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Research article

Acute Modification of Cardiac Autonomic Function of High-Intensity Interval Training in Collegiate Male Soccer Players with Different Chronotype: A Cross-Over Study

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Abstract
The purpose of this study was to evaluate if the time of the day (8.00 a.m. vs 8.00 p.m.) and chronotype could influence autonomic cardiac control in soccer players in relation to an acute session of high-intensity interval training. The morningness-eveningness questionnaire was administered to recruit Morning-type and Evening-type collegiate male soccer players. Therefore, 24 players (12 Morning-types and 12 Evening-types) were randomly assigned, to either morning (n = 12; age 23 ± 3 years; height 1.75 ± 0.07 m; body mass 73 ± 10 kg; weekly training volume 8±2 hours), or evening (n = 12; age 21 ± 3 years; height 1.76 ± 0.05 m; body mass 75 ± 11 kg; weekly training volume 8 ± 3 hours) training. Heart Rate Variability vagal and sympatho/vagal indices were calculated in time, frequency and complexity domains at rest, before, after 12 and 24 hours of high-intensity interval training. Before evening training session, a higher resting heart rate was observed which was determined by a marked parasympathetic withdrawal with a sympathetic predominance. Moreover, Evening-type subjects during morning training session, present a significant higher heart rate that corresponded to significant higher vagal indices with a significant lower parasympathetic tone that returned to the rest values after 24 hours of the cessation of high-intensity interval training exercise. On the contrary, Morning-type subjects did not reveal any significant differences with Evening-Type subjects during evening high-intensity interval training session. Stress response of high-intensity interval training is influenced by both the time of the day and by the chronotype. Understanding the Heart Rate Variability response to high-intensity interval training can be an additional important procedure for evaluating of cardiovascular recovery in soccer players. Moreover, these results suggest that an athlete's chronotype should be taken into account when scheduling a high-intensity interval training exercise.

Key words: Autonomic nervous system, heart rate variability, circadian rhythms, interval training.

Introduction

Soccer is one of the most played sports in the world, where players need technical, tactical, and physical skills to succeed. Cardiorespiratory fitness and therefore maximal oxygen uptake (VO_{2max}), is one of the most important parameters affecting soccer performance with a clear relationship with the distance covered, work intensity, number of sprints and involvements with the ball during a match (Helgerud et al., 2001). Helgerud et al. (2007) clearly demonstrated that a high-intensity interval training (HIIT) is significantly more effective than performing the same total work at either lactate threshold or at 70% of maximal heart rate (HR_{max}), in improving VO_{2max}. However, this type of protocol is physically demanding and it does not allow for complete recovery after training. In fact, according to Seiler et al., (2007) elite soccer athletes during their training session perform very high training loads, submitting the body to a very high stress which could induce adaptive effects which are cumulative and incomplete recovery from them can lead to non-functional overreaching or overtraining. For this reason, the day-to-day distribution of training intensity may be a crucial variable to effectively balance positively and negative stress effects so that performance development is achieved without stagnation or overtraining (Uusitalo, 2001).

One of the most non-invasive reliable methods to assess cardiovascular recovery after training is to evaluate the time course of the heart rate variability (HRV), which is the natural fluctuation of HR in time which reflects the neural control of the heart via sympathetic and parasympathetic innervation on the sinus node (Aubert et al., 2003). The quantification of HRV is one of the key aspects of this stress response, consisting in activation of the sympathetic arm of the autonomic nervous system (ANS) and a shift in autonomic balance. Generally, sympathetic predominance has been observed 1 hour after the cessation of exercise and pre-exercise levels appeared to be regained only 24 hours after the exercise bout (Bernardi et al. 1997; Furlan et al. 1993). In addition, a rebound of the parasympathetic activity was also found two days after prolonged exercise (Hautala et al. 2001). On the other hand, the exercise performed in the aforementioned studies for their length and environmental conditions differ from those that athletes would routinely perform in a training program. Indeed, changes in HRV were reported after 30 min of maximal exercise leading to exhaustion (Furlan et al. 1993), 46 km of running at a mean altitude of 2,500 m (Bernardi et al. 1997), and 75 km of cross-country skiing (Hautala et al. 2001). Regarding HIIT Al Haddad et al. (2009) found that a single supramaximal exercise bout, lasting no more than 12 min, can perturb the ANS for about 36 hours, confirming that there is also a strong influence of exercise intensity on short- and long-term post exercise HRV recovery. For what concern team

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sport activities, Flatt et al. (2017) observed in a group of female collegiate soccer players that an intense training was associated with a reduced parasympathetic activity which was associated with a higher training load due to an increased training stress. Moreover, Esco et al. (2016) found an association between the change in weekly parasympathetic activity and the adaptation of aerobic power following an off-season program suggestion that this could be an efficient method to evaluate the aerobic adaptation in female soccer players.

