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ORIGINAL ARTICLE

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High-frequency oscillations of the heart rate during ramp load reflect the human anaerobic threshold

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Abstract The aim of this study was to analyze the dynamic behavior of the high-frequency component ($HF > 0.15$ Hz) of heart rate variability (HRV) and the respiratory frequency in relation to the anaerobic threshold (AT). Twenty-two healthy subjects [mean (SD) age: 24 (6) years, height: 175 (10) cm, body mass: 65 (11) kg] completed a ramp load, with increments of $20\ \mathrm{W} \cdot \mathrm{min}^{-1}$, on a cycle ergometer. The AT was determined by the V-slope-method. Respiratory movements of the thorax, and the electrocardiogram were monitored. The instantaneous frequency of the HF component of HRV and of the respiratory signal were obtained by the Hilbert transformation. Both frequencies were closely related, the cross correlation coefficient being between 0.84 and 0.99. Various patterns of HRV and respiration were observed during the protocol. Remarkably, however, in over 90% of these cases, a shift in the instantaneous frequency of the HF component occurred during the transition from aerobic to anaerobic work. The difference between the AT determined by gas analysis and the AT evaluated as the power output (AT_f), calculated using the approximation of the curve of the instantaneous frequency of HF by hyperbolic tangent functions, varied between 2 and 14%. In conclusion, the present study demonstrates significant changes in the behavior of the instantaneous frequency of HF in the region of the AT.

Key words Exercise · Heart rate variability · Respiration · Cardiorespiratory interaction · Hilbert transformation

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Introduction

In the 1960s, concepts were developed regarding a critical threshold during exercise, which is characterized by an inadequate oxygen supply of the muscle, leading to an increase in anaerobic metabolism and lactate accumulation (Wasserman and McIllroy 1964). Since then, the work rate at the aerobic-anaerobic transition has been used more and more as a marker for muscular and cardiovascular capability, although the underlying physiological mechanisms of the "anaerobic threshold" (AT) are not fully understood. Thus, there is a large demand for reproducible and robust methods for the determination of the AT (e.g., in sports medicine, cardiology, and clinical rehabilitation).

Increasing work above the AT work rate is accompanied with marked physiological effects such as metabolic acidosis, changes in the gas exchange ratio or in ventilation. Consequently, changes in the parameters of breathing and heart rate control have been found in relation to the aerobic-anaerobic transition. A nonlinear behavior that is dependent upon load has been demonstrated for the respiratory frequency (f_R; James et al. 1989; Cheng et al. 1992). f_R markedly increases while surpassing the AT. A deflection point was also described for the heart rate, which was associated with the AT during running field tests (Conconi et al. 1982; Ballarin et al. 1996). Unfortunately, as shown by various studies, a significant number of subjects do not exhibit such a deflection point (Jones and Doust 1995; Hofmann et al. 1997).

Studies employing spectral analysis reveal a strong correlation between the instantaneous frequency of the high-frequency (HF) component of heart rate variability (HRV) and the f_R in non-rapid-eye-movement sleep, quiet wakefulness, or during metronome-paced breathing (Saul et al. 1989; van de Borne et al. 1995). Only little data, however, exists regarding the interaction between f_R and heart rate during physical activity. It has been shown that the overall HRV is drastically diminished during exercise (Perini et al. 1990). However, it seems from that study that the respiratory component of HRV is maintained even at high loads.

The present study has two purposes. First, to examine whether exercise-related changes in f_R are mirrored by changes in the HRV. The second question focuses on the behavior of the HF component of HRV during the transition from aerobic to anaerobic work.

Most of the commonly employed methods of spectral analysis rely on stationary time series. Since the time series of cardiorespiratory parameters are highly non-stationary during increasing load, special algorithms for the analysis of HRV in the frequency domain are necessary. Therefore, in this study the analysis was performed using the Hilbert transformation, which allows us to investigate the behavior of instantaneous phase and frequency for non-stationary time series (Panter 1965; Rosenblum et al. 1996).

Methods

Subjects

A total of 22 subjects were examined. All volunteers were untrained, having less than 4 h physical training per week, and none of them had taken part in competitive sports training at any time before. The anthropometric and functional data of this group are presented in Table 1. The volunteers were physically examined and the medical history was recorded to exclude illness. They were informed about the objectives of the study, and provided written consent before participation. The local ethical committee approved of the study.

