ORIGINAL ARTICLE

Autonomic modulations of heart rate variability and performances in short-distance elite swimmers

Giampiero Merati · Martina Anna Maggioni · Pietro Luigi Invernizzi · Claudio Ciapparelli · Luca Agnello · Arsenio Veicsteinas · Paolo Castiglioni

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Abstract

Purpose Endurance exercise is associated with high cardiac vagal tone, but how the cardiac autonomic control correlates with shorter anaerobic performances is unknown. Therefore, the aim of this study was to evaluate how autonomic modulations of heart rate (HR) variability (V) correlate with performances of short- (<1 min) and very short (<30 s) duration in elite athletes.

Method Thirteen male swimmers, national-level crawl specialists in short (100-m) and very short (50-m) distances, were enrolled. HR was recorded during 15-min supine rest: (1) in the morning after wake up, (2) in the afternoon before sprint-oriented training sessions, (3) few minutes after training (first recovery phase after swimming cooldown). Heart rate variability (HRV) vagal and sympatho/vagal indices were calculated in time, frequency and complexity domains. Correlations of best seasonal times on 100- or 50-m distances with HRV indices and the

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G. Merati · M. A. Maggioni (🖂) · P. L. Invernizzi · L. Agnello · A. Veicsteinas Department of Biomedical Sciences for Health, University of Milan, Via Colombo, 71, Milan, Italy e-mail: martina.maggioni@unimi.it		
G. Merati · P. Castiglioni IRCCS Fondazione Don Carlo Gnocchi, Milan, Italy e-mail: pcastiglioni@dongnocchi.it		
M. A. Maggioni Department of Physiology, Center for Space Medicine and Extreme Environments, Charité University of Medicine, Berlin, Germany		
C. Ciapparelli DDS Sport Center, Settimo Milanese, Milan, Italy		

velocity at blood lactate accumulation onset ($V_{\rm OBLA})$ were calculated.

Results and conclusion Vagal indices were highest in the morning where they positively correlated with very short-distance times (higher the index, worse is the 50-m performance). Sympatho/vagal indices were highest after training where they negatively correlated with short-distance times (higher the index, better is the 100-m performance). V_{OBLA} did not correlate with the performances. Therefore, autonomic HRV indices and not V_{OBLA} predict short and very short, most anaerobic, performances. Results also suggest that a strong cardiac vagal control has no effect on short performances, and that the capacity to powerfully increase the sympathetic tone during exercise may improve short, but not very short performances.

Keywords Anaerobic exercise · Entropy · Detrended fluctuation analysis · Power spectral analysis · Autonomic nervous system

Abbreviations		
HF	High-frequency power	
HR	Heart rate	
HRV	Heart rate variability	
LF _{nu}	Normalized low-frequency power	
NN	Normal-to-normal R-R intervals	
NN50+	Hourly number of increases between consecu-	
	tive NN intervals larger than 50 ms	
NN50-	Hourly number of decreases between consecu-	
	tive NN intervals larger than 50 ms	
pNN50+	Proportion of beats with increases larger than	
	50 ms	
pNN50-	Proportion of beats with decreases lower than	
	50 ms	

SampEn	Sample entropy
SD ₁	Dispersion of Poincarè plots around the iden-
	tity line
$V_{\rm OBLA}$	Velocity at the onset of blood lactate
	accumulation

Introduction

Physical fitness has been associated with cardiac vagal modulation (Goldsmith et al. 1997). Cardiac vagal tone has been also reported to increase after aerobic training (Aubert et al. 2003), shifting the autonomic control of heart rate (HR) toward parasympathetic predominance. Together with morphological cardiac changes also caused by aerobic training, this results in lower HR at rest and during submaximal exercise (Ekblom et al. 1973; Kasikcioglu 2011; Wilhelm et al. 2011). The decreased cardiac rhythm for a given workload is usually associated with an increased stroke volume in response to training, and this is a positive factor in endurance competitions.

On performances lasting less than 1 min the relative importance of aerobic power is low and the possible benefit of an enhanced vagal tone is less clear. Therefore, it can be hypothesized that the development of a hypervagotonic status may not result in any substantial improvement of the performance. As regards swimming competitions of short (less than 1 min) or very short (less than 30 s) duration, like 100 or 50-m front-crawl events, the athlete may have to overwhelm the vagal predominance on HR and to increase the sympathetic tone quickly to rapidly develop maximal cardiac outputs, to sustain the systemic arterial blood pressure, increase muscle perfusion and efficiently remove the metabolic end products of the anaerobic metabolism (Nobrega et al. 2014). In these cases, it can be even hypothesized that a hypervagotonic status has a detrimental effect on the performance.