Another important variable which may determine different autonomic responses during recovery from a demanding training bout, are circadian rhythms. In fact, individual differences in these chronobiological rhythms can be summarized under the concept of Morningness/Eveningness, also termed chronotype. These rhythms influence our daily behavior. People typically, based on one's innate circadian rhythm, display preferences for activity at different time of day (Montaruli et al., 2017; Roveda et al., 2017). These individual differences in the phase of biological and behavioral patterns are genetically based and controlled by an endogenous circadian pacemaker, although other factors such as social and cultural influences might contribute to determining differences in behavioral rhythms. This circadian phenotype differs among individuals and may be classified as: Morning-type (M-type), Evening-type (E-types) and Neither-type (N-type) (Natale and Cicogna, 2002). Research often focused on the differences between M- and E-type individuals. M-types wake up early and perform mentally and physically at their best in the morning hours, compared to E-types who plan their daily activities for the afternoon or evening and prefer to stay out late (Bonato et al., 2017). Recent studies clearly showed large differences among chronotypes for several physiological variables: sleep behaviour (Vitale et al., 2015; 2017), hormones secretions (Bailey and Heitkemper, 2001), physical performance (Rossi et al., 2015; Vitale et al., 2013), and HRV (Roeser et al., 2012).

Regarding HRV, previous findings support the assumption that E-types presents a significantly higher HR and systolic blood pressure but lower HRV than M-types both at baseline and during mental stress conditions (Roeser et al., 2012), demonstrating that stress induced in the evening had a significantly higher impact on cardiovascular responses than stress induced in the morning independent of chronotype. On the other hand, Roeser et al., (2012) induced stress using an arithmetical task. Proven that supramaximal intermittent exercise perturbed the ANS for more than 24 hours (Al Haddad et al., 2009), we hypothesized that there could be differences in post exercise autonomic control according to the time of the day at which this training is performed and chronotype. Therefore, the purpose of this study was to evaluate the influence of the time of the day (8.00 a.m. vs 8.00 p.m.) and chronotype on pre- and post-exercise autonomic cardiac control in soccer players in relation to an acute session of HIIT.

Methods

Participants were recruited among Bachelor's students of the School of Sport Science of Università degli Studi di Milano, Milan, Italy during the academic year 2015-2016. Inclusion criteria for subject's participation to the study were: age ≥ 18 years; male; being soccer players; 6 hours of training a week and with a morning or evening chronotype scores (see "assessment of circadian typology"). Exclusion criteria were smoking, use of medications and any other medical condition contraindicating physical exercise. Twenty-four collegiate male soccer players (12 M-Types and 12 E-Types) were therefore deemed eligible and enrolled in the study. Before entering the study, the participants were fully informed about the study aims and procedures, and written informed consent was obtained before testing. The study protocol was approved by the Institutional Ethics Review Committee of the Università degli Studi di Milano (approved on 12/10/15, prot. N. 52/15) in accordance with current national and international laws and regulations governing the use of human subjects (Declaration of Helsinki II). This trial was registered at Australian New Zealand Clinical Trials Registry (ACTRN12617000432314). After a baseline assessment consisted of an anthropometric evaluation, subjects underwent a Yo-Yo Intermittent Recovery Test Level 1 and then they were randomly assigned in a 1:1 ratio according to their chronotype to either morning training (Group A: n = 12; age 23 ± 3 years; height 1.75 ± 0.07 m; body mass 73 ± 10 kg, weekly training volume 8 ± 2 hours) that started performing the high intensity interval training protocol at 8.00 am or evening training (Group B: n = 12; age 21 ± 3 years; height 1.76 ± 0.05 m; body mass 75 ± 11 kg, weekly training volume 8 ± 3 hours) that started performing the high intensity interval training protocol at 8.00 pm. Both groups were blinded about the aim of the study.

