurs with the LF band, the power in the overlapped band contributes to $P_{\mathrm{LF}}(n)$ only, although its origin may not be purely sympathetic or parasympathetic but also due to the SF coupling. When the overlapping occurs with the HF band, the power in the overlapped band contributes both to $P_{\mathrm{HF}}(n)$ and $P_{\mathrm{S}}(n)$. In this case, it is impossible to distinguish if its origin is in the respiratory or SF coupling. The overlapping between the HF and SA bands occurs intermittently and occasionally during the exercise phase for some of the subjects. The overlapping between the LF and SA bands centered at $-d_{\mathrm{HRM}}(n) + 2F_{\mathrm{ST}}(n)$ occurs during most of the exercise phase for many of the subjects. Moreover, when the LF and SA bands do not overlap, the power in the LF band is usually higher than the power in the SA band. It was, therefore, decided that in the first case the power in the overlapped band contributes to both $P_{\mathrm{HF}}(n)$ and $P_{\mathrm{S}}(n)$, while in the second case the power in the overlapped band contributes to $P_{\mathrm{LF}}(n)$ only.

In any case, this overlapping phenomenon imposes a problem when the evolution of ANS activity, namely, the sympathetic activity and the RSA, is to be assessed from $P_{\mathrm{LF}}(n)$ and $P_{\mathrm{HF}}(n)$. In fact, it may introduce bias into the results presented in Fig. 4. Thus, the statistical analysis was repeated excluding from the interval analysis those subjects for which the overlapping between any SA band with the HF band was higher than 40% for more than 60% of the analyzed interval. The results were similar to those reported in Fig. 4. Statistical significance was maintained, except for the increase in $\overline{P^{\mathrm{I}}_{\mathrm{HF}}}$ from interval 0–20% Δs with respect to intervals 20–40% Δs and 40–60% Δs (the new p-values being in the range 0.02–0.05). In this case, the increase in $\overline{P^{\mathrm{I}}_{\mathrm{HF}}}$ from interval 40–60% Δs to interval 80–100% Δs was also significant (p-value$<$0.02 while the previous p-value was in the range 0.02–0.05). The main drawback of this approach is the reduced number of subjects included in the analysis. The overlapping between the LF and SA bands centered at $-d_{\mathrm{HRM}}(n) + 2F_{\mathrm{ST}}(n)$ occurred during the exercise phase for the majority of the subjects (16 out of 20). Therefore, statistical analysis excluding those subjects was not repeated.

While analysis according to percentage of speed excursion was performed in five 20% Δs regular intervals, having approximately the same duration in time, analysis according to percentage of $\dot{\mathrm{V}}\mathrm{O}_2$ was performed only in intervals 40–60% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$, 60–80% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$, and 80–100% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$, with different time durations. The reason for this is that, in contrast to speed, $\dot{\mathrm{V}}\mathrm{O}_2$ does not increase linearly, but very rapidly at the beginning of the exercise and progressively more slowly as the exercise intensity increases. Intervals 0–20% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ and 20–40% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ are too short to obtain reliable measurements. Moreover, when the exercise begins there is a transitory period until the treadmill reaches the initial speed of 8 km·h^{-1} and the stride sensor gives stable measurements. Intervals 0–20% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ and 20–40% $\Delta\dot{\mathrm{V}}\mathrm{O}_2$ usually lie within this transitory period.

In this study, peak stress is defined as the instant when MHR is achieved. Other possibilities could be considered, such as the instant of maximum SF or $\dot{\mathrm{V}}\mathrm{O}_2$, respectively. In the database analyzed, the instants of MHR and maximum SF differ, in absolute value, by 8 $\pm$ 4 s (median $\pm$ MAD). The instants of MHR and maximum $\dot{\mathrm{V}}\mathrm{O}_2$ differ by 30 $\pm$ 24 s. Since HRV measurements are averaged in a 1-min interval, we consider that the different definitions of peak stress would not change the results presented in this study.