Protocol

The subjects exercised on an electrically braked cycle ergometer (CPE 2000, Medical Graphics Minnesota, USA). The test protocol consisted of a ramp load. The incremental test started after the subjects performed a 3-min warm-up, cycling with a load of 5 W. The exercise intensity was increased at a rate of 20 W · min^{-1}. The pedaling frequency amounted to 50 revs · min^{-1}. The criteria for exhaustion were the inability to maintain the cycling frequency and a lack of an increase in oxygen uptake ($\dot{V}O_2$) within a 30 s-period. Some subjects finished the ramp load without reaching the exhaustion criteria. In these cases, and in the other cases showing a plateau, the highest value for $\dot{V}O_2$ ($\dot{V}O_{2peak}$) and minute ventilation ($\dot{V}_{Epeak}$) were obtained (Table 1). The volunteers breathed through a mouthpiece. $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) were measured breath-by-breath using an open system (Medical Graphics System CRX). $\dot{V}O_2$ and $\dot{V}CO_2$ served for the determination of the AT using the V-slope method (Beaver et al. 1986). During the test, respiratory thorax excursions were obtained by the Respitrace system, and heart rate was obtained from a bipolar electrocardiogram (ECG) lead of the chest.

Data analysis

The ECG was A/D converted at a rate of 1000 Hz, and the signal of the respiratory effort was sampled at a rate of 10 Hz (b-Scope, MedNatic, Germany). The HRV time-series were constructed from R-R interval sequences. The R-waves were identified using a template-based algorithm and the results were inspected visually. In less than

Table 1 Anthropometric and functional data of the volunteers. (*AT* anaerobic threshold computed using the V-slope method, AT_f anaerobic threshold computed from the deflection of the instantaneous frequency of the high frequency component of the heart rate variability, *E* difference between AT and AT_f, $\dot{V}O_{2peak}$ peak oxygen uptake, $\dot{V}_{Epeak}$ peak minute ventilation)

Subject no.	Gender	Age (years)	Height (cm)	Mass (kg)	$\dot{V}O_{2peak}$ (l · min^{-1})	$\dot{V}_{Epeak}$ (l · min^{-1})	Maximum power (W)	AT (W)	AT_f (W)	*E* (%)
1	F	20	171	58	1809	45	139	75	77	2.7
2	F	20	164	52	1595	45	127	69	78	13.0
3	F	20	173	58	1647	46	150	91	89	2.2
4	F	20	159	54	1993	49	143	90	–	–
5	F	20	162	58	1556	44	98	76	66	13.2
6	F	21	161	58	2194	53	177	95	99	4.2
7	F	21	176	60	1360	32	104	78	80	2.6
8	F	21	188	81	3121	75	227	134	131	2.2
9	F	22	164	50	1271	41	103	57	58	1.8
10	F	22	168	57	2114	48	154	88	–	–
11	F	23	172	60	2022	56	171	100	98	2.0
12	F	23	170	53	1637	46	118	69	66	4.3
13	F	23	170	64	2075	43	164	114	111	2.6
14	M	22	187	82	2942	58	205	101	87	13.9
15	M	22	184	74	2774	73	239	116	132	13.8
16	M	22	191	80	3052	82	279	141	146	3.5
17	M	22	183	68	2836	75	229	114	99	13.2
18	M	25	176	66	2114	72	172	70	75	7.1
19	M	28	193	85	2960	75	184	100	90	10.0
20	M	38	180	70	2911	66	200	93	100	7.5
21	M	40	182	62	2288	70	186	82	89	8.5
22	M	40	172	73	2732	78	201	87	85	2.3
Mean		24.3	174.8	64.7	2227.4	57.8	171.4	92.7[a]	92.8[a]	6.5
SD		6.4	10.0	10.5	595.5	14.9	47.8	21.3[a]	22.9[a]	4.7

[a] The mean (SD) value of AT from 22 subjects, whereby the mean (SD) of AT_f is computed from just 20 subjects. In two subjects (nos. 4 and 10), AT_f was not detected by the applied method

10% of the segments it was necessary to correct the time series of R-R intervals on account of the technical artifacts or ectopic beats. Not more than one transient event per minute was allowed in the time series, which had to be corrected. This criterion was fulfilled in all analyzed segments. The correction of the R-R interval series was carried out in two steps. First, the statistical distribution was calculated for each time series. The patterns of R-R interval distribution are normally unimodal and continuous. R-R intervals outside of this distribution were considered to be too short or too long. In a second step, these intervals were examined with the help of heuristic algorithms regarding their potential technical or physiological origin. The time series were then corrected taking the total duration into consideration (Patzak et al. 1996).