Study design

This was a randomized crossover study which was carried out in spring, between March and April 2016, over a period of four weeks and the experimental design consisted of the following: Group A performed the morning training session at 08.00 am while Group B performed the evening training session at 08.00 pm; after a recovery period of 7 days in which subjects maintained their habitual lifestyle without performing physical training, Group A performed in the evening while Group B performed the training session in the morning. One participant in the Group B dropped out because he did not perform the second training session (Figure 1). In each test session, the measurement of HRV (see "heart rate variability assessment") was performed at REST, before (T0), and 12 (T12) and 24 (T24) hours after the training session. All subjects were familiarized with all testing procedures.

Procedures

Assessment of subject's circadian typology

Participants' circadian typology was assessed by the use of the Horne-Ostberg MEQ by placing the scores on the Morningness-Eveningness scale (Horne and Ostberg, 1976). According to the MEQ-score participants were categorized as Morning-type, scoring 59 and above;

Evening-type, scoring 41 and below and Neither-type, scoring 42 to 58. Individual chronotype scores and categories were communicated to the participants only after the completion of the experimentation.

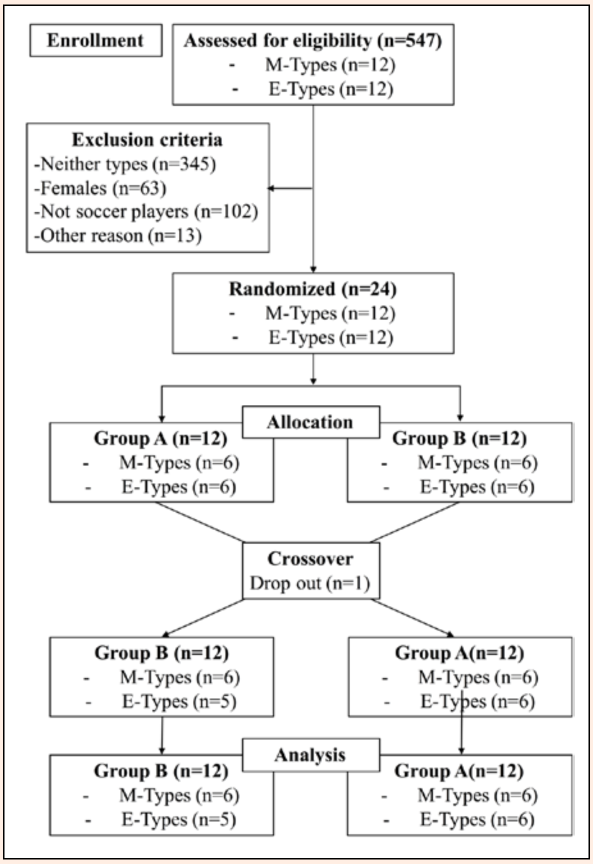

Figure 1. Protocol overview.

Anthropometric assessment

Anthropometric variables included body mass and stature. Stature was measured with a stadiometer and body was with a portable scale to the nearest 0.5 cm and 0.1 kg, respectively (Seca 217, Vogel & Halke, Hamburg Germany). Body mass index (BMI) was then calculated using the standard formula.

Yo-Yo intermittent recovery test Level 1

The Yo-Yo intermittent recovery test Level 1 (Yo-Yo IR1) consisted of repeated 2 X 20-m runs back and forth between the starting, turning, and finishing line at a progressively increased speed controlled by audio bleeps from a tape recorder (Bangsbo, 1994). The test protocol started with 4 running bouts at 10-13 km · h^{-1} (0-160 m) and another 7 runs at 13.5-14.0 km · h^{-1}, then it continues with stepwise 0.5 km · h^{-1} speed increments after every 8 running bouts (i.e., after 760, 1080, 1400, 1720 m etc.) until exhaustion. Between each running bout, the subjects had a 10 second active rest period, consisted of 2 X 5 m of jogging. When the subjects failed to reach the finishing line in time on two occasions, the distance covered was recorded and represented the final result. Test was performed outdoor on athletic track, marked by cones having a width of 1.22 m and a length of 20 m. Another cone placed 5 m behind the finished line marked the running distance during the active recovery period. All tests were conducted at 12 ± 2 h which is considered an intermediate time of day, and in dry, windless weather conditions with a temperature about 10°. Before the test, all subjects carried out a warm-up period consisting of the first four running bouts in the test. The total duration of the test was 6-20 minutes. All subjects were familiarized to the test by at least one pre-test. Heart rate was recorded beat-to-beat using a Polar RS800 heart rate monitor (Polar, Kempele, Finland) in order to measure directly the HR_{peak} reached during the test.

