



## Original Article

# Influence of biological maturation on cardiac autonomic recovery in female volleyball players during & after repeated sprints training: An experimental trial



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## ABSTRACT

Previously, it was suggested that biological maturation (BM) could be linked to cardiac autonomic recovery (CAR) in the pediatric population. However, this influence hasn't been confirmed yet. Our aim was to investigate the impact of BM on CAR in female volleyball players. Experimental study with a sample of 38 volleyball players, comprising 20 girls (age: [11.6 ± 2.1] years) and 18 women (age: [24.5 ± 5.5] years), we analyzed BM, comparing maturing subjects (girls) with mature subjects (women). Additionally, we assessed peak height velocity (PHV) in girls. We conducted a training session involving repeated sprints (3 rounds of 6 sprints interspersed by 5 min [min] of passive rest). Using short-range radio telemetry, we analyzed CAR during (at the end of the 1<sup>st</sup> and 2<sup>nd</sup> rounds) and after (following the 3<sup>rd</sup> round) the training session of repeated sprints by applying the 60-s to 300-s heart rate recovery index (HRR-Index). Girls exhibited superior CAR compared to women (round 2: 60-s, 120-s, 240-s, and 300-s,  $p < 0.005$ ). Subgroup analyses of BM indicated that individuals in the Late-PHV stage demonstrated superior CAR compared to those in the Early-PHV and During-PHV groups. (60-s to 300-s,  $\eta^2 p > 0.4$ ,  $p < 0.05$ ). Subjects in the During-PHV stage were superior to those in the Early-PHV stage (240-s to 300-s,  $\eta^2 p > 0.4$ ,  $p < 0.05$ ). We have concluded that biological maturation has a significant impact on cardiac autonomic recovery.

## Key-points

1. This study provides crucial information for prescribing recovery periods during training for pediatric volleyball players, particularly in high-intensity training like repeated sprinting (RST).
2. The present study has identified that cardiac autonomic recovery during and after RST appears to be dependent on biological maturation in female pediatric volleyball players. Furthermore, we offer reference values for the minimum and maximum recovery periods for each stage of biological maturation.

## 1. Introduction

Volleyball is an intermittent sport that requires athletes to perform several sprints and jumps during a match.<sup>1</sup> The series of actions performed with the ball in play is called a rally, which, in children and youth categories, can last an average of (8 ± 6) seconds (s).<sup>2</sup> During an official volleyball match, child and youth athletes tend to maintain a rest pattern approximately 2.5 times longer than the duration of the rally.<sup>3</sup> This duration may vary based on the player's position on the court, as cardiovascular responses depend on the player's position (e.g., libero and lifter).<sup>4</sup> However, cardiac autonomic recovery is shown to be the primary factor in reducing the physical strain that occurs during and after exercise.<sup>5</sup>

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**List of abbreviations & acronyms**

ANS	Autonomic nervous system
BMI	Body mass index
BM	Biological maturation
BSA	Body surface area
CA	Chronological age
CAR	Cardiac autonomic recovery
cm	Centimeters
CO <sub>2</sub>	Carbon dioxide.
COMT	Catechol-Omethyltransferase
CV	Coefficient of variation
°C	Temperature in degrees Celcius
HR	Heart rate

HRR	Heart rate recovery
ISAK	International Society of the Advancement of Kinanthropometry
kg	Kilogram
m	Meters
MAO	Monoamine oxidase
<i>n</i>	Absolut number
O <sub>2</sub>	Oxygen
PHV	Peak height velocity
RPE	Rating of perceived exertion
RST	Repeated sprints training
s	Seconds
%	Percentage
*	Statistical significance ( $p < 0.05$ )

Cardiac autonomic recovery is associated with the return to homeostasis (to basal levels), which is one of the responses modulated by factors from the external and internal environments of the body.<sup>6</sup> This mechanism is coordinated by the autonomic nervous system (ANS).<sup>6</sup> The ANS is divided into the parasympathetic nervous system (which predominates during rest) and the sympathetic nervous system (which predominates during activity).<sup>7</sup> A physiological marker responsive to the ANS is heart rate (HR) behavior, which indicates specific cardiac ANS activity.<sup>8</sup> HR increases (indicating sympathetic activity) during physiological stress and tends to decrease (indicating parasympathetic activity) during the reduction of such stress, behaviors that occur during and immediately after physical activity.<sup>9,10</sup>