B. Physiological Aspects

The hypothesis of our study was that a component centered at SF would appear due to cardiolocomotor coupling in the HRV of subjects during maximal treadmill exercise test, similar to the pedaling frequency component described in subjects performing maximal cycle ergometer exercise test [16]. This paper demonstrates not only the presence of this component at SF, but also the presence of SA due to the undersampling of the SF component and its harmonics when the mean HR does not satisfy the Nyquist criterion.

The consideration of the SF component as well as its aliases is very important when the evolution of the ANS activity is to be assessed from HRV analysis. SA may overlap with the LF and HF components (see Figs. 2 and 3), and it is impossible to distinguish in such cases if the power measured in the LF and HF bands have an autonomic and respiratory origin, or a cardiolocomotor origin. It is, therefore, essential to monitor both respiratory and stride frequencies in order to identify TF areas in which the SF and SA bands overlap with the LF and HF bands. The analysis proposed in this study is able to identify those TF areas but it cannot separate components into those with autonomic, respiratory, or cardiolocomotor origin. These TF areas can be excluded from the analysis of the ANS response to exercise, or analyzed with the knowledge that cardiolocomotor

coupling is present and may confuse the interpretation of HRV results.

Consideration of SF coupling is also important in the redefinition of the HF band during exercise. If the HF band is extended to include the whole range of physiological respiratory frequencies, as in [4] and [9], the power due to the SF coupling would be added to the HF power. This may explain, for example, the difference in the diagnostic performance of coronary artery disease achieved by HRV indices from exercise stress testing reported in [35] when considering either an extended HF band or an HF band centered at the respiratory frequency.

SF coupling was present during exercise in the case of all the subjects analyzed in this study. The percentage of the total HRV power contributed by power associated with the SF coupling averaged over the whole exercise phase has a median $\pm$ MAD value of $22 \pm 7\%$ across subjects. The relative contribution of the SF component and each of its aliases to the total SF coupling power varies substantially among subjects depending on the instantaneous HR, the SF and the particular way of running of each subject.

The percentage of the total HRV power contributed by SF coupling power has been averaged in different intervals defined according to physiological standardized exercise intensity (including ventilatory thresholds), percentage of $\dot{V}O_2$ and percentage of speed excursion. There were no statistical differences in the median values of the normalized SF power between the different intervals analyzed in any of the three categories. These results contrast with those reported in [16] where the amplitude of the pedaling frequency component increased significantly when the percentage of maximal power output increased from 60% to 100%, and was minimal at 50%. Note that in our study total SF coupling power is computed summing the power in the SF and SA bands, while only the power of the component centered at the pedaling frequency is considered in [16].

However, these studies should be compared with caution because although the SF coupling and the pedaling frequency component may both be due to cardiolocomotor coupling and have similar origins, there are important differences between both studies. First, there is the type of exercise, cycling, or running, which differs greatly from the physiological point of view (maximum $\dot{V}O_2$, MHR, ventilatory response [36], [37]). Second, the fixed and constant pedaling frequency in [16] may affect cardiolocomotor coupling in HRV differently than the spontaneous SF in our study, as occurs with controlled versus spontaneous breathing [38]. Third, SF coupling in our study includes not only the power in the SF band but also the power in the bands of the SA.

SF coupling has been observed not only during exercise but also during recovery when subjects were running at a speed of 8 $km \cdot h^{-1}$. This is of particular importance when HRV indices are studied during recovery. The overlapping between SA and LF and HF components is frequent during recovery because of the reduction of the frequency range with physiological meaning due to the fall in the mean HR.

