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OPEN Heart rate variability in female soccer players, before, during, and after a COVID-19 positive test

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The purpose of this study is to examine the impact of COVID-19 infection on heart rate variability (HRV) in female soccer players, with a focus on identifying changes in autonomic regulation before, during, and after a COVID-19 positive test. Seven elite female soccer players (age: 20.14 ± 6.41 years, height: 162.43 ± 4.32 cm, weight: 51.73 ± 5.65 kg) were included in the analysis after consistently recording their HRV during the specified period. Morning HRV measures were completed using photoplethysmography via the HRV4 training smartphone application, a validated tool for field-based monitoring, along with self-reported data. The players were tracked for at least 30 consecutive days before testing positive for COVID-19 using a polymerase chain reaction (PCR) test. They were also requested to record their HRV while they had COVID-19 until a negative PCR result was obtained. The study presents data on RMSSD, LnRMSSD and HR for the 30 days prior to COVID-19, as well as for the 3 days (day - 3), 2 days (day - 2), and 1 day (day - 1) leading up to COVID-19. Also, data for the first 5 days following a positive COVID-19 test are included. The results of this study indicated that LnRMSSD measurements were significantly lower 2 days (day - 2) and 1 day (day - 1) before the onset of COVID-19, as well as during the first four days following a positive COVID-19 test, compared to baseline. In addition, RMSSD measurements were significantly lower during the first 4 days after a positive COVID-19 test, while resting heart rate was significantly higher during the first and second days following a positive COVID-19 test, compared to baseline. Our findings suggest that reductions in LnRMSSD, reflecting decreased parasympathetic activity, may serve as early indicators of COVID-19 infection in elite female soccer players, potentially allowing for pre-symptomatic detection through daily HRV monitoring.

Keywords Autonomic regulation, Infection, Heart rate variability, Football players, Elite athletes, Wearable technology

Coronavirus (COVID-19), known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has impacted numerous aspects of human life, including sports¹. Although COVID-19 primarily presents as a respiratory disease, research has demonstrated that it affects multiple body systems and may cause autonomic dysfunction². Specifically, a meta-analysis revealed that acute SARS-CoV-2 infection can result in autonomic dysfunction during the acute stages, affecting cardiovascular, sudomotor, and pupillometric functions³. Concurrently, the aforementioned meta-analysis indicated that a complex autonomic nervous system imbalance is a prominent feature of acute COVID-19, often associated with a poor prognosis³. Moreover, autonomic dysfunction related to post-acute SARS-CoV-2 can present with symptoms such as dizziness, tachycardia, syncope, exercise intolerance and brain fog⁴, all of which are important to recognize, particularly in high-demand sports such as soccer.

Heart rate variability (HRV) is frequently used to analyze autonomic parameters and dysfunction as it reflects the balance between the sympathetic and parasympathetic activity of the autonomic nervous system that controls the homeostasis of all vital organs⁵. HRV is assessed by measuring the variation between the R-R intervals, where a change in the duration of the R-R intervals demonstrates an altered autonomic activity⁶. Athletes and coaches

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have traditionally utilized HRV not only to evaluate athletic performance and monitor training⁷ but also as an indicator of psychological stress⁸, reflected in reduced parasympathetic activity^{9,10}. Concurrently, HRV has been associated with fatigue, overtraining^{9,10}, and excessive sedentary time¹¹.