The time series of HRV were interpolated with a cubic spline function method and resampled at a rate of 10 Hz to obtain equidistant time series. The analysis was performed for the time series of the HRV as well as for the respiratory signal. Before this, the low-frequency trend of the HRV time series was removed by the procedure of least fitting of a polynomial function of 2nd order in a moving window. The width of the window was 10 s. After this procedure, the HF component was selected by Gauss-filter, with the maximum at the base f_R and a level of 0.1 at the frequency of 0.15 Hz, to decrease the influence of the low-frequency component of HRV. The figure of 0.15 Hz was taken as the low-frequency limit of the HF component of the HRV.

Calculation of the instantaneous phase deflection and instantaneous frequency of the HF component of HRV and f_R

Ramp loading leads to non-stationary time series of cardiorespiratory data. Thus, algorithms based on the Fourier analysis, which require stationary time series, are not applicable. For calculation of the instantaneous phase and frequency of the HF component of HRV, the analytic signal approach was used (Panter 1965; Rosenblum et al. 1996). Let $z(t)$ represent an arbitrary signal. The analytic signal, a complex function of time, is defined by: $Z(t) \equiv z(t) + iz_1(t) \equiv A(t)\exp[i\Psi(t)]$, where $z_1(t)$ is the Hilbert conjugate function to $z(t)$, $A(t) \equiv \sqrt{z^2(t) + z_1^2(t)}$, and $\Psi(t) = \arctan[z_1(t)/z(t)]$, are the instantaneous amplitude and phase of the signal $z(t)$, and i is the imaginary unit. Since respiratory signals and the HF component of HRV contain an essential harmonic component, the instantaneous phases of both signals can be represented by a linear part, $\omega_0 t$, and by a deflection from the linear function, $\varphi(t)$:$\Psi(t) = \omega_0 t + \varphi(t)$, where ω_0 is the base frequency. The linear component of the instantaneous phase was determined by the procedure of least fitting of a linear function. The instantaneous phase deflection was calculated as a difference between the instantaneous phase and the linear part of instantaneous phase: $\varphi(t) = \Psi(t) - \omega_0 t$.

The instantaneous frequency $f(t)$ can be calculated as the derivative of the instantaneous phase: $f(t) = (1/2\pi)\mathrm{d}\Psi(t)/\mathrm{d}t$. For the diminution of the digital derivation noise, the instantaneous frequency was calculated after smoothing the instantaneous phase by moving average with a window width of 30 s.

The instantaneous phase deflection and instantaneous frequency were calculated for the time series of the HF component of HRV as well as for the respiratory signal.

Calculation of the cross correlation coefficient

Calculating the cross correlation coefficient enables estimation of the degree of linear co-ordination between the f_R and the HF component of the HRV. The cross correlation coefficient, R was calculated for the instantaneous phase deflection as well as for the instantaneous frequency, according to the following formula:

$$R = \int_{-\infty}^{\infty} x(t)y(t)\,\mathrm{d}t \Bigg/ \sqrt{\int_{-\infty}^{\infty} x^2(t)\mathrm{d}t \int_{-\infty}^{\infty} y^2(t)\,\mathrm{d}t} \tag{1}$$

where $x(t)$ is the instantaneous phase deflection $\varphi_{\mathrm{HRV}}(t)$, or frequency $f_{\mathrm{HRV}}(t)$, of the HF component of HRV, and $y(t)$ is the instantaneous phase deflection $\varphi_{f_R}(t)$, or frequency $f_{f_R}(t)$, of respiration.

Determination of AT-related changes of the instantaneous frequency

For the determination of AT-related changes of the HF component of HRV, the curves of the instantaneous frequency were approximated by the sum of two shifted hyperbolic tangent functions:

$$f(t) = k_1 f_1(t) + k_2 f_2(t) + C = k_1 \tanh[a_1(t - \tau_1)] + k_2 \tanh[a_2(t - \tau_2)] + C \tag{2}$$

where k_1 and k_2 are weighting coefficients, a_1 and a_2 are scaling factors, τ_1 and τ_2 are time shifts and C is a constant. AT is evaluated as the power output (ATf), where the function $f_2(t)$ reaches level L (Fig. 1). The AT revealed individual errors, which were calculated using the formula: $E = \frac{\mathrm{abs(AT - AT}_f)}{\mathrm{AT}} 100\%$, where abs symbolizes the absolute value.

Results

Synchronization of the respiration and HF component of HRV

In terms of the instantaneous phase deflection and the instantaneous frequency, the ramp-load experiments showed a strong correlation between the HF component of HRV and respiration. Both parameters changed in parallel with increasing exercise intensity (Figs. 2–6). The cross-correlation coefficient of these signals ranged between 0.84 and 0.99.