Thus, HR recovery immediately after exercise is used as a noninvasive procedure to assess cardiovascular parasympathetic function.<sup>10–13</sup> The mechanism that controls such function depends on the release of acetylcholine in the vagus nerve, which promotes the binding of muscarinic receptors, generating hyperpolarization of the cardiac muscle to reduce depolarization and cause a reduction in HR.<sup>14,15</sup> Recovery HR (HRR) can be measured by the absolute difference between peak HR (i.e., achieved during exercise) and HR recorded during a given period of time after exercise (e.g., 30 s, 60 s, and 300 s).<sup>10,11,16</sup> In pediatric patients, parasympathetic nervous system functions exhibit high individual variability, even when analyzed over a narrow age range.<sup>17</sup>

This can be explained by biological maturation (BM), which is the improvement of the body's systems (e.g., nervous and metabolic systems).<sup>18,19</sup> The effects of BM can be analyzed based on age groups, comparing an organism in the maturation process (e.g., children and adolescents) to a mature organism (e.g., adults). Additionally, it can be analyzed by comparing the different levels of BM present in mature organisms. BM is not always in synchrony with chronological age (CA) and can occur early or late relative to CA.<sup>20</sup> One of the most common methods of assessing BM levels is by checking the occurrence of Peak Height Velocity (PHV).<sup>18,21</sup> The PHV can be classified into early-PHV, during-PHV, and Late-PHV.<sup>22</sup> PHV is a measure of somatic maturation and is associated with the development of body tissues (i.e., muscle, fat, bone, etc.).<sup>20</sup> Therefore, PHV is related to overall body size, which, in turn, is associated with the efficiency of the oxidative system in the human organism.<sup>23</sup> Thus, it is likely that PHV has an influence on cardiac autonomic recovery in pediatric patients.

Previously, through the evaluation of variables involved in HR variability, it has been suggested that in pediatric participants, substantial changes in parasympathetic nervous system activity could be due to BM.<sup>24–28</sup> Recently, it has been pointed out that the cardiac autonomic system shows a tendency toward a plateau phase during middle childhood, suggesting that the maturation process of this system in the pediatric population may be associated with or dependent on BM.<sup>17</sup> The aforementioned studies evaluated the BM of pediatrics by comparing age groups (e.g., children, adolescents, and adults). PHV stages were not

taken into consideration to determine BM levels among pediatrics. Likewise, the use of the heart rate recovery index (i.e., HR peak - HRR after exercise) to analyze cardiac autonomic recovery in this population was not taken into consideration.

Thus, the present study hypothesizes that cardiac autonomic recovery in pediatrics is dependent on biological maturation. With that said, the objective was to investigate the impact of biological maturation on cardiac autonomic recovery, as evaluated through the heart rate recovery index, in female volleyball players.

## 2. Methods

Experimental trial, with sample composed by 38 volleyball players, 20 girls (age: [11.6 ± 2.1] years; stature: [149.5 ± 11.4] cm; weight: [46.7 ± 15.8] kg; BMI: [20.5 ± 5.5] kg/m<sup>2</sup>; PHV: [−0.28 ± 1.8]) and 18 women (age: [24.5 ± 5.5] years; stature: [165.8 ± 8.3] cm; weight: [62.5 ± 8.5] kg; BMI: [22.8 ± 3.11] kg/m<sup>2</sup>). The sample size was determined from a pilot study with nine girls and nine women, the variable analyzed was the 60-s HRR index after repeated sprints training (RST). Thus, we identified an effect size of 1.2 (Cohen's *d*), then with the help of the software G\*Power (Version 3.1, Düsseldorf, Germany), we considered an  $\alpha = 0.05$  and a  $\beta = 0.8$ . Thus, a minimum sample size of 14 subjects per group was indicated (critical *t*: 2.0. Power: 0.81). In the same pilot study, we performed analyses of variance for the PHV variable in the girls' sample, from this we identified an effect size of 2.3 ( $\eta^2 p$ ). Then, we considered the G\*power software according to the aforementioned specifications. Subsequently, for the PHV, a minimum sample size of six subjects per stage was pointed out (critical *F*: 9.55; Power: 0.80).