The role of HRV in COVID-19 has been relatively understudied, with few studies investigating its potential application. Research on seventeen patients using wearable technology demonstrated significant decreases in HRV 72 h prior to the elevations in C-reactive protein, suggesting the potential value of short-segment, intermittent HRV analysis in COVID-19 patients¹². In another pilot study of fourteen patients admitted to the intensive care unit, high HF-HRV (a purely vagal index) and low HRV time-domain parameter of SDNN (reflecting mostly vagal but also sympathetic activity) were associated with worse prognosis, higher mortality, as well as higher IL-6 levels¹³. While these studies were conducted with small sample sizes, a larger-scale study ($n=271$) also found that low HRV predicted intensive care unit admission during the first week after hospitalization, especially in patients aged 70 years and older, while a higher HRV was associated with greater chances of survival¹⁴. Furthermore, a longitudinal study examining HRV metrics from commonly worn wearable devices (Apple Watch) indicated that these parameters could predict COVID-19 diagnosis and identify COVID-19-related symptoms in healthcare workers¹⁵. While the above studies focused on older and non-athletic populations, only one recent study examined HRV metrics derived from wearable technology in NCAA Division 1 female athletes¹⁶. The investigators observed decreases in HRV one day before a positive COVID-19 test compared to baseline values. They concluded that wearable technology was successful in predicting COVID-19 infection through changes in respiration rate three days before a positive test and changes in HRV and resting heart rate the day prior to the positive test¹⁶.

For athletes, especially those engaged in high-intensity sports such as soccer, optimal autonomic function is important for recovery, readiness and overall performance^{7,9}. Considering that HRV provides a non-invasive measure of autonomic nervous system function, it becomes particularly useful for detecting COVID-19-related effects. Viral infections, including SARS-CoV-2, may compromise autonomic regulation¹⁷, potentially impair performance, delay return to play, or increase the risk of complications such as myocarditis or post-viral syndromes. Thus, monitoring HRV in athletes offers a practical tool not only for detecting signs of infection but also for guiding individualized return-to-play protocols based on physiological recovery rather than relying on symptom resolution or polymerase chain reaction (PCR) tests.

Although preliminary research suggests that HRV may serve as a tool for predicting COVID-19, further research is needed to explore the impact of COVID-19 infection on HRV before, during and after a positive test, particularly in athletic populations, where early detection and informed return-to-play decisions are essential. Considering the well-documented sex-based differences in autonomic nervous system function, including the higher parasympathetic activity observed in females¹⁸, this study focused on female soccer players to ensure more accurate and sex-specific results, rather than combining data from both sexes. Therefore, the purpose of this study is to examine the impact of COVID-19 infection on HRV in female soccer players, with a focus on identifying changes in autonomic regulation before, during, and after a positive test.

Methods

Participants

Seven elite female soccer players (age: 20.14 ± 6.41 years, height: 162.43 ± 4.32 cm, weight: 51.73 ± 5.65 kg) were included in the analysis after consistently recording their HRV during the specified period. Although ten players from two teams were initially recruited for the study, only seven tested positive for COVID-19 and provided sufficient data.

The players were tracked for at least 30 consecutive days before testing positive for COVID-19 using a polymerase chain reaction (PCR) test. They were also requested to record their HRV while they had COVID-19 until a negative PCR result was obtained. It should be noted that the data were collected at the beginning of the regular in-season period, before the implementation of the lockdown measures, and none of the players had been vaccinated at that time. Female players who reported musculoskeletal injuries and did not participate in the in-season training program or official games were considered ineligible to participate in the study. In addition, non-starters were not eligible to participate. Non-starters were excluded as their training load, game-time exposure, and recovery demands differed from those of starting players. Furthermore, a preliminary comparison of baseline data (collected over the 30 days prior to a COVID-19 positive test) indicated differences in autonomic responses between starters and non-starters, likely due to the lower physical and psychological stress experienced by non-starters. Therefore, to ensure consistency and maximize internal validity, only starters were included in the analysis.

Both players and their legal guardians were informed about the study procedures and provided written informed consent. The study adhered to ethical guidelines according to the Helsinki Declaration and was accepted by the National Committee of Bioethics.