High-intensity endurance interval training protocol

The high-intensity endurance interval training protocol consisted of 4 bouts of 4 minutes (4X4) at 90-95% HR_{peak} with 3 min of active recovery at 50-60% HR_{peak} (Helgerud et al., 2001). The calculation of the training percentages was carried out using the HR_{peak} achieved during the Yo-Yo IR1. The training intervention started with a standardized 10-min warm-up and ended with a 3-min cool-down period at a self-selected intensity. Before, during, and after the test HR was recorded beat-to-beat using a Polar RS800 heart rate monitor (Polar, Kempele, Finland). All training sessions were conducted outdoor on an athletic track in dry, windless weather conditions with a temperature about 15-20°C. All subjects completed training sessions without complications. The high-intensity endurance interval training protocol was generally well tolerated and subjects did not report dizziness, light-headache or nausea, symptoms that occasionally occur during this type of training.

Heart rate variability assessment

Heart variability measurements were performed at REST, before (T0), after 12 (T12) and after 24 (T24) hours of the high-intensity endurance interval training protocol. Subjects assumed a supine position in a quiet light dimmed room. After HR had stabilized, R-R intervals were recorded continuously for 10 minutes with a beat-to-beat HR monitor (Polar, RS800CX; Polar Electro, Kempele, Finland) validated for heart rate variability (HRV) analysis (Pu and Patterson, 2003). The R-R interval series were subsequently visually inspected by expert operators who identified and edited premature beats of 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). Data were then processed by using Kubios HRV software (version 2.0; Department of physics, University of Kuopio, Finland). Artefact correction was carried out using Kubios' artefact correction option; very low correction level was used as default and visual inspection showed noticeable changes in very few cases. Low quality beat-to-beat data were discarded from the analysis. HRV was quantified by validated indices of autonomic cardiac modulation applied to the NN interval series. The following indices based on time domain, frequency domain and complexity analyses were used (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996)**:**

Time-domain analysis: The time-domain methods were applied directly to the series on successive RR interval values (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). We evaluated the square root of the mean of the sum of the squares of differences between adjacent normal R–R intervals (RMSSD; units ms), as index of cardiac parasympathetic activity.

Frequency-domain analysis: The frequency-domain methods, were derived from the NN series spectral power in a high frequency (HF) band, between 0.15 and 0.40 Hz, reflecting respiratory oscillations mediated by the cardiac vagal drive, and in a low frequency (LF) band, between 0.04 and 0.15 Hz, which 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).

Non-linear domain: Complexity analysis was performed assuming that the autonomic tone may also influence the short-term self-similarity coefficient (α1) of NN intervals as assessed by detrended fluctuation analysis (DFA) (Peng et al., 1995). It has been shown that α1 increases when the sympatho/vagal balance increases or the vagal tone decreases (Tulppo et al., 2001).

Statistical analysis

Descriptive statistics (mean ± SD) for the outcome measures were calculated. The normality of the distribution of the anthropometric (weight, height, and BMI), background (age, training hours per week, and years of practice), and Yo-Yo IR1 (total distance and HR_{peak}) variables were checked using the D'Agostino Pearson test. Since all variables were normally distributed differences between Group A and Group B were checked using unpaired t test. Parametric statistical tests were applied to compare HRV indices when the hypothesis of Gaussian distribution could be assumed. Gaussianity was assumed for α_1DFA (Castiglioni et al., 2011) and after a log-transformation, for RMSSD, HF power (ms^2) and LF power (ms^2). Intra- and inter- group differences in HRV between morning and evening HIIT for the 23 subjects were evaluated using mixed ANOVA with Tuckey's multiple comparisons test. Moreover, to test intra- and inter- group differences in HRV between the 12 M-Type and 11 E-Types during morning and evening training session another mixed ANOVA with Tuckey's multiple comparisons test was performed. The level of statistical significance was set at $P < 0.05$. Statistical analysis was performed using GraphPad Prism version 6.00 for Mac OSX (GraphPad Software, San Diego, CA, USA). Standardized changes in the mean values were used to assess magnitude of effects (Effect Size, ES). Values <0.2, <0.6, <1.2 and >2.0 were interpreted as trivial, small, moderate, large and very large, respectively (Betterham and Hopkins, 2006)

Results

The pre-training parameters of Group A and Group B, are presented in Table 1 respectively. Unpaired t-test showed no significant differences in age, height, body mass, BMI, MEQ-Score, Yo-Yo IR1 distance, Yo-Yo IR1 HR_{peak}, and weekly training volume between the two groups.