An easy and validated way to study cardiac autonomic activity is through the analysis of spontaneous heart rate variability (HRV). So far, however, no information is available on the relationship between vagal or sympathetic modulations of HR and swimming performances on short or very short distances. In fact, the only studies evaluating the influence on swimming performances of autonomic HRV modulations considered relatively long performances (Garet et al. 2004) or events of different swimming styles on non-homogeneous distances (Atlaoui et al. 2007).

The aim of this study was therefore to assess whether a relation exists between autonomic modulations of HR and the performances of shorter duration in elite swimmers. In particular, we tested the hypothesis that in elite athletes, an elevated cardiac vagal activity may have a neutral or even detrimental effect on short- or very short-distance performances. This was done by correlating the best seasonal times over 50 and 100-m front crawl in a homogeneous population of Italian nationallevel swimmers with a number of HRV indices known to be associated with cardiac vagal tone or with cardiac sympatho/vagal balance.

Materials and methods

Subjects

Thirteen Italian national-level male swimmers aged between 22 and 32 years were enrolled. Height, weight and body mass index ranged as 175–192 cm, 68–82 kg, and 20.8–24.8 kg/m². All athletes belonged to the same regional team (DDS, Milan, Italy), had the same training history in the previous 3 years, and were specialized in 50- and 100-m front crawl. They usually trained two times daily, 6 days per week, with swimming distances between 5,500 and 7,500 m per training session. Their best seasonal times ranged between 23.47 and 25.95 s for 50-m front crawl, and between 51.48 and 56.30 s for 100-m front crawl. All athletes were no smokers. No one reported taking any drug or presented any sign of cardiorespiratory pathology.

Experimental protocol

The experimental design was approved by the Ethical Committee of the Faculty of Exercise Sciences, University of Milan. All swimmers signed an informed consent to participate in the study. For each athlete, data were collected in a 2-day experimental session. The session was performed during the same agonistic season (from September to December) where the best seasonal times on 50- and 100-m front crawl were recorded (see "Subjects"). The experimental session was not held close to agonistic events to reasonably exclude the effects of psychological stress preceding the competition. Data were collected during the same training phase for all the subjects. This phase started at around the fifth month of the training season and consisted of a 6-week period devoted to technical work and to specific training for the 100-m race. Training load distribution in this phase was: 40 % of time for exercises at critical speed; 20 % for exercises at lactate threshold (race tests with recovery); 20 % for aerobic endurance exercises (to enhance recovery); 15 % for training over short distances (25 m, to improve maximal speed); 5 % for exercises improving technical skills. Exercises to induce functional overreaching were excluded in this phase. Training of the previous 4 months included: an initial work to build a strong aerobic base during the first month; lactic threshold training, sprint and resistance training during the second and third months; a series of tests for races followed by 15-day tapering during the fourth month.

In one of the 2 days of the experimental session, anthropometric data were measured and training data were collected by an interview. Then the swimming velocity corresponding to the onset of blood lactate accumulation (V_{OBLA}) was assessed by an incremental swimming test (Bonifazi et al. 1993). V_{OBLA} is a physiological parameter widely employed to assess the effects of training, set the swimming velocity for aerobic training and provide information on the fraction of aerobic energy that contributes to the overall energy consumption also during short-distance performances (Zamparo et al. 2011). Briefly, theoretical velocities at 2, 4 and 8-mmol lactate concentrations were estimated based on the differential time between 100- and 200-m performances. A series of 5×100 m at each of the three theoretical velocities was then performed and the lactate concentration (Lactate Pro LT-1710, Arkray, USA) was measured after each series. Finally, V_{OBLA} was estimated by interpolating the linear relationship between swimming velocity and lactate concentration at 4 mmol of lactate.