Procedures and instrumentation

All the participants completed laboratory testing at the beginning of the season as part of their regular testing routine. During the testing, they were instructed on how to download the HRV4 training application (<https://www.hrv4training.com>) on their phones and how to record their HRV each morning. Morning HRV measures were completed using photoplethysmography (HRV4training) along with self-reported data on fatigue levels, sleep quality, soreness, perceived exertion, physical condition, mental energy, menstrual cycle status, and lifestyle information. The HRV4training application uses photoplethysmography (PPG) to obtain R-R intervals from a continuous pulse rate reading¹⁹. While electrocardiography (ECG) remains the gold standard for HRV analysis, recent validation studies have demonstrated that photoplethysmography-based methods, such as those used in the HRV4Training application, provide reliable and valid estimates in short-term HRV parameters,

especially when testing is done at rest and in the supine position¹⁹. Considering that photoplethysmography is more susceptible to motion artifacts and may have a lower precision than ECG for frequency-domain measures, only the time-domain measures were used in this study. Furthermore, to enhance data quality, participants received a familiarization session and specific instructions during their visit to the laboratory.

The protocol required a 1-minute measurement²⁰ to be recorded upon waking, within the same time frame each day, in a supine position. If they had to empty their bladder upon waking, they were required to rest for at least one minute to allow for stabilization of heart rate before the measurement. This brief stabilization period has been shown to produce reliable HRV measurements, even from recordings as short as one minute²¹. Players were instructed to remain in a supine position and breathe normally during the measurement. If the application reported that the quality of the signal was low, the measurement was repeated.

HRV was tracked using time domain parameters of the root mean square of successive differences (RMSSD-describing the cardiac vagal activity), natural log-transformed RMSSD (LnRMSSD) and standard deviation of N-N intervals (SDNN). Measurements were also obtained for pNN50, LF and HF domains; however, these were not reported in this study as research indicated the need for longer recordings for accurate results²². Heart rate was also tracked before, during and following COVID-19 infection. At least 30 days were used to establish a normal range for each female athlete. The principal investigator was able to access all the saved data through the coach's platform, as recommended by the manufacturer. Compliance with daily HRV recordings was monitored through the HRV4Training Coach platform, which allowed the principal investigator to track measurement frequency and self-reported data daily. In addition, the players were instructed to email the exported Excel file to the principal investigator at the end of each week. The study presents data on RMSSD, LnRMSSD and HR for the 30 days prior to COVID-19, as well as for the three days (day - 3), two days (day - 2), and one day (day - 1) leading up to COVID-19. Also, data for the first 5 days (days 1, 2, 3, 4, 5) following a positive COVID-19 test are included. All seven athletes provided HRV measurements for the 3 days leading up to a positive COVID-19 test, as well as the five days following the positive test (Fig. 1).

Statistical analysis

Analysis was performed using SPSS, version 28.0, for Windows (SPSS Inc., Chicago, IL, USA). The assumption of normality was assessed using the Shapiro-Wilk test. All parameters are presented as means and standard deviations. Time differences (before, during, and after COVID-19 infection) were analyzed using one-way analysis of variance (ANOVA). Pairwise comparisons were conducted using Bonferroni post hoc analysis. Cohen's *d* was calculated to determine the effect size. Effect sizes were interpreted as follows: small (0.2–0.4), medium (0.5–0.7), and large (0.8–1.4)²³. Cohen's *d* was determined between the baseline measurements and the rest of the days. The level of significance was set at $p < 0.05$.

Results

Based on the players' subjective reports, six out of the seven players experienced moderate symptoms, including fever, cough, headache, aches, sore throat, chest pain and pressure (especially while coaching) and fatigue. One player reported only mild symptoms, limited to a sore throat (for only two days).

One-way analysis of variance ANOVA indicated significant differences in HRV measures of LnRMSSD between the different measurements [$F(8) = 41.20$, $p < 0.05$, Table 1]. Specifically, it was demonstrated that the LnRMSSD measurements were significantly lower two days (day - 2) and one day (day - 1) prior to the onset of COVID-19, as well as during the first four days following a positive COVID-19 test, compared to the baseline measurements (30 days) (Fig. 2).