Morning vs evening HIIT

Table 2 presents the results of mixed ANOVA with Tuckey's multiple comparisons, with associated p-values of the HRV during REST, T0, T12 and T24, of the 23 subjects that performed the morning (8.00 a.m.) and the evening (8.00 p.m.) HIIT. Significantly interaction for HR, LnRMSSD, LnLF power (ms^2), LnHF power (ms^2), and α_1 DFA was noted, with significantly differences regarding the effect of time and chronotype at the different time points. Post-hoc inter-group differences were noted at T0 for what concerns HR (p = 0.017; ES = 1.0), LnRMSSD (p = 0.009; ES = 0.8), LnLF power (ms^2) (p = 0.006; ES = 1.8), LnHF power (ms^2) (p = 0.001; ES = 1.0), LF (n.u.) (p = 0.0006; ES = 0.6), α_1 DFA p = 0.029; ES = 1.0).

M-Types vs E-Types

Table 3 shows the mixed ANOVA results with the associated p values of the HRV at REST, T0, T12 and T24 in relation to morning and evening session of HIIT related to M-types and E-Types. According to chronotype no significantly interaction was observed between M-type and E-types. On the other hand, significantly differences regarding the effect of time and chronotype were detected. Figure 2 and Figure 3 shows the results of HR, LnRMSSD, and LF power (n.u.), of the M-Types and E-Types performing the morning and the evening HIIT session respectively. Significant post-hoc intergroup differences for HR (REST: p = 0.024, ES > 2.0; T0: p < 0.0001, ES > 2.0; T12: p = 0.014; ES = 1.9; T24: p = 0.040; ES = 1.6), LnRMSSD (REST: P=0.022, ES=1.6; T0: p = 0.041, ES = 1.7; T12: p = 0.049; ES = 1.2; T24: p = 0.042; ES = 1.3) and LF power (n.u.) (REST: p < 0.0001, ES = 1.8; T0: p < 0.0001, ES > 2.0; T12: p < 0.0001; ES > 2.0; T24: p = 0.037; ES = 1.5) were noted in Figure 2. On the contrary, no significant post-hoc intergroup differences in Figure 3 were detected.

Table 1. Subjects characteristics. Data are means (±SD).

Parameter	Group A	Group B	ES
Age (years)	23 (3)	21 (3)	.6
Height (m)	1.75 (.07)	1.76 (.05)	<.2
Body Mass (kg)	73 (10)	75 (11)	.2
BMI (kg · m^{-2})	23 (2)	23 (3)	<.2
MEQ-score (points)	45 (16)	43 (16)	.2
Yo-Yo IR1 distance (m)	2045 (312)	1987 (302)	.2
Yo-Yo IR1 HR_{peak} (bpm)	192 (7)	190 (6)	.3
Weekly training volume (hours · week^{-1})	8 (2)	8 (3)	<.2

MEQ: morningness eveningness questionnaire; **Yo-Yo IR1:** Yo-Yo intermittent recovery test Level 1; **ES:** Effect Size.

Table 2. Results of mixed ANOVA with Tuckey's multiple comparisons, with associated p-values of the HRV during REST, T0, T12 and T24, of the 23 subjects that performed the morning (8.00 a.m.) and the evening (8.00 p.m.) HIIT. Data are means (±SD).

	HR ($b \cdot min^{-1}$)	LnRMSSD (ms)	LnLF power (ms^2)	LnHF power (ms^2)	LF power (n.u.)	HF power (n.u.)	α_1 DFA
			Morning (n = 23)				
Rest	55 (8)	4.2 (.4)	7.4 (.8)	7.2 (.7)	70.7 (14.5)	29.3 (14.5)	1.0 (.2)
T0	60 (7)	4.0 (.4)	7.9 (.5)	7.7 (.9)	70.5 (16.4)	42.6 (20.0)	1.2 (.2)
T12	60 (5)	4.1 (.3)	7.4 (.6)	7.4 (.6)	58.9 (18.4)	41.1 (18.4)	1.0 (.2)
T24	54 (7)	4.2 (.3)	7.9 (.7)	7.2 (.7)	67.6 (12.7)	32.4 (12.8)	1.2 (.2)
			Evening (n = 23)				
Rest	57 (7)	4.2 (.3)	7.5 (.9)	7.1 (.6)	70.7 (14.5)	31.1 (13.1)	1.1 (.2)
T0	67 (8)	3.7 (.3)	8.8 (.7)	6.8 (.6)	79.3 (7.2)	42.6 (20.0)	1.4 (.3)
T12	57 (7)	4.2 (.4)	7.8 (.7)	7.2 (.8)	65.2 (15.9)	39.7 (15.8)	1.1 (.2)
T24	57 (6)	4.2 (.4)	7.6 (.7)	7.0 (.2)	63.9 (13.4)	32.4 (12.7)	1.1 (.2)
Interaction	p = .010	p = .012	p = .001	p = .002	p =.017	n.s.	p = .014
Effect of time	p < .0001	p < .0001	p < .0001	p = .040	p = .002	n.s.	p = .022
Morning vs Evening	p = .029	p = .048	p = .004	p = .049	p = .021	n.s.	p < .0001