### 2.1. Ethical approval

This study was submitted and approved by the Ethics Committee of the Federal University of Rio Grande do Norte/Brazil (#5.792.835/2022) and followed the standards of the Helsinki declaration.<sup>29</sup> The design of the present study was publicly available a priori on the Open Science Framework Registries platform (DOI: 10.17605/OSF.IO/53PBV). All participants and their respective guardians (in the case of minors) were introduced to all research procedures and those who agreed to participate in the research signed the informed consent form (assent - in the case of children and adolescents).

### 2.2. Participants

All participants were recruited in the same club, located in the city of Natal, Brazil. The girls were members of the sports initiation team and as an inclusion criterion we adopted having age between 8 and 16 years old. The women were volleyball players at regional level and were in pre-season (not performing routine training), as inclusion criteria, we

adopted having age equal to or > 18 years, not using contraceptives and not undergoing any type of hormone therapy. For all participants, we have adopted as exclusion criteria: (i) taking any drug that could interfere with cardiac autonomic recovery (e.g. beta-blockers); (ii) taking any supplement that could act as a stimulant (e.g., caffeine and taurine) or that could interfere with cardiac recovery (e.g., vasodilators); and (iii) having suffered any musculoskeletal injury in the six months preceding the study. The sample characteristics are exposed in Table 1.

The women’s group showed lower resting heart rate ( $p < 0.001$ ), larger body surface area ( $p < 0.001$ ) and higher workload per training session ( $p = 0.002$ ). No significant differences were noted for weekly training load and the other variables ( $p > 0.055$ ) (see Table 1).

### 2.3. Blinding

The evaluators did not know the biological maturation stages of the girls who participated in the study, nor did the participants. The principal investigator did not have access to the data collection regarding recovery heart rate. Finally, statistical analyses were performed in a blinded fashion by an external research collaborator.

### 3. Procedures

Twenty-four hours after signing the informed consent form, we have performed the anthropometric evaluations. Next, blood pressure and resting HR assessments were performed (Fig. 1-A). Subsequently, after a brief warm-up (5-min) involving jumping and short walks, the RST was initiated (Fig. 1-B). During the RST, HR behavior was monitored and during the rest between RST series HRR was monitored, both by short-range telemetry (Polar®, Model H10, Kempele, Finland).

#### 3.1. Anthropometry

With the participants barefoot and wearing light clothing, body mass was measured using a Filizola® digital scale with a capacity of 150 kg and a variation of 0.10 kg (São Paulo, Brazil). Height was determined by a Sanny® stadiometer (0.1 mm precision) (São Paulo, Brazil). For such procedures, we used the protocols of the International Society for the Advancement of Kinanthropometry (ISAK).<sup>30</sup> All assessments were performed by a single examiner and for the technical error of the intra-observer anthropometric measurements, we adopted  $\leq 1.0\%$  as an acceptable margin.<sup>31</sup> From the height and body weight data we have calculated the body surface area (BSA) using the formula:<sup>32</sup>  $BSA = (Body\ weight_{[kg]}^{0.5378}) \times (Stature_{[cm]}^{0.3964}) \times 0.024265$ .