On a different day, beat-by-beat series of R-R intervals were recorded by an HR monitor (S810, Polar Electro Oy, Finland), validated for HRV analysis (Pu and Patterson 2003). Each recording was performed continuously for 15 min with the subject lying supine, at rest, in a quiet environment. Recordings were repeated three times on the same day: (1) in the morning, a few minutes after waking up (baseline); (2) just before the afternoon training session (before training); and (3) 1–2 min after getting out of water at the end of the afternoon training session, which was concluded by warm-down slow swimming for 600-800 m (after training). Athletes were asked not to speak during the recording and to avoid consumption of caffeine and alcoholic drinks on the recording day. The day before the recording, the athletes performed two training sessions as usual. The time interval between the end of the afternoon training session and the morning recording of the following day was about 14 h. On the day of recording, there was a break of 6 h between the morning and afternoon training sessions.

HRV analysis

The R–R interval series were visually inspected by expert operators who identified and edited premature beats or artefacts to obtain the normal-to-normal (NN) interval series (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). The software Kubios HRV ver. 2.0 (Kuopio, Finland) was used to remove the identified beats and interpolate them with cubic splines, setting the detection threshold at a "low" level (Tarvainen and Niskanen 2012). However, the total number of edited beats was actually very low, and abnormal beats appeared to be due to few movement artefacts only.

Heart rate variability (HRV) was quantified by validated indices of autonomic cardiac modulation applied on the NN interval series. The following indices based on time domain, frequency domain, geometrical and complexity analysis were used.

Time domain analysis

We calculated the hourly number of increases (NN50+) or decreases (NN50-) between consecutive NN intervals larger than 50 ms (Ewing et al. 1984), and the percentage of such differences with respect to the total number of NN intervals (pNN50+ and pNN50-) (Bigger et al. 1988). All these indices have been proposed as measures of cardiac parasympathetic activity. Under the hypothesis that bursts of vagal outflow on the sinus node produce NN changes larger than 50 ms, the NN50 statistics may reflect the rate of "vagal bursts". It should be noted that accordingly the pNN50 statistics depends not only on the hourly number of "vagal bursts", but also on the mean HR, being pNN50 = NN50/(HR × 60).

Geometrical analysis

A Poincarè plot graphically describes HRV as a cloud of points. The Cartesian coordinates of each point are the NN interval of each given beat and of its previous beat. The dispersion of points around the identity line, SD₁, is a validated index of cardiac vagal activity (Penttila et al. 2001). It should be noted that SD₁ is related to another popular measure of vagal activity, the root mean square of consecutive NN differences, RMSSD, through the relation: RMSSD² = $2 \times SD_1^2$ (Brennan et al. 2001, 2002).

Frequency domain analysis

The spectral power in a high-frequency (HF) band, between 0.15 and 0.40 Hz, reflects respiratory oscillations mediated by the cardiac vagal drive, while the power in a lowfrequency (LF) band, between 0.04 and 0.15 Hz, is generated by both the sympathetic and parasympathetic outflows to the sinus node (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). The LF power normalized by the sum of LF and HF powers, LF_{nu}, is a validated index of cardiac sympatho/vagal balance. The HF power and LF_{nu} were assessed by calculating the Welch periodogram of NN intervals resampled at 4 Hz, over 256 s-long, 50 %-overlapped, Hann data windows. Other spectral indices of sympatho/vagal balance, such as LF/HF powers ratio or normalized HF power (HF_{nu}) can be derived from LF_{nu} by simple mathematical manipulations: LF/HF = LF_{nu}/ $(1 - LF_{nu})$ and HF_{nu} = $1 - LF_{nu}$.

Complexity analysis

The HR sample entropy (SampEn) measures the level of irregularity of the NN interval series. Vagal activations or sympathetic deactivations may increase SampEn (Porta et al. 2007). SampEn is the negative logarithm of the probability that segments of m consecutive beats which are similar remain similar when the length of the segments increases by one beat (Richman and Moorman 2000). Two segments are defined to be similar if their distance in an m-dimension space is lower than a given threshold r. As suggested previously (Richman and Moorman 2000), SampEn was estimated by setting m = 2 and r = 20 % of the standard deviation of NN intervals.

The autonomic tone may also influence the short-term self-similarity coefficient (α_1) of NN intervals as assessed by detrended fluctuation analysis (Peng et al. 1995). It has been shown that α_1 increases when the sympatho/vagal balance increases or the vagal tone decreases (Tulppo et al. 2001; Castiglioni et al. 2009, 2011a).

The software Kubios HRV ver. 2.0 (Kuopio, Finland) was also used to calculate all HRV indices, with the exception of NN50 and its derived indices, which were directly calculated from the data.