HR: heart rate; RMSSD: square root of the mean of the sum of the squares of differences between adjacent normal R–R intervals; LF: low frequencies; HF: high frequencies; DFA: detrended fluctuation analysis; n.s.: not significant

Table 3. Results of mixed ANOVA with the associated p values of the HRV at REST, T0, T12, T24 in relation to morning and evening session of HIIT related to M-types and E-Types.

	Effect of time	M-Type vs E-Type	Interaction
	Morning Training		
HR rest (bpm)	p < .0001	p < .0001	n.s.
Ln RMSSD (ms)	p < .0001	p = .0005	n.s.
Ln LF power (ms^2)	p < .0001	p < .0001	n.s.
Ln HF power (ms^2)	p = .0140	p = .004	n.s.
LF (n.u.)	p < .0001	p < .0001	n.s.
HF (n.u.)	p < .0001	p < .0001	n.s.
α1 DFA	p < .0001	p < .0001	n.s.
	Evening Training		
HR rest (bpm)	p < .0001	n.s.	n.s.
Ln RMSSD (ms)	p = .003	n.s.	n.s.
Ln LF power (ms^2)	p < .0001	n.s.	n.s.
Ln HF power(ms^2)	p = .038	n.s.	n.s.
LF (n.u.)	p = .011	n.s.	n.s.
HF (n.u.)	p = .007	n.s.	n.s.
α1 DFA	p = .0002	n.s.	n.s.

HR: heart rate; RMSSD: square root of the mean of the sum of the squares of differences between adjacent normal R–R intervals; LF: low frequencies; HF: high frequencies; DFA: detrended fluctuation analysis; n.s.: not significant

Discussion

To our knowledge, this was the first study using HRV methods to study the possible ANS adaptations related to HIIT performed at different time of the day (8.00 a.m. vs 8.00 p.m.). In addition, the novelty of this research was to study the effect of chronotype (M-types vs E-types) on the ANS responses to HIIT. The main findings of this study were the following: 1) before (T0) HIIT soccer players that trained at 8.00 p.m. had a significant increase of parasympathetic tone with a sympathetic predominance before exercise; 2) according to subject's circadian typology, E-Types during morning training presented a significant parasympathetic withdrawal with a sympathetic predominance at REST and before (T0) HIIT compared to M-Types; this activation also remained in the 24 hours the cessation of the exercise; 3) During evening training both groups showed and higher predominance of the sympathetic tone (T0), but no significant differences between M-Types and E-Types were observed.

The effects of physical activity on HR circadian rhythm are of particular interest. In this respect, temporal programming of physical activity can modify temporal structure of the physiological variables, although the effects depend on duration, intensity and frequency (Montaruli et al., 2009). Soccer matches are played at various times of the day, ranging from morning, to midday and to night-time matches under floodlights, in which athletes are constantly striving for high levels of performance. While morning matches may be rare for professional soccer players than for collegiate soccer players, the former group conventionally trains in the morning. Competition time is a critical factor affecting performance. Generally, physical performance is affected by diurnal variation with reference to circadian rhythms (Vitale et al., 2013). In particular, soccer match time schedules are often out of synchrony with the typical time for training, and this separation might affect performance in competition and also apply to training at atypical times. This lack of synchrony might affect both performance in competition and internal circadian rhythm synchronization.