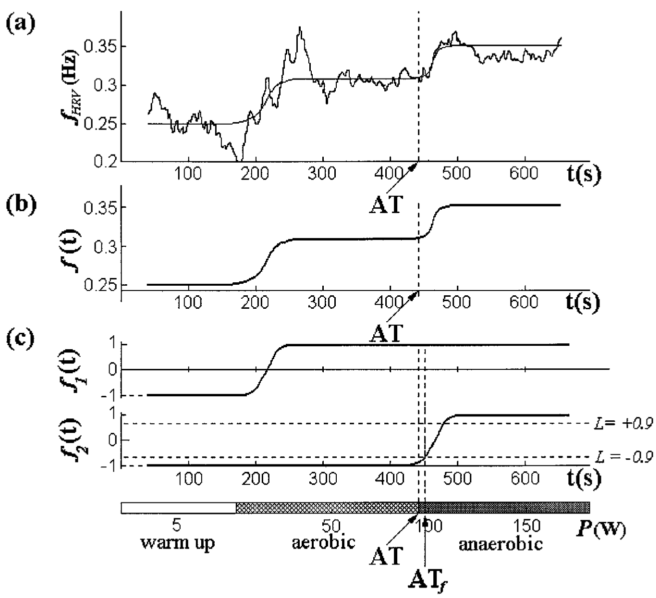

Fig. 1 Curve of instantaneous frequency of the high-frequency (*HF*) component of heart-rate variability (*HRV*) (**a**), the approximation function $f(t)$ (**b**), and the approximation functions $f_1(t)$ and $f_2(t)$ (**c**). L is the level at which the anaerobic threshold *ATf* was estimated from the approximated function. Here, P (W) is the exercise scale and *AT* is the anaerobic threshold determined by gas analysis

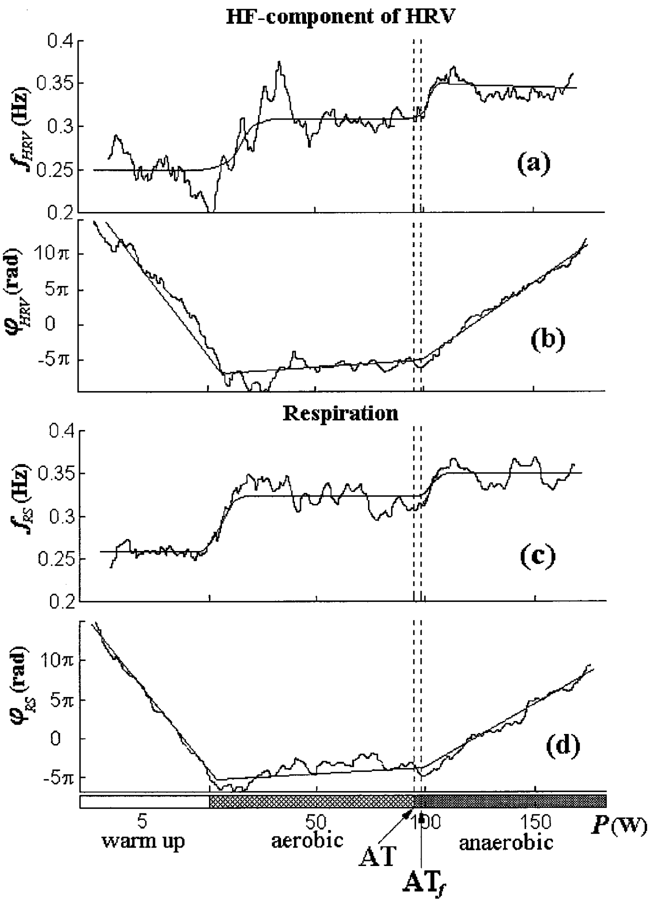

Fig. 2a–d Example of a step change from a short stationary state to another stationary in relation to AT (subject no. 6). (**a**) Instantaneous frequency f_{HRV} (Hz), and (**b**) instantaneous phase deflection φ_{HRV} (rad) of the HF component of HRV; (**c**) instantaneous frequency f_{RS} (Hz) and (**d**) instantaneous phase deflection φ_{RS} (rad) of respiration. The interpolated curves are the approximations by hyperbolic tangent functions