Furthermore, results indicated significant differences in RMSSD between the different measurements [$F(8) = 23.85$, $p < 0.05$, Table 2].

Specifically, it was demonstrated that the RMSSD measurements were significantly lower during the first four days following a positive COVID-19 test, compared to the baseline measurements (30 days) (Fig. 3).

Additionally, results indicated significant differences in resting heart rate values between the different measurements [$F(8) = 13.93$, $p < 0.00$, Table 3]. It was demonstrated that the resting heart rate was significantly higher during the first and second days following a positive COVID-19 test, compared to the baseline values.

Lastly, Table 4 indicates the absolute magnitude of change across those days to provide a direct numerical difference between the baseline values and the days leading up to and following a COVID-19 positive test, making it easier to interpret how much a variable has increased or decreased.

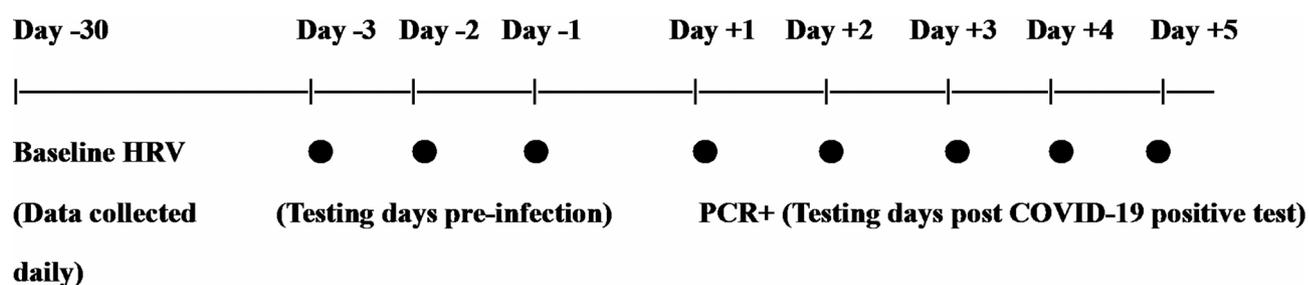


Fig. 1. HRV data collection timeline.

LnRMSSD (ms)	Mean ± SD	Minimum	Maximum	p-value	Cohen's d
Baseline values	8.48 ± 0.41	8.14	9.22		
Day - 3	7.98 ± 0.38	7.50	8.64	0.21	
Day - 2	7.47 ± 0.36*	7.10	8.10	<0.01	2.62
Day - 1	6.77 ± 0.35*	6.20	7.10	<0.01	4.48
Day 1	6.51 ± 0.32*	6.10	6.90	<0.01	5.35
Day 2	6.31 ± 0.13*	6.10	6.50	<0.01	7.13
Day 3	6.46 ± 0.36*	6.10	7.00	<0.01	5.23
Day 4	7.01 ± 0.33*	6.40	7.50	<0.01	3.95
Day 5	8.08 ± 0.23	7.75	8.40	0.94	

Table 1. LnRMSSD measurements, which include baseline values, day - 3, day - 2, day - 1, and 5 days following a positive COVID-19 test. * $p < 0.05$ comparisons of different days to baseline values.