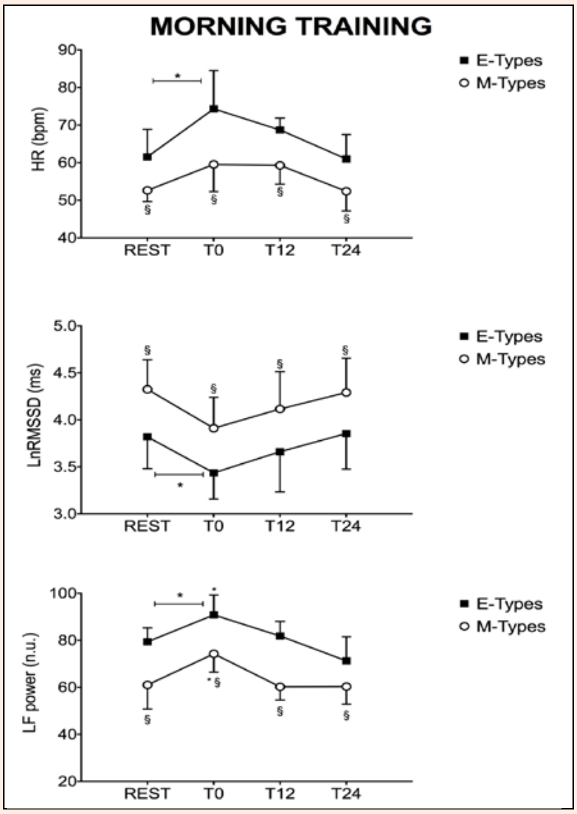

Figure 2. Results of HR, LnRMSSD, and LF power (n.u.), of the M-Types and E-Types performing the morning HIIT.
*: Intra group difference $p < 0.05$; §: Inter group difference, $p < 0.05$

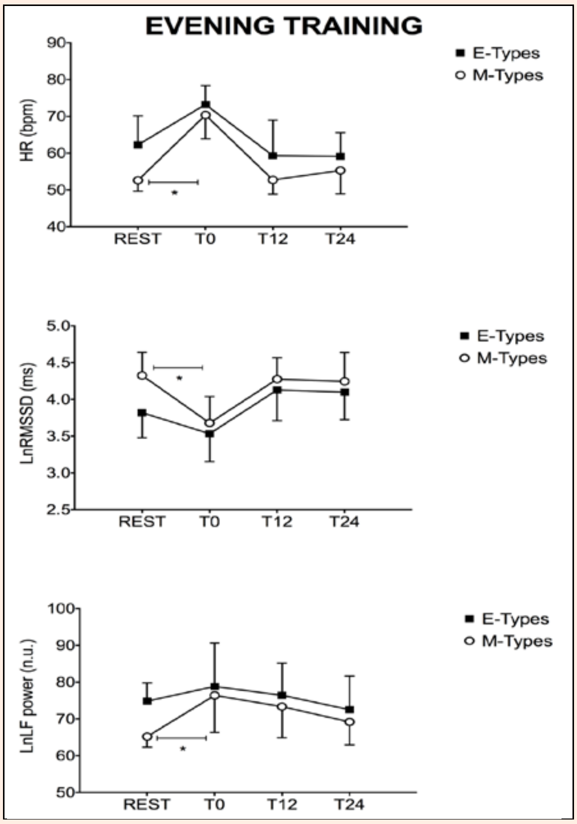

Figure 3. Results of HR, LnRMSSD, and LF power (n.u.), of the M-Types and E-Types performing the evening HIIT.
*: Intra group difference $p < 0.05$