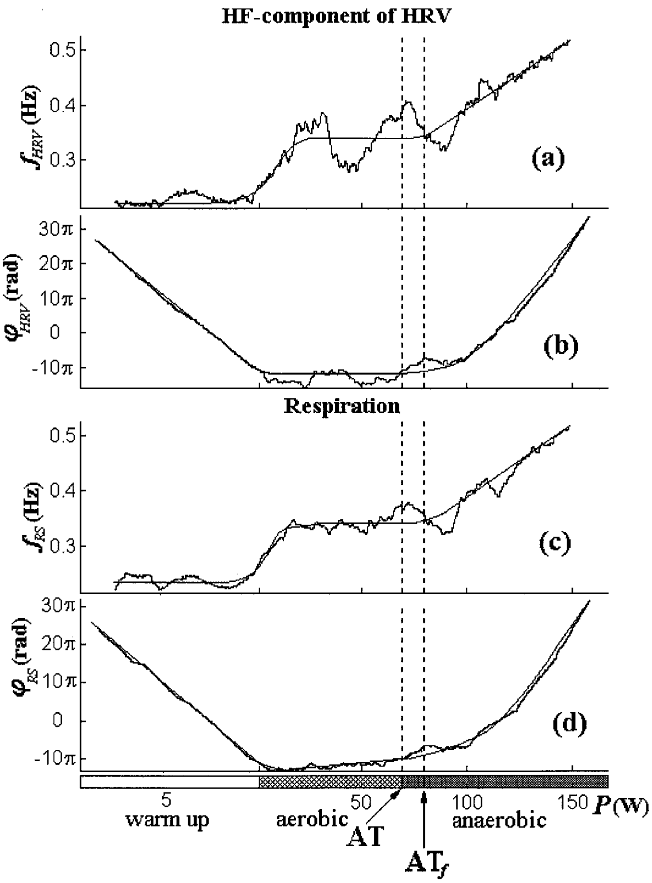

Fig. 3a–d Example of a transition from a short stationary state to another state with a continuous increase of the instantaneous frequency in relation to AT (subject no. 2). (**a**) Instantaneous frequency f_{HRV} (Hz) and (**b**) instantaneous phase deflection φ_{HRV} (rad) of the HF component of HRV; (**c**) instantaneous frequency f_{RS} (Hz) and (**d**) instantaneous phase deflection φ_{RS} (rad) of respiration. The interpolated curves are the approximations by hyperbolic tangent functions

Behavior of the instantaneous frequency and phase during increasing load

The instantaneous frequency of respiration and of the HF component of HRV followed different patterns during the test period. In most cases, the first change in dynamics was observed at the onset of the incremental test. This was characterized either by a corresponding step change of the instantaneous frequency of respiration and of the HF component of HRV (parts a and c of Figs. 2, 3, 5, 6), or by a gradual increase of these two measures (Fig. 4a, c).

For most of the cases (91%), a second change in the dynamic state was observed in the region of the AT: the instantaneous phases and frequencies showed a deflection that was correlated to AT.

The pattern of the behavior of both the instantaneous frequency of the HF component and the respiration of the 22 subjects, can be classified into three main groups. In 8 cases (36.4%) a step change was observed resulting in a higher baseline value of the frequency. In these cases, the mean value of the instantaneous frequency was increased by 10–15% over a period of less than 50 s. The step was recorded as an abrupt change in the function of the instantaneous phase (Fig. 2). In 8 cases (36.4%), a transition was observed from a stationary state to a state with a constant increase of instantaneous frequency. In this pattern, the deflection of the instantaneous phase varied from a linear function to a quadratic function (Figs. 3–5). Other subjects showed: (1) an increase in instantaneous frequency up to a saturation level (Fig. 4), along with a deflection of the instantaneous phase, which changed from a quadratic function to a linear function, and (2) a step decrease and subsequent sharp increase when passing the AT (18.2%).

The difference between the power output AT$_f$ (i.e., calculated from the instantaneous frequency of the HF component) and the AT determined by gas analysis revealed individual errors of 2–14%. For the calculation of AT$_f$, the level L was fixed at −0.9 (10% increase of the hyperbolic tangent function) for the frequency patterns