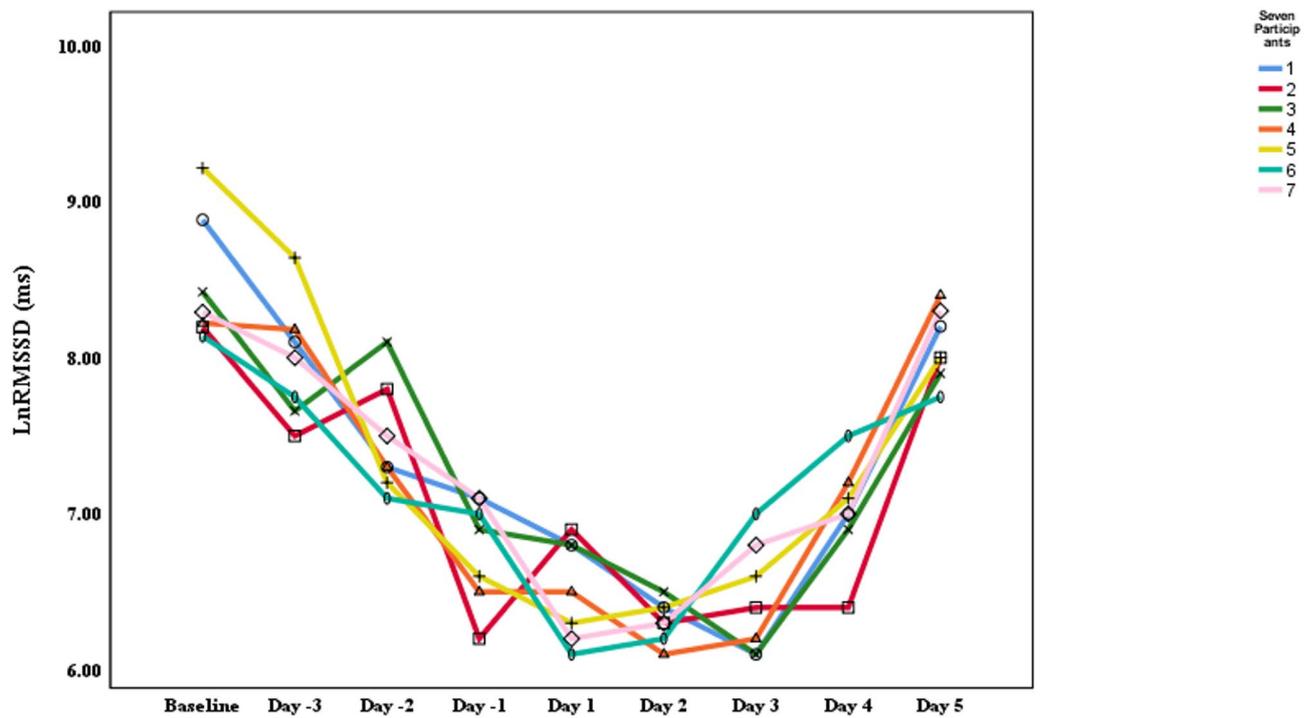


Fig. 2. LnRMSSD values for the seven players.

RMSSD (ms)	Mean ± SD	Minimum	Maximum	p-value	Cohen's d
Baseline values	80.51 ± 10.18	68.87	95.10		
Day - 3	98.86 ± 25.87	55.00	125.00	0.81	
Day - 2	93.29 ± 19.09	57.00	109.00	1.00	
Day - 1	60.86 ± 15.89	37.00	89.00	0.54	
Day 1	33.91 ± 9.63*	27.80	55.00	<0.01	4.73
Day 2	34.59 ± 5.41*	29.20	45.70	<0.01	5.67
Day 3	40.30 ± 7.22*	30.60	52.00	<0.01	4.60
Day 4	52.20 ± 2.82*	49.00	56.10	0.02	3.84
Day 5	96.00 ± 18.76	55.00	109.00	1.00	

Table 2. RMSSD measurements, which include baseline values, day-3, day-2, day-1, and 5 days following a positive COVID-19 test. * $p < 0.05$ comparisons of different days to baseline values.