Even if circadian rhythms are genetically determined by a core set of circadian clock genes, they are influenced by exogenous synchronizers. In particular, Nédélec et al. (2015), in a population of soccer players, stated that circadian rhythmicity can be modified by some external factors like changes related to environmental conditions and alterations of sleep-wake behavior. For this reason, players are frequently exposed to various situations and conditions that can interfere with recovery. This could have a practical relevance for coaches, both in preparing teams for matches at different times of the day and in optimizing training programs. To achieve this, athletes must work together with coaches and support staff to ensure that there is an appropriate balance between training and recovery, particularly during competitive season (Barnett, 2006). Consequently, it has been hypothesized that professional soccer players might potentially benefit from training in the morning or evening in anticipation of a soccer match (Hérbert et al., 2002). On the other hand, training in the morning or in the evening could lead different magnitude of the stress response and the time required for recovery. Therefore, HRV monitoring could help athletic and medical staff to provide useful methodological indications on training scheduling and competition monitoring. In fact, one key aspect of this stress response is the activation of the sympathetic arm of the ANS, and a shift in autonomic balance. Quantifying HRV, we found that soccer players who performed the HIIT at 08.00 p.m. showed a significant higher HR which was determined by lower activation of the parasympathetic tone with a sympathetic predominance respect to morning training. HR in normal adults depends on the pacemaker activity of the sinoatrial node cells and constantly varies under the influence of a number on unmodifiable and modifiable factors. Factors like posture, and physical fitness levels are primarily responsible of the circadian variation of HR (Palatini & Julius, 1997) and therefore of HRV. In fact, during daytime, HR shows substantial variation being higher in evening (Montaruli et al., 2009; Mancia et al., 1983). In addition, epidemiologic, clinical, and laboratory studies had repeatedly demonstrated a relationship between emotional cognitive and physical stress (Schwartz et al., 2003). For this reason, performing a single HIIT in the evening could produce the activation of the sympathetic nervous system that seems to be activated by a higher mental stress accumulated during the day that triggers a rather consistent increase of plasma catecholamines, HR and blood pressure that has been showed to be associated with an increased sympathetic tone and parasympathetic withdrawal (Arai et al. 1989; Nakamura et al. 1993; Yamamoto et al. 1991). These findings suggest that the stress induced in the evening had a significantly higher impact on cardiovascular responses than stress induced in the morning independent of chronotype. Our observation is consistent with the study of Fürholz et al. (2013) in which they clearly showed that there is an increase in markers of vagal tone time with higher HF and lower LF power during nighttime. In addition, we detected that this single bout of HIIT induced an acute stress response that returned to the baseline values after 24 hours for both morning and evening training session, suggesting that this type of very physically demanding training need and adequate period of recovery. These findings are also in accordance with the study of Jelinek et al (2015) in which they found an increase of HR one-day post-exercise suggesting greater vagal predominance and increases temporal dynamics. The results of present work indicate that vagal modulation of HRV could provide guidance for choosing the most appropriate time of the day to perform a HIIT giving useful indication for successfully monitoring the effects of exercise loads by HRV analysis

In addition to this, we wanted to investigate the stress response of HIIT in accordance of the diurnal preference of our sample. We observed that subject circadian typology determined different autonomic responses during recovery from a demanding training bout performed in the morning instead of evening. In particular, E-types during morning training presented a significant higher HR caused by a significant parasympathetic withdrawal with a sympathetic predominance at rest and before exercise. Several studies have shown differences between M-Types and E-Types. According to Taillard et al. (2004) and Vitale et al. (2013) M-Types wake up and go bed early and have their best performances in the first part of the day, whereas E-Types go to bed and wake up late and have their peak performance in the evening. Moreover, E-Types had a reduced sleep quality and quantity compared with the M-Types during the weekdays, whereas the E-Types reached the same levels as E-Types during the weekends. In addition, it has been assumed that E-types seems to be less emotionally stable and therefore less efficient in self-regulating in coping with environmental and social demands (Mecacci and Rocchetti, 1998). Therefore, we assumed that E-types had greater problems to self-regulating comparing to M-types for the morning training session producing therefore an increased sympathetic activity of the autonomic nervous system. This lack of self-regulatory behavior in E-type produces a significant higher sympathetic activation that remained higher also in the 24 hours the cessation of the exercise. For this reason, assessing chronotype in professional soccer teams, that used to train twice a day, is crucial in order to better scheduling HIIT and our results specifically suggest that E-types should avoid performing vigorous and all-out interval training early in the morning. From a practical point of view, strength and conditioning coaches of soccer teams with a large number of evening-oriented athletes should select intermediate time of day (from 10.00 a.m. to 16.00 p.m.) to perform high intensity exercise bouts.

Conclusion

Based on our findings, we can conclude that stress response of HIIT is influenced by both the time of the day and by the chronotype. In perspective, our study provides methodological indications on training management and monitoring. Further studies are required to investigate the time course of HRV of this training performed during a complete soccer training session. It would be of interest to investigate the influence of the severity of the exercise overload on the post-exercise response. Although the simple use of questionnaire to determine chronotype has long been established, we suggest that the assessment of HRV can be an additional important procedure for evaluating cardiovascular recovery. An understanding of the post-exercise HRV response is necessary in the monitoring of athletes in general and the process of recovery in particular.

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Key points

- The influences of chronotype on HRV and recovery process from HIIT are unknown.
- Generally, a significant parasympathetic withdrawal with a sympathetic predominance before evening training was detected
- During morning training session E-types had significant higher vagal indices with a significant lower parasympathetic tone that returned to the rest values after 24 hours the cessation of HIIT.
- M-types did not reveal any significant differences compared to E-types during both morning and evening HIIT.
- Stress response of HIIT is influenced by both the time of the day and by the chronotype.

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