Statistics

Comparison of the mean HR among the three conditions (baseline, before training and after training) was performed by repeated measure ANOVA at the p < 0.05 statistical threshold with Newman–Keuls post hoc analysis. The same statistical test was applied to compare HRV indices when the hypothesis of Gaussian distribution could be assumed. Based on previous evidence, Gaussianity was assumed for α_1 (Castiglioni et al. 2011b) and, after log-transformation, for SD₁ and the HF and LF_{nu} spectral indices (Castiglioni et al. 1999). SampEn, NN50+, NN50-, pNN50+ and pNN50- were tested by the nonparametric Friedman test. In this case, when the difference among conditions was significant at p < 0.05, post hoc analysis was performed by the nonparametric Wilcoxon's matched pairs test with Holm's correction for multiple comparisons.

The relationships between times of best performance and HRV indices were assessed by Spearman's rank correlation. Since in the normal population (1) body mass index and age influence the sympatho/vagal balance, (2) height is an advantage in swimming and (3) the training volume (i.e., number of swimming hours per week) improves swimming performances, the Spearman's rank correlation was also calculated between these variables and best performance times to evaluate whether they represent confounding factors.

Independent predictors were identified by multiple regression analysis with forward stepwise procedure, considering as possible predictors only indices with significant (p < 0.05) Spearman's rank correlation. The number of possible predictors was preliminary reduced by calculating the correlation matrix among predictors and removing highly correlated (|r| > 0.90) indices. Analyses were performed by Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA).

Results

The mean HR (upper left panel of Fig. 1) significantly increased by 20 %, from the morning recording (baseline) to the first afternoon recording (before training). It significantly increased by a further 15 % after training.

HRV indices

Differences between after training (condition expected to have the lowest vagal tone) and baseline or between after training and before training were always significant for vagal HRV indices, with the lowest values measured after training (Fig. 1). Differences between baseline and before training were significant only for pNN50+ and pNN50-, with higher values in baseline. The indices of sympatho/ vagal balance (LF_{nu} and α_1) were significantly higher after training compared to baseline or before training (Fig. 2).

Correlation with best seasonal times The mean HR did not correlate with 50- or 100-m best seasonal time, neither at baseline nor Before or after training. Moreover, no correlations were found (p always greater than 0.10) between best seasonal times over 50 or 100 m and age, height, weight, body mass index and training volume.

By contrast, all the HRV indices of vagal modulation at baseline were significantly and positively correlated with the 50-m best seasonal time (Fig. 3, left). This means that the higher the vagal index, the higher is the time to swim 50 m and therefore the worse the performance. As to the indices of sympatho/vagal balance, LF_{nu} was negatively correlated to the 50-m best seasonal time (i.e., the higher the value of LF_{nu} , the lower is the time to swim 50 m and therefore the better the performance), and α_1 showed the same tendency (p = 0.06). All the correlations were lost when HRV indices were measured in the afternoon, both before and after training.

Significant correlations between HRV indices and 100-m best seasonal times were found after training only, and only for indices of sympatho/vagal balance (Fig. 3,

Fig. 1 HR and vagal indices of HRV (mean \pm SD) in the three experimental conditions. *Single asterisks* and *Double asterisks* indicate significant differences at p < 0.05 and p < 0.01 (see "Materials and methods" for abbreviations)



right). The negative correlation coefficients indicated that the better performances on 100 m were associated with the higher LF_{nu} and α_1 values.

Multiple regression analysis identified in NN50+ the only independent predictor of 50-m best seasonal time among all the HRV indices that were significantly correlated at baseline. This means that the prediction of swimming performances on 50 m cannot be improved by considering other HRV vagal indices in addition to NN50+. Multiple regression analysis also indicated that LF_{nu} was the only independent predictor of 100-m best seasonal time considering LF_{nu} and α_1 after training as predictors.

Fig. 2 HRV indices of cardiac sympatho/vagal balance (mean \pm SD) in the three experimental conditions (see "Materials and methods" for abbreviations). *Double asterisks* indicate significant differences at p < 0.01



Figure 4 illustrates clearly that the best seasonal times over 50 and 100 m were, respectively, linked to NN50+ at baseline and to LF_{nu} after training, but not to V_{OBLA} .