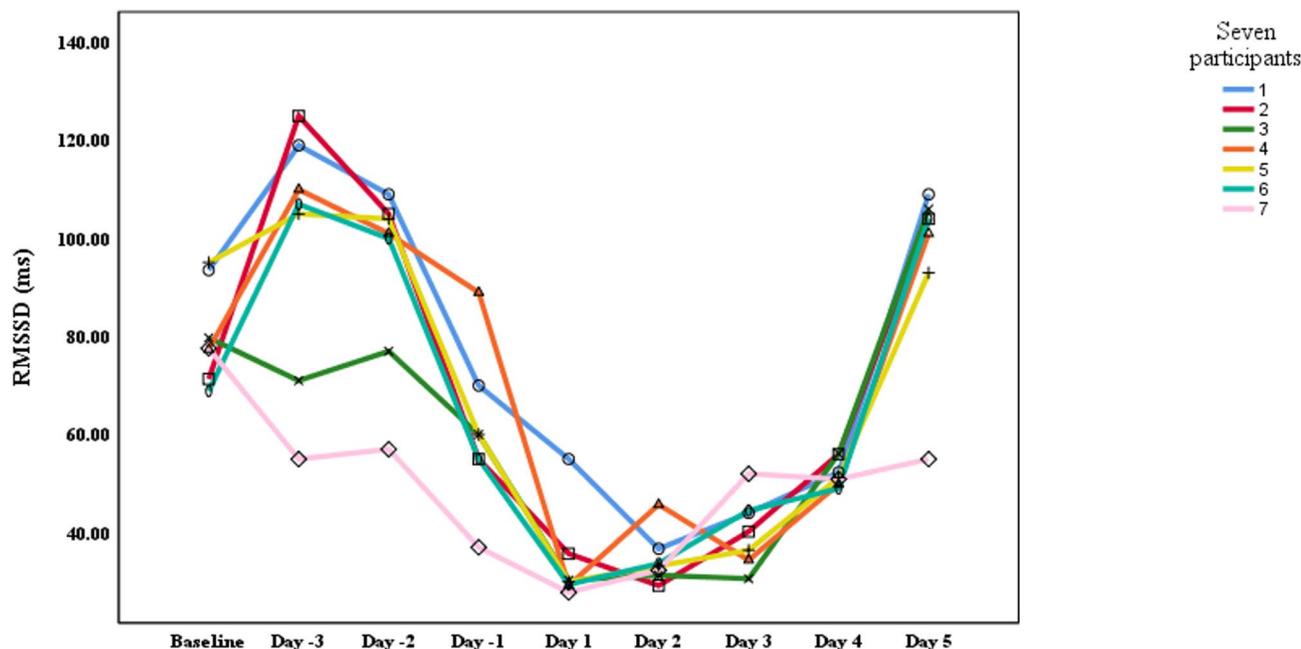


Fig. 3. RMSSD values for the seven players.

Heart rate (beats/min)	Mean ± SD	Minimum	Maximum	p-value	Cohen's d
Baseline values	67.14 ± 2.55	65	72		
Day - 3	69.29 ± 3.10	66	75	1.00	
Day - 2	71.57 ± 3.16	68	78	0.22	
Day - 1	71.00 ± 2.77	69	77	0.58	
Day 1	77.86 ± 3.81 *	72	84	<0.01	3.30
Day 2	78.29 ± 4.61*	72	85	<0.01	2.99
Day 3	70.14 ± 1.35	68	72	1.00	
Day 4	68.29 ± 1.11	67	70	1.00	
Day 5	68.00 ± 1.92	66	72	1.00	

Table 3. Resting heart rate measurements, which include baseline values, day-3, day-2, day-1, and 5 days following a positive COVID-19 test. * $p < 0.05$ comparisons of different days to baseline values.