#### 3.2. Blood pressure and resting heart rate

For sample characterization purposes, the resting blood pressure was measured by a single rater with extensive experience. The participant rested for 10 min, sitting in a comfortable chair with backrest and knees positioned at 90°. Then, the evaluator used a manual sphygmomanometer (BIC®, São Paulo, Brazil) with a stethoscope (Premium®, São Paulo,

**Table 1**  
Characteristics of study participants.

Variables	Girls (n = 20)	Women (n = 18)
	Median (Minimum; Maximum)	
Training weekly load (days)	2.0 (2.0; 4.0)	2.0 (2.0; 5.0)
Training load per day (min)	60.0 (60.0; 120.0)	90.0 (60.0; 240.0)
Body surface area (m <sup>2</sup> )	1.3 (0.9; 1.8)	1.7 (1.4; 1.9)
Heart rate at rest (bpm)	98.0 (58.0; 118.0)	74.5 (64; 98)
Resting blood saturation (%)	98.0 (96.0; 100)	99.0 (97.0; 100)
Systolic blood pressure (mmHg)	100.0 (80.0; 111.0)	100.0 (90.0; 120.0)
Diastolic Blood Pressure (mmHg)	70.0 (60.0; 90.0)	77.0 (58.0; 90.0)

PHV: Peak Height Velocity. n: Absolute number. bpm: Beats per minute. %: Percent. mmHg: Millimeters of mercury. RST: Repetitive sprints training.

**Table 2**  
Comparisons between girls and women in relation to cardiac recovery index.

Moment	Girls	Women	Effect size		p
	Median (Interquartile Ranger)		Cohen-d	95 % CI	
HRR Index in Round 1 RST (n = 20 girls and 18 women)					
60-s (bpm)	26.0 (20.5)	22.0 (19.8)	0.4	-0.2; 1.1	0.2
120-s (bpm)	49.5 (17.8)	42.0 (21.0)	0.4	-0.2; 1.0	0.3
180-s (bpm)	56.5 (18.3)	50.0 (19.3)	0.2	-0.4; 0.8	0.5
240-s (bpm)	62.0 (20.0)	58.5 (13.5)	0.0	-0.6; 0.7	0.6
300-s (bpm)	63.5 (18.0)	59.0 (14.5)	-0.0	-0.6; 0.6	0.7
HRR Index in Round 2 RST (n = 17 girls and 18 women)					
60-s (bpm)	35.0 (18.0) *	22.0 (7.5)	1.2	0.4; 2.0	<b>0.002</b>
120-s (bpm)	54.0 (17.0) *	42.5 (12.7)	1.1	0.4; 1.9	<b>0.005</b>
180-s (bpm)	62.0 (16.0)	54.0 (14.0)	0.6	-0.0; 1.3	0.07
240-s (bpm)	67.0 (11.0) *	59.0 (15.0)	0.7	0.0; 1.5	<b>0.01</b>
300-s (bpm)	69.0 (10.0) *	60.0 (12.5)	0.7	0.0; 1.4	<b>0.01</b>
HRR Index in Round 3 RST (n = 14 girls and 18 women)					
60-s (bpm)	38.0 (12.2) *	23.0 (6.5)	1.2	0.3; 1.9	<b>0.004</b>
120-s (bpm)	56.0 (17.0)	49.0 (13.7)	0.6	-0.2; 1.2	0.07
180-s (bpm)	66.5 (14.2)	58.5 (13.7)	0.4	-0.3; 1.1	0.1
240-s (bpm)	68.5 (11.7)	61.0 (11.2)	0.5	-0.2; 1.3	0.05
300-s (bpm)	72.0 (4.5) *	61.0 (7.5)	0.7	-0.0; 1.4	<b>0.01</b>

HRR: Heart Rate Recovery. RST: Repeated sprints training. (-s): Seconds. Analyses: \* Statistical superiority.

Brazil) to measure blood pressure (systolic and diastolic). Resting HR was checked with the aid of a portable oximeter (G-Tech®, São Paulo, Brazil), positioned on the index finger of the left hand of the assessed, the percentage of blood saturation at rest was also acquired.

#### 3.3. Biological maturation

In girls, the stage of biological maturation (BM) was assessed by the mathematical model proposed by Moore et al.,<sup>20</sup> to determine maturity offset from peak height velocity (PHV) (for girls aged eight to 16 years). The equation explains 89.8 % of the variation of PHV in females ( $r^2 = 0.898$ , standard error = 0.528), proving valid for the determination of the BM. The mathematical model consists of:

$$\text{Maturity offset in females} = -7.709133 + [0.0042232 \times (\text{Age [years]} \times \text{Stature [cm]})]$$

Legend: (cm): centimeters.