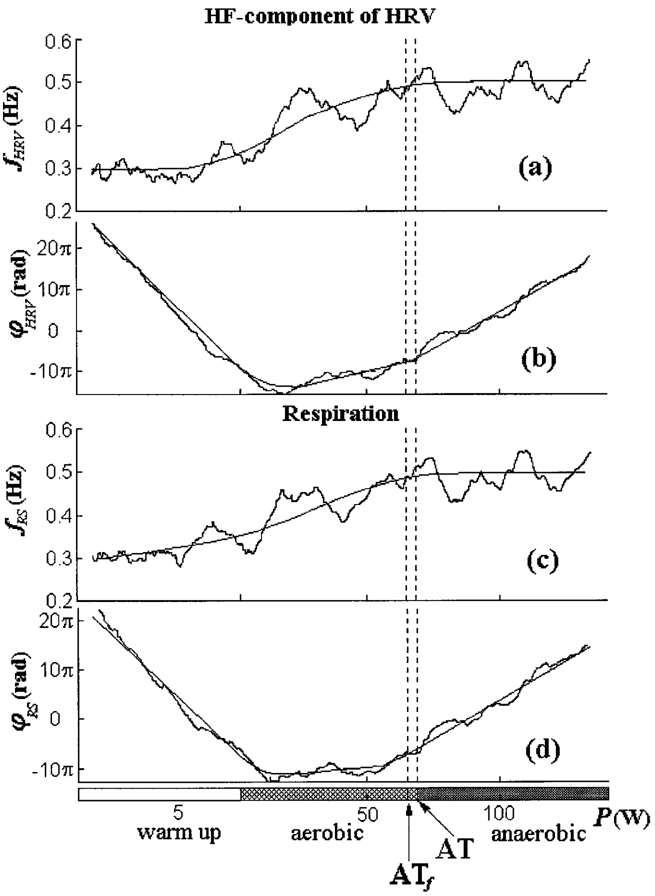

Fig. 4a–d Example of a change from a state characterized by an increase in instantaneous frequency to a short stationary state in relation to AT (subject no. 12). (**a**) Instantaneous frequency f_{HRV} (Hz) and (**b**) instantaneous phase deflection φ_{HRV} (rad) of the HF component of HRV; (**c**) instantaneous frequency f_{RS} (Hz) and (**d**) instantaneous phase deflection φ_{RS} (rad) of respiration. The interpolated curves are the approximations by hyperbolic tangent functions

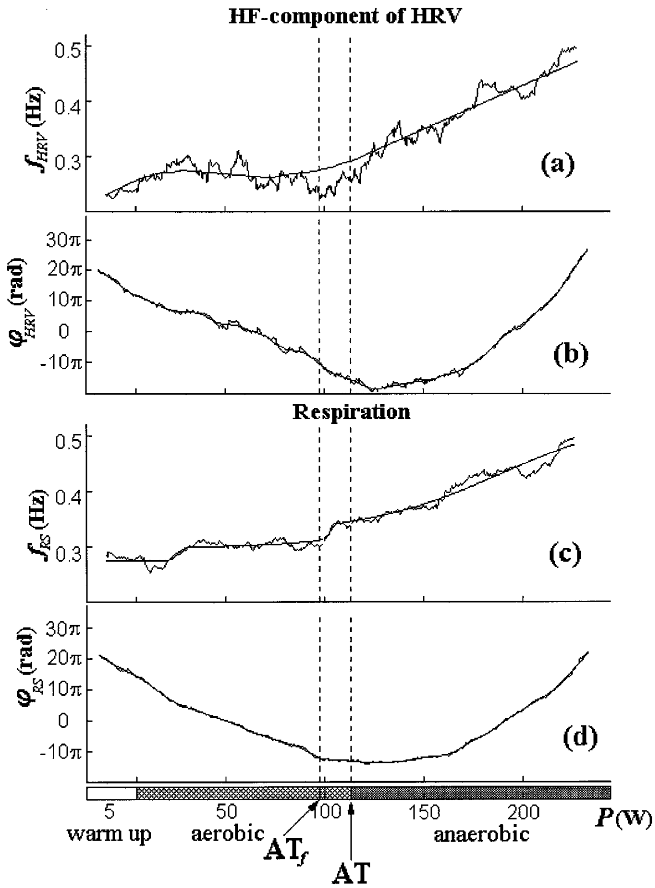

Fig. 5a–d Example showing the similar behavior of instantaneous frequency in relation to AT as in Fig. 3 (subject no. 17), but with a higher difference between AT and AT_f. (**a**) Instantaneous frequency f_{HRV} (Hz) and (**b**) instantaneous phase deflection φ_{HRV} (rad) of the HF component of HRV; (**c**) instantaneous frequency f_{RS} (Hz) and (**d**) instantaneous phase deflection φ_{RS} (rad) of respiration. The interpolated curves are the approximations by hyperbolic tangent functions

showed in Figs. 2, 3 and 5. For the patterns presented in Fig. 4, the level L was fixed at $+0.9$ (10% decrease of the hyperbolic tangent function). In Fig. 7, AT_f is plotted against AT, showing a high correlation between both parameters.

In two cases (9%) no changes in the instantaneous frequency were observed in relation to the AT. An example for this case is presented in Fig. 6.