Variable	Δ Day - 3	Δ Day - 2	Δ Day - 1	Δ Day 1	Δ Day 2	Δ Day 3	Δ Day 4	Δ Day 5
LnRMSSD (ms)	- 0.51 ± 0.28	- 1.01 ± 0.61*	- 1.7 ± 0.51*	- 1.97 ± 0.51*	- 2.17 ± 0.35*	- 2.02 ± 0.60*	- 1.47 ± 0.52*	- 0.40 ± 0.47
RMSSD (ms)	18.35 ± 26.99	12.77 ± 19.49	- 19.65 ± 16.90	- 49.60 ± 9.99*	- 45.93 ± 10.91*	- 40.21 ± 13.31*	- 28.31 ± 10.61*	15.49 ± 20.91
Heart rate (beats/min)	2.14 ± 2.19	4.43 ± 2.30	3.86 ± 1.46	10.71 ± 4.03*	11.14 ± 4.34*	3.00 ± 1.63	1.14 ± 2.79	0.86 ± 3.63

Table 4. Mean ± SD of change (Δ) from baseline in the days leading up to and following a COVID-19 positive test. * $p < 0.05$.

Discussion

The results of this study indicated that LnRMSSD measurements were significantly lower two days (day - 2) and one day (day - 1) before the onset of COVID-19, as well as during the first four days following a positive COVID-19 test, compared to baseline (Tables 1 and 4; Fig. 2). In addition, RMSSD measurements were significantly lower during the first four days after a positive COVID-19 test (Tables 2 and 4; Fig. 3), while resting heart rate was significantly higher during the first and second days following a positive COVID-19 test, compared to baseline (Tables 3 and 4). These findings align with a study on Division 1 NCAA athletes, which indicated that wearable technology (WHOOP band) was successful in predicting COVID-19 infection through changes in respiration rate three days before a positive test and changes in HRV and resting heart rate the day before the positive test¹⁶. Our findings suggest that LnRMSSD may have potential as an early indicator of COVID-19 infection. However, this observation should be interpreted with caution due to the small sample size. Further research with larger

cohorts is needed to validate its predictive ability for viral infection in athletic populations. Conversely, RMSSD and resting heart rate did not have significant changes until after the detection of COVID-19 and may instead be useful for monitoring athlete recovery. Although RMSSD and LnRMSSD are mathematically related, LnRMSSD is statistically more stable and less affected by external factors such as respiration rate, making it a preferred index for day-to-day monitoring in athletes²⁴. The early decline in LnRMSSD observed in our study may reflect initial vagal withdrawal in response to systemic stress or immune system activation before the appearance of any clinical symptoms. This response has been described in endurance athletes undergoing heavy training or fatigue, where reductions in vagally-derived HRV measures were observed before performance decrements or illness²⁴. On the contrary, RMSSD may only detect more pronounced autonomic changes that occur after the onset of the symptoms. This supports the potential of LnRMSSD as a more sensitive early marker of autonomic disturbance and infection in athletes.

Despite the challenges and limitations in interpreting the different time and frequency domains of HRV measures, our study revealed altered autonomic function shortly before the detection of COVID-19 and during the first four days following detection. These findings are consistent with previously published studies that demonstrated autonomic dysfunction in other infectious diseases such as the Epstein-Barr virus²⁵, human immunodeficiency virus²⁶, and human T cell lymphotropic virus²⁷. Lower RMSSD values have also been associated with higher scores on the risk inventory of sudden death and epilepsy²⁸. Furthermore, a study on HRV (SDNN) and COVID-19 reported autonomic dysfunction as early as seven days before a positive COVID-19 test in healthy healthcare workers¹⁵.

Interestingly, another study on nine men and five women, which extracted data from the Welltory app, did not reveal any statistical difference between HRV metrics before, during, and after COVID-19 when time domain indices (SDNN and RMSSD) were assessed for the group as a whole. However, individual-level analysis demonstrated significant individual changes during COVID-19 for some of the participants²⁹. These discrepancies emphasize the importance of considering the methodological differences and study designs when evaluating HRV. Also, it appears that HRV results should be assessed on an individual basis rather than as group averages, considering the great variability observed among individuals (see Figs. 2 and 3). Furthermore, the results of our study cannot be directly compared to those of studies conducted on clinical populations^{12–14}, as the severity of COVID-19 differed significantly between those populations and the female soccer players included in our study. Also, variations in symptoms caused by different viral strains (mutations) should be considered when evaluating HRV. It is important to note that all the participants in our study were likely infected with the Alpha variant. Therefore, consideration should be given when evaluating Beta, Delta and Omicron variants as well as individuals who were vaccinated.