After the equation it is possible to classify the PHV stage into early (results > 1), during (results between -1 and 1) and late (results < -1).<sup>20</sup> Thus, six girls were classified in early-PHV stage, six in during-PHV stage, and eight in late-PHV stage.

#### 3.4. Repeated sprints training

The repeated sprint training (RST) was conducted in the afternoon (ambient temperature 28 °C) on an official athletics track. For RST the procedure consisted of performing three rounds of six maximal horizontal sprints over a 35-m distance, with 10-s passive rest intervals between each sprint. The rounds were interspersed with 5 min (300-s) of passive rest (participant sitting in a chair with knees at 90°) (Fig. 1-B). For this, an automated photocell kit (Model Speed Test Fit 6.0, Cefise®, São Paulo, Brazil) connected to the Speed test software (Version 6.0, Cefise®, São Paulo, Brazil) was used. The rest time between sets was monitored by a semi-automated digital stopwatch (Monalisa®, São Paulo, Brazil). During the RST, the behavior of HR was monitored by short-range radio telemetry, using a Polar® belt-type equipment (Model H10, Kempele, Finland). A descriptive visualization of the behavior of HR during the RST is available in Fig. 2.

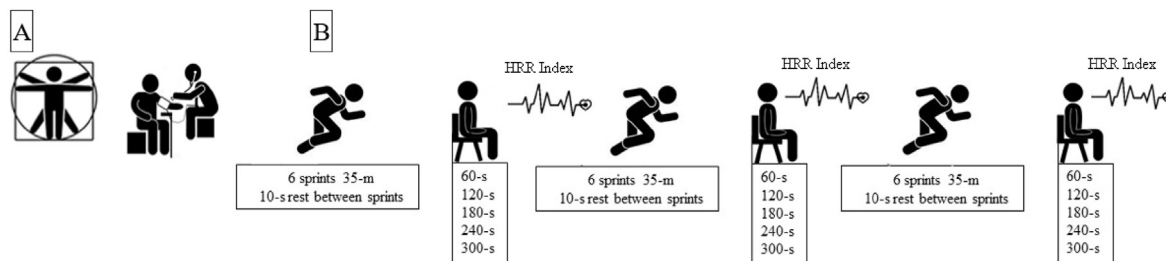


Fig. 1. Study procedures. A: Anthropometric evaluation (height and body weight), blood pressure and resting heart rate evaluation. B: Repeated sprints training divided into three rounds interspersed by recovery heart rate assessment. –s: seconds. –m: meters. HRR: Heart Rate Recovery.

3.5. Cardiac autonomic recovery

For the cardiac autonomic recovery we have analyzed the HRR index during (after rounds 1 & 2) and immediately after RST (after round 3) (Fig. 1-B). For this, A short-range radio telemetry was used (as described in the previous topic). Thus, HRR data was assimilated at different times of cardiac autonomic recovery: (i) fast (at the end of 60-s rest), (ii) intermediate (at the end of 120-s, 180-s, and 240-s rest), and (iii) slow (at the end of 300-s rest).<sup>10</sup> Participants were seated with knees at 90° on a comfortable chair with a backrest in a tented environment. Subsequently as per recommendations from previous studies,<sup>10–12</sup> we subtracted the HRR (from each recovery time [60-s, 120-s, 180-s, 240-s and 300-s]) from the peak HR assimilated during RST. Thus, we acquired the number of recovered heartbeats at each cardiac autonomic recovery phase (fast, intermediate, and slow). According to the equations:

- 1) **HRR Index 60-s** = HRR post 60-s – HRR peak in RST
- 2) **HRR Index 120-s** = HRR post 120-s – HRR peak in RST
- 3) **HRR Index 180-s** = HRR post 180-s – HRR peak in RST
- 4) **HRR Index 240-s** = HRR post 240-s – HRR peak in RST
- 5) **HRR Index 300-s** = HRR post 300-s – HRR peak in RST

Legend: HRR: Heart Rate Recovery. -s: seconds.