Discussion

To our knowledge, this is the first work using HRV methods to study a homogeneous group of short-distance swimmers. The main findings are the following: (1) vagal modulations of HR, measured at rest in the morning, positively correlate with 50-m performance times, indicating that the lower the vagal outflow, the better the 50-m performance; (2) cardiac sympatho/vagal balance, measured after training in the afternoon, negatively correlates with 100-m times, indicating that the higher the cardiac sympatho/vagal balance in response to the exercise session, the better is the 100-m performance. These results allow us to better understand the role of the autonomic cardiovascular control on performances over short and very short distances in elite swimmers.

Endurance training is believed to shift the cardiac autonomic control toward a parasympathetic predominance. While this may be beneficial to perform long-lasting aerobic exercises, according to our data a high vagal tone may be useless or even detrimental (Fig. 3) for mostly anaerobic exercises. In particular, our data suggest that the ability to quickly depress the cardiac vagal outflow to increase HR and cardiac output is critical for very short performances, such as 50-m front crawl.

However, vagal indices did not correlate with 100-m performances (Fig. 3). This also suggests that the increased duration of the performance allows the positive effect of a high vagal tone on the aerobic component of the exercise to compensate for the detrimental effect on the anaerobic component. Extrapolating to more aerobic events, one might expect vagal indices to positively correlate with performances of longer duration. This was actually found comparing HF power with performances over 400-m front stroke in seven regional-level swimmers (Garet et al. 2004), and with performances over 2,000-m distances in eight national-level swimmers (Schmitt et al. 2006).

Furthermore, 100-m performance times negatively correlated with indices of sympatho/vagal balance after training (Fig. 3). Since in this case vagal indices were not correlated with the performance, results indicate that the athletes with best 100-m performances are those with the higher cardiac sympathetic tone after training, likely because they activated the sympathetic system more in response to exercise during the preceding training session. In this regard, a residual long-lasting sympathetic activation has been reported following the training session (Dixon et al. 1992; Arai et al. 1989) and an increased sympathetic tone coexisting with vagal bradycardia in elite athletes at training peaks (Iellamo et al. 2002; Pichot et al. 2000; Furlan et al. 1993), suggesting that intense training loads may increase substantially the sympathetic activity. This is in line with the alterations in catecholamine concentrations after marked increases in training load reported in different studies (Bosquet et al. 2008).

Therefore considering our findings on both short and very short-term distances, it is conceivable that the capacity to produce high sympathetic outflows results in a greater cardiovascular activation that allows better performances in short-term competitions like 100-m events, while on very short-term competitions like 50-m events, the sympathetic control does not have enough time to produce significant effects.

Additional aspects deserve attention. First, the autonomic tone was assessed by a series of indices quantifying



Fig. 3 Spearman's rank correlation coefficients between best seasonal times on very short (*left*) and short (*right*) performances vs. resting vagal and sympatho/vagal indices of HRV in the three experimental conditions (see "Materials and methods" for abbreviations).

Dashed bars indicate rank coefficients significantly (p < 0.05) different from 0; # points out a difference at p = 0.06. Error bars are the standard errors

different aspects of HR dynamics. Although derived from different algorithms, they were similarly related to the performances, emphasizing their overall consistency as estimators. However, their sensibility to changes among experimental sessions differed. In particular, among vagal HRV indices, only pNN50+ and pNN50- differed significantly between "baseline" and "before training" (Fig. 1). It is likely that the pNN50 indices detected a significant difference because they also reflect changes in the mean HR (see "Materials and methods"). This would explain why, **Fig. 4** Best performances vs. V_{OBLA} or vs. HRV indices. *Upper panels* relationships between 50-m best seasonal times and V_{OBLA} (*left*) or NN50+ at baseline (*right*); *lower panels* relationships between 100-m best seasonal times and V_{OBLA} (*left*) or LF_{nu} after training (*right*). Spearman's *R* and its significance *p* are reported; for reference, a linear regression line is plotted when *p* < 0.05



on the other side, the NN50 statistics was a stronger predictor of 50-m performance, being a purer index of cardiac vagal modulation. Moreover, NN50+ predicted 50-m performances better than NN50-. While NN50+ quantifies the rate of increases in successive R-R intervals, i.e., HR decelerations, NN50- quantifies the rate of HR accelerations. HR decelerations (following vagal activation and sympathetic withdrawal) and HR accelerations (following sympathetic activation and vagal withdrawal) are likely characterized by the different time constants of vagal and sympathetic activations and therefore they may differently reflect the vagal modulations of HR. Actually, differences in the distributions of HR decelerations and accelerations have been associated with the presence of sympathetic modulations (Porta et al. 2008) and the HR decelerations have been identified as a better marker of vagal activity than HR accelerations in a clinical setting (Bauer et al. 2006).