Discussion

In this study, we demonstrate for most of the cases that the phase and frequency of the HF component of HRV change significantly if the workload exceeds the AT. This occurs irrespective of the various different behavioral patterns of the frequency of the HF component of HRV. Furthermore, we were able to show that the instantaneous frequency of the HF component of HRV and of the respiratory signal develop in parallel during a ramp-test load. Both signals are closely linked, the cross correlation coefficient between both signals was between 0.84 and 0.99. The high correlation can be interpreted as a strong co-ordination between respiration and heart rate. This is associated with a significant respiratory sinus arrhythmia of the heart rate. The modulation of HRV, in terms of its frequency, is strong even at high physical activity levels. This is remarkable, since the absolute power of HRV is clearly reduced at high work loads (Perini et al. 1990; Shin et al. 1995). The diminution of the HF modulation is due to a reduction in the vagal activity to the heart (Warren et al. 1997). It has also been shown that respiratory sinus arrhythmia becomes smaller when the f_R increases (Sanderson et al. 1996), or when tidal volume decreases (Brown et al. 1993). The genesis of respiratory sinus arrhythmia is not yet fully understood. Central rhythmic generation in the cardiorespiratory network of the brainstem (Richter et al. 1991), modulation of the baroreflex sensitivity (Eckberg et al. 1980), reflexes via pulmonary stretch receptors (Taha et al. 1995), and direct influence of intrathoracic pressure on the pacemaker cells (Bernardi et al. 1992) have all been considered as possible mecha-

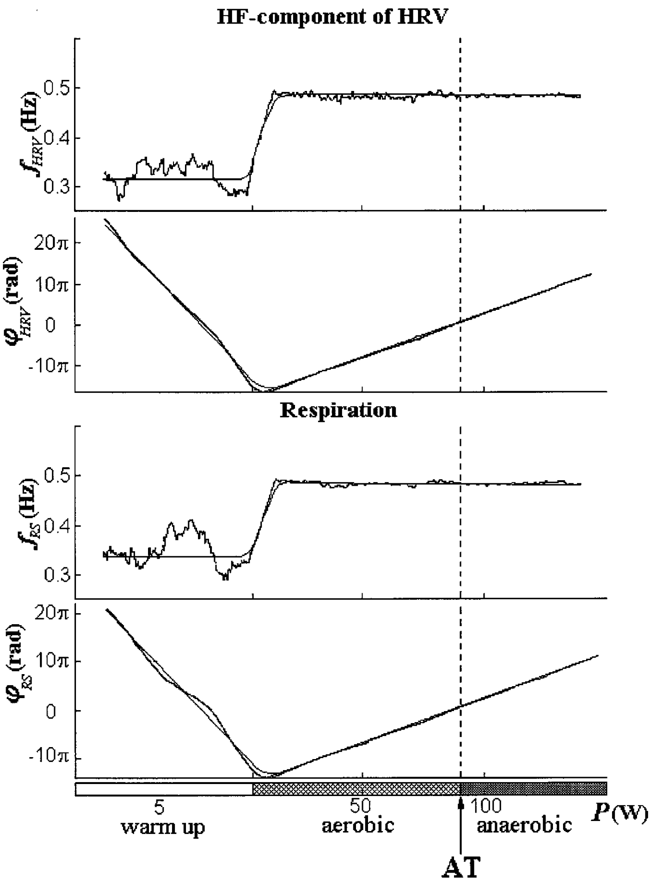

Fig. 6a–d Example of a lack of change in respiratory frequency and the HF component of HRV in the region of the AT (subject no. 4). (**a**) Instantaneous frequency f_{HRV} (Hz) and (**b**) instantaneous phase deflection φ_{HRV} (rad) of the HF component of HRV; (**c**) instantaneous frequency f_{RS} (Hz) and (**d**) instantaneous phase deflection φ_{RS} (rad) of respiration. The interpolated curves are the approximations by hyperbolic tangent functions

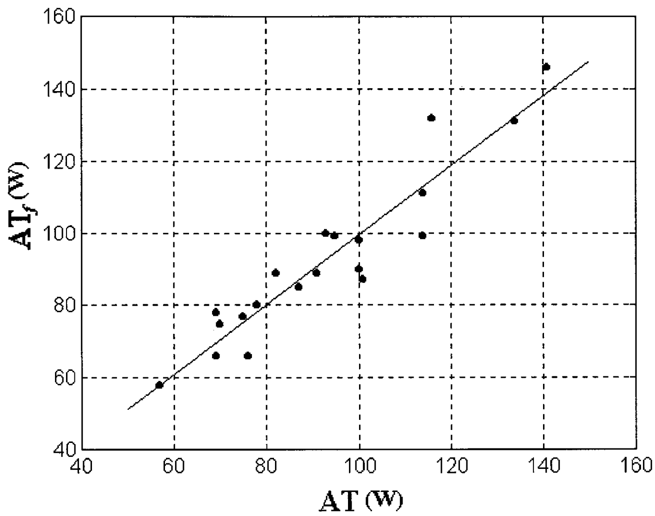

Fig. 7 Correlation between AT and AT_f for 20 of the 22 subjects studied. The equation for the linear regression is $AT_f = 0.97 \cdot AT + 2.94$, with $r = 0.94$, $P < 0.001$. In two subjects, AT_f was not detectable by the applied method