The origin of the observed SF coupling is not clear but we hypothesize that it has the same origin as the pedaling frequency component described in [16]. It was postulated that the pedaling frequency component may have a mechanical origin, coming from the dynamic modulation of the venous return by the rhythmic limb muscle contractions, similar to the mechanical effect of breathing on the sinus node reported in [4], [7], and [12]–[15]. The SF coupling is not likely to have an autonomic origin since vagal tone progressively withdraws with exercise [3], [7] and neither sympathetic nor parasympathetic systems respond to frequencies as high as SF [39]. Moreover, lack of baroreflex control of carotid baroreceptor stimulation by neck suction at respiratory frequencies has been reported during exercise [40]. However, according to [7], a homeostatic origin cannot be rejected since it is speculated that baroreflex sensitivity at respiratory frequencies can be maintained during heavy exercise by a local baroresponse presumably determined by mechanoelectric feedback of hyperpnea to the sinus node.

Although considerable power has been measured in the bands around aliased harmonic stride frequencies, experimental conditions cannot be created physiologically where the harmonics which become aliased are present and absent under otherwise identical conditions. However, if we assume that venous return modulation induced by limb muscle contraction originates the SF coupling, and that it has a component periodic with stride period, it is reasonable to expect the presence of harmonic components in the input which generates the SF coupling in HRV. If venus return modulation does not deviate much from a sinusoidal signal, the first and second harmonics are expected to contain most of the power of the signal.

Finally, the contribution of power in the LF and HF bands to the total HRV power has been analyzed in the different intervals defined according to physiological standardized exercise intensity, percentage of $\dot{V}O_2$, and percentage of speed excursion. The influence of exercise on HRV has been studied in the literature using either a single intensity steady-state exercise, a number of steady-state intensities, incremental stages, ramping and even simulated altitude [41]. Several common patterns clearly emerge from the analysis of HRV during exercise. First, HRV is greatly reduced during exercise as a function of exercise intensity. When expressed in absolute values, both LF and HF powers decrease as a function of exercise intensity. When expressed in normalized units, LF power increases initially but then falls until almost disappearing. The HF power shows an initial decrease but then increases in parallel with exercise intensity [41]. Most studies analyzing HRV during exercise use a bicycle ergometer in the laboratory. Although the type of exercise may have an influence on HRV parameters, a study of HRV during field running trails also reported a sharp decrease in LF power with exercise and a significant decrease in HF power just after the start, with the values being maintained throughout the rest of the race [42]. Our results are consistent with this. They show a statistically significant decrease in normalized LF power as exercise intensity increases, concomitant with an increase in normalized HF power. These results are in agreement with [4], where normalized LF power was found to be significantly higher and normalized HF power significantly lower during exercise performed below the anaerobic threshold (VT2) compare with above. In addition, below the anaerobic threshold there was a prevalence of LF compared with HF power, whereas the balance

was reversed above the anaerobic threshold. This can also be appreciated in our results. Normalized LF and HF powers have median $\pm$ MAD values of $48 \pm 14\%$ and $9 \pm 4\%$ during interval VT1, of $14 \pm 10\%$ and $13 \pm 7\%$ during interval VT2, and of $4 \pm 2\%$ and $21 \pm 10\%$ during interval MHR.

The decrease in normalized LF power, despite the increase in sympathetic activity with exercise intensity, may be explained by vagal withdrawal during moderate exercise and by the saturation of the cardiac β-receptors by catecholamines [7].

The increase in normalized HF power, despite vagal withdrawal with exercise and near disappearance during heavy exercise, may be explained by the large hyperventilation induced by exercise above the anaerobic threshold, which provokes a mechanical stretching of the sinus node [4], [7], [12]. (During the inspiration phase, the expansion of the ribcage induces a decrease in intrathoracic pressure that in turn increases the venous return and consequently increases the filling of the right pump. This increase induces a stretch of the sinus node, provoking an increase in the sinus node activity and an increase in HR. During the expiration phase, the emptying of the lungs provokes the opposite effect with a decrease in HR.) These results are in agreement with [13], [14], which showed the persistence of HRV during exercise in humans after complete ganglion autonomic blockade, which inhibits the autonomic control of HR. In addition, studies with heart transplant patients showed a reappearance of HRV during exercise [15]. Although the HF power is significantly lower in heart transplant patients than in controls at rest, there is no significant difference between the two during exercise, suggesting that the observed HF power resulted from a mechanical effect of breathing on the sinus node.