Second, HRV indices correlated to seasonal 50- and 100-m performances much better than measures of blood lactate concentration (Fig. 4). A weak correlation between best seasonal performances and V_{OBLA} could be expected because the fraction of aerobic energy spent in short-distance races is relatively small, estimated at around 33 % of the overall energy expenditure on 100-m events, and at around 15 % on 50-m events (Zamparo et al. 2011). In any case, it should be considered that HRV indices are very easy to assess compared to the complex procedure

required for V_{OBLA} determination. This may be an advantage in terms of reproducibility of results and reliability of estimates.

Third, the time of the day when HRV indices are measured is critical. Although HR was always recorded in supine resting position in a quiet environment, only morning measures predicted short-term performances. Unlike the afternoon recordings, the morning recording was preceded by hours of night sleep with a relatively high vagal tone (Di Rienzo et al. 1989) not disturbed by autonomic activations due to daily activities and postural changes. This would explain why morning and afternoon HR values markedly differed, and why HRV indices provide different information when measured in the morning than in the afternoon. Previous observations (1) that the time period immediately after wake up reflects the effects of aerobic training on HR better than other moments during the 24 h (Shiotani et al. 2009), and (2) that vagal HRV indices are better indicators of aerobic fitness when measured in the morning than in other time periods (McNarry and Lewis 2012) support our finding.

HRV indices measured in the afternoon before training did not correlate with 50- or 100-m times, suggesting that in this period of the day neither the vagal nor the sympathetic cardiac controls were sufficiently elicited to produce a significant correlation with the best swimming performance, on short or on very short distances. The lack of correlation could be due to a residual effect of fatigue resulting from the morning training session, which may have slightly increased the sympatho/vagal balance masking the relation with the vagal control in some of the athletes. However, even if a residual fatigue may be present, its effects should be very low, because neither NN50+ nor LF_{nu} differed significantly between the "baseline" session in the morning and the "before training" session in the afternoon (Figs. 1, 2). Blurring effects in the afternoon could be also ascribed to other psychological, environmental or physiological influences that activated the autonomic tone, such as competitive stress, effects of meal consumption at noon or of activities like driving a car. All these factors may have influenced the autonomic profile of each athlete differently. In this regard, Fig. 5 shows values of LF_{nu} , the index better predicting the 100-m best performances, before and after training in each athlete separately. Only values after training correlate with the performance, because values before training are more dispersed. Therefore we may conclude that a proper autonomic activation produced by the training session is needed to remove possible masking influences and to elicit a sympathetic response to exercise that can predict the short-term performance.

We acknowledge some limitations of our study. First, we cannot exclude that the total number of years that each athlete spent training may have influenced his autonomic



Fig. 5 Relation between best seasonal time on 100-m distance and LF_{nu} before (*open circle*) and after (*solid circle*) training separately in each athlete. The *dotted line* shows the linear regression between best seasonal time and LF_{nu} after training, which was significant at p < 0.05

cardiovascular profile. However, it is likely that older athletes spent more years training and we did not find a relation between the athlete's age and his performances. Second, the athletes followed their usual daily training program also the day before the recording. In this way, the HRV indices can be considered to be representative of the actual athlete's condition at the fifth month of the training season. The resting period spent between the afternoon training session and the morning recording of the successive day (14 h) appears to be long enough to assure recovery from training, also considering that in this training phase functional overreaching was not intentionally induced and actually no athlete showed any sign of overreaching. However, a residual carryover effect of fatigue cannot be completely excluded.

In perspective, our study provides methodological indications on training management and monitoring. Training approaches in competitive swimmers are under debate (Faude et al. 2008) and although a mix of low- and highintensity exercise has been suggested in short and very short distances (Laursen 2010), elite swimmers often undergo high volume aerobic training. In this regard, our results support the hypothesis of a beneficial effect of high-intensity training loads in preparation to short-term competitions, because such loads likely allow producing greater sympathetic activations during the performance. Furthermore, our work indicates that vagal modulations of HRV could provide guidance for choosing, at a given time, the most appropriate distance between short-term and very short-term competitions, and that some indices (like HR decelerations) and recording periods (i.e., after wake up in the morning) better detect the association between HRV and performances. These findings represent useful indications for successfully monitoring the effects of exercise loads on training or rehabilitation programs by HRV analysis.

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