nisms. The individual contribution of these mechanisms changes during exercise. This relies on baroreceptor resetting (Potts et al. 1993) and modified ventilation.

As mentioned above, an essential modification of the instantaneous frequency and of the instantaneous phase were observed in the region of the AT. Although both parameters display different patterns, the approximation of the curve of the instantaneous frequency by hyperbolic tangent functions enabled us to estimate the AT with an error of 2–14%. Thus, the results of this investigation show that for most cases, the AT can be detected satisfactorily from analyses of dynamics of the frequency of the HF component of HRV. To our knowledge, this is the first study focusing on the instantaneous frequency of the HF component of HRV during ramp load. The high correlation between the instantaneous frequencies of the HF component of HRV and respiration allows a comparison of the present results with previous studies dealing with f_R. In a study utilizing an incremental test in physically active adults, James et al. (1989) observed a high correlation between a disproportionate increase in minute ventilation ($\dot{V}_E$) and in f_R at AT. Cheng et al. (1992) inaugurated the D_{max}-method for the analysis of ventilatory and metabolic parameters in incremental tests. These authors showed that in adult cyclists, it is feasible to use the f_R for the determination of AT. In contrast to these studies, however, Shimana et al. (1991) performed incremental tests in healthy adults and reported significant differences between AT and the work corresponding to the inflection point of the f_R. The latter authors speculate that the differences are due to an entrainment of the f_R to the pedaling rhythm. There is hitherto no agreement regarding the entrainment of f_R during load experiments in the literature. It seems more likely to occur during treadmill running (Paterson et al. 1986) than in cycling experiments. Paterson et al. (1986), Jasinskas et al. (1980), and Bechbache and Duffin (1977) reported the occurrence of entrainment during cycling in steady-state conditions, but the incidence varied between these studies. During ramp load, as in the present study, f_R and the frequency of the HF component of HRV change continuously. That is why entrainment is not readily recognized. It cannot be excluded, however, that changes in the f_R at AT are due to entrainment phenomena, at least in single cases. Furthermore, the potential coupling could also mask changes in f_R that are due to the metabolic and respiratory gas status.

Ventilation parameters change during ramp load due to different mechanisms. During exercise, lactate acid, which stimulates peripheral chemoreceptors, potassium as a humeral factor, and alterations in neural activity, are linked to the metabolic processes. This can lead to more or less abrupt changes in respiratory parameters (Myers and Ashley 1997). For $\dot{V}_E$, it has been shown that this parameter accelerates with respect to the $\dot{V}CO_2$ (Wasserman 1987). Therefore, ventilatory parameters are potential markers of AT, provided the subject's respiratory control is sensitive to carbon dioxide. Interestingly, some healthy subjects are insensitive to carbon dioxide (Beaver et al. 1986). This may be one reason for the lack of changes in f_R and frequency of HF component of HRV in the two subjects in the present study. Moreover, the patterns seen at AT vary, since the f_R is more variable than $\dot{V}_E$. There is not a single, typical pattern of f_R. An acceleration after exceeding AT may occur, or a relative decline (Fig. 4). An advantage of the method demonstrated in the present study is the detection of transients nearly independently of their pattern. This algorithm is more robust regarding variations in control, which are subjected mainly to the f_R.

The various behaviors of HRV and f_R as the AT is exceeded, suggest the existence of different adaptation patterns of the cardiorespiratory system. In this context, it is noteworthy that individual differences are seen, as well as differences within subjects (unpublished observation).

Conclusion

The instantaneous frequency of the HF component of HRV is strongly correlated with the f_R. The HF component is modified during ramp load and, in most cases, can be used for the detection of the AT. Since most studies employ heart rate measurements, but often do not include respiratory parameters, this study provides a possibly useful tool for identifying AT. Furthermore, the successful use of the Hilbert transformation demonstrates a possible way of circumventing the pitfall of non-stationarity, which often flaws the spectral analysis of cardiovascular measures.

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