Differences between studies examining HRV responses to COVID-19 may be attributed to several factors. First, variability in study design, such as group-level versus individual-level analysis, can affect whether meaningful patterns can be detected. Second, population characteristics differ significantly, as some studies included sedentary individuals or a clinical population, while others included elite athletes. These groups differ significantly in their autonomic responses due to variations in fitness levels and immune function. Third, methodological variations including the use of different devices (e.g. ECG vs. photoplethysmography), HRV parameters and recording durations can influence outcomes. In addition, the strain of the virus and vaccination status may alter the physiological response to infection. Lastly, individual-level factors such as menstrual cycle phase, prior illness, psychological stress and sleep quality may contribute to intra- and inter-study differences in HRV results. Therefore, all of the above should be considered when interpreting and comparing findings across studies investigating HRV responses to COVID-19 infection.

To the best of our knowledge, this is the first study to assess the impact of COVID-19 infection on HRV before and during infection in female soccer players. Our findings highlight the potential of these highly accessible HRV applications, specifically of the LnRMSSD metric, for predicting COVID-19 before a positive clinical test. While these results are promising, this study is limited by a small sample size. With only seven participants, the results are more susceptible to individual variability and the likelihood of Type II errors is increased. Also, the generalizability of the findings is limited, as the sample may not fully represent the broader population of female soccer players. Although large effect sizes were observed in some variables, these should be interpreted with caution as they may be inflated in small samples due to variability. Therefore, future multicenter studies with larger and more diverse cohorts should be conducted to validate these preliminary findings, enhance statistical power and improve the applicability of the results across different athletic populations. Also, in this study, we did not control for the menstrual cycle of the players, which might have influenced the LnRMSSD values. Although there is no clear agreement on how HRV changes across the menstrual cycle, with some studies reporting increased sympathetic activity during the luteal phase³⁰, others indicating higher parasympathetic activity³¹, and some finding no significant changes³², it is recommended that future HRV research consider the menstrual cycle for more accurate interpretation. Furthermore, we reported only the RMSSD and the natural logarithm of RMSSD (to achieve normal distribution). Future research should incorporate both time and frequency domains of longer recordings for more detailed results. Lastly, it is important to note that all participants in this study were unvaccinated at the time of data collection. As COVID-19 vaccination can alter the immune response of the individuals, the autonomic responses indicated by HRV measurements may differ in vaccinated individuals. Thus, the applicability of our findings to vaccinated female soccer players remains uncertain.

A key strength of the study was the 30 days of HRV measurements, which provided an accurate baseline for comparison with the COVID-19 data. Furthermore, despite the above limitations, our findings carry important practical implications for coaches, medical staff of the teams and sports scientists. Daily monitoring of LnRMSSD using accessible wearable tools such as the HRV4Training may help detect early physiological changes associated with viral infections such as COVID-19, even before the onset of symptoms or a positive test result. This early warning could be used to modify training loads or initiate precautionary isolation to protect the rest of the

players and minimize disruption of team activities. Also, the observed changes in RMSSD and resting heart rate following infection may be used for tracking recovery and guiding individualized return-to-play decisions.

Data availability

Data will become available upon request to the corresponding author.

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Conceptualization, K.P.; M.M.; A.C.P.; methodology, M.M.; K.G.; K.P.; G.B.; A.C.P.; formal analysis, M.M.; K.G.; G.B.; K.P.; investigation, K.P.; G.B.; A.C.P.; data curation, M.M.; K.G.; writing—original draft preparation, K.P.; writing—review and editing, M.M.; K.G.; G.B. and A.C.P.; All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Institutional review board statement

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Informed consent

Informed consent was obtained from all subjects involved in the study and legal guardians.

Additional information

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