Normalized LF, HF, and SF coupling power are lower than those reported in other studies [4]. Note that in [4], normalized LF and HF power are computed dividing absolute LF and HF power by the summation of absolute LF and HF powers, while in our study absolute LF and HF powers are divided by the total HRV power (after high-pass filtering the modulating signal at 0.04 Hz). Thus, total HRV power contains not only LF and HF power but also SF coupling power as well as other components that we have observed to appear during exercise in some of the subjects analyzed, such as respiratory frequency harmonics and a suspicious component centered around 1 Hz whose origin is uncertain. Which normalization (by the summation of absolute LF and HF powers or by total HRV power above 0.03 Hz) is more meaningful clinically is an open question.

The 1-Hz-component appeared in 60% of the analyzed subjects. It may have a physiological origin or may be induced by the HR monitor. To solve this uncertainty, the simultaneous recording of an alternative ECG signal is needed. Respiratory frequency harmonics may also be considered to contribute to HF power, in the same way that SF harmonics aliases contribute to the power associated with the SF coupling.

One limitation of the study is that only male subjects have been analyzed. Due to the differences in cardiac autonomic regulation between men and women [43], [44], it would be interesting to repeat the study in a female population to test whether the results are gender-dependent or not.

V. Conclusion

The consideration of SF component as well as its aliases is very important when the evolution of the ANS activity is to be assessed from HRV analysis, since SA may overlap with the LF and HF components. This makes it impossible to distinguish whether the power measured in the LF and HF bands has an autonomic and respiratory origin, or a cardiolocomotor origin. In this paper, we have demonstrated the appearance due to cardiolocomotor coupling of components centered at running SF as well as it aliases, representing the $22 \pm 7\%$ (median $\pm$ MAD) of the total HRV power in all the subjects, which overlap with the LF and HF bands during exercise and recovery and complicate the interpretation of HRV during treadmill exercise testing.

Appendix

Consider a periodic signal $d_{\mathrm{ST}}(t)$ with fundamental frequency F_{ST}. A periodic signal can be represented as a linear combination of harmonically related complex exponentials which is referred to as the Fourier series. The Fourier series of $d_{\mathrm{ST}}(t)$ can be written as

$$d_{\mathrm{ST}}(t) = \sum_{k=-\infty}^{+\infty} a_k e^{-jk2\pi F_{\mathrm{ST}} t} \tag{3}$$

where coefficients a_k determine the amplitude of the corresponding harmonic components.

The Fourier Transform of $d_{\mathrm{ST}}(t)$ is

$$D_{\mathrm{ST}}(j\Omega) = \sum_{k=-\infty}^{+\infty} 2\pi a_k \delta\left(\Omega - k2\pi F_{\mathrm{ST}}\right). \tag{4}$$

Thus, $D_{\mathrm{ST}}(j\Omega)$ is a train of impulses occurring at the harmonically related frequencies kF_{ST}, whose area is determined by the coefficients a_k.

Consider now that the signal $d_{\mathrm{ST}}(t)$ is amplitude modulated by the signal $v(t)$. The Fourier Transform of the modulated signal $m_{\mathrm{ST}}(t) = v(t)d_{\mathrm{ST}}(t)$ is

$$\begin{aligned} M_{\mathrm{ST}}(j\Omega) &= \frac{1}{2\pi}\left[D_{\mathrm{ST}}(j\Omega) * V(j\Omega)\right] \\ &= \sum_{k=-\infty}^{+\infty} a_k V(j(\Omega - k2\pi F_{\mathrm{ST}})) \end{aligned} \tag{5}$$

where $*$ represents the convolution operation and $V(j\Omega)$ the Fourier Transform of $v(t)$. Thus, $M_{\mathrm{ST}}(j\Omega)$ consists of the superposition of $V(j\Omega)$ shifted to the harmonically related frequencies kF_{ST} and scaled by the coefficients a_k.

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Authors' photographs and biographies not available at the time of publication.