3.6. Rating of Perceived Exertion

During the RST, the Rating of Perceived Exertion (RPE) scale proposed by Borg<sup>33</sup> was used. The scale was shown to the participants at the end of each rest between sets (at the end of the 5 min). Subsequently, the RPE of the session was asked, and the participant was asked to point out the effort of the training session as a whole. The scale was made up of numerical values between 6 and 20, where 6 indicates rest and 20 maximum effort. We emphasize that there was a previous familiarization with the RPE. The behavior of the RPE during the RST is displayed descriptively in Fig. 3.

We calculated the coefficient of variation (CV) for RPE in each round and for the entire session using the formula:  $CV(\%) = (standard\ deviation / mean) \times 100$ . We observed that in the first round, there were variations

of over 15% for both groups (Girls: 15.2%. Women: 15.9%); in the second round, the coefficient of variation decreased to approximately 12% for both groups (Girls: 12.2%. Women: 12.5%); in the third round, the variation was 9.6% for the girls and 15.4% for the women. Regarding the total session, the variation was 10% for the girls and 14.2% for the women. This suggests that a greater number of girls achieved intense RPE in round 3 and at the end of the session, while the women indicated a more consistent effort in round 2 of RST.

4. Statistical analysis

The normality of the data was tested by Shapiro-Wilk, z-score tests for skewness and kurtosis (–1.96 to 1.95) and by QQ-line plotting. The assumption of normality was rejected, so we carried out the analysis using non-parametric tests. Before all comparative analyses we applied Bonferroni correction. Comparisons between groups (girls vs. women) were performed using the Mann-Whitney U test for independent samples, the effect size between differences was checked by Cohen-d test, considering the magnitude<sup>34</sup>: Small ≤ 0.10 to 0.23; average: 0.24 to 0.34; large: 0.35 to 0.44; very large ≥ 0.45). Sub analyses considering biological maturation stages in the group of girls (condition effect: early-PHV × during-PHV × late-PHV) and time effect (60-s to 300-s) were done by Kruskal-Wallis test, point differences were checked by Bonferroni post-hoc. The effect size was checked by partial eta-square ( $\eta^2 p$ ), considering magnitude, supra cited.<sup>34</sup> All analyses were performed in the open source software JASP® (Version 0.15.0.0; University of Amsterdam, Holland) considering  $p < 0.05$ . All figures analyses were performed in GraphPad Prism software (Version 8.01 244, California, USA).

5. Results

We have point out that during the RST, six girls did not complete the training due to exhaustion. Three of them did it two rounds and three did it only one round (Fig. 4). However, the sample size remained within the minimum required by the a priori calculation. In addition, out that in the entire sample only one participant in the women’s group was during her menstrual cycle.

Comparisons between the groups indicated that after the first round

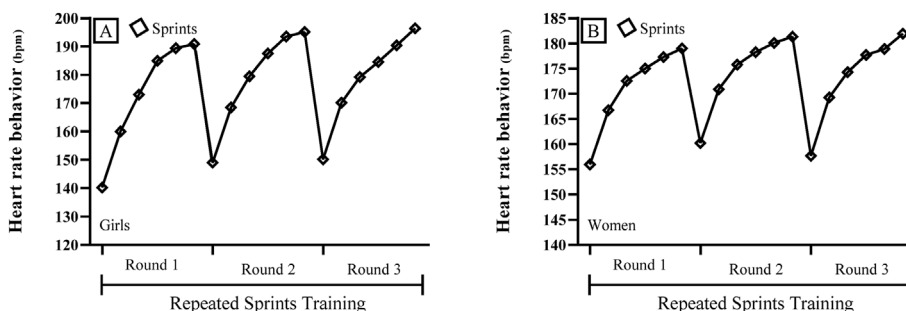


Fig. 2. Heart rate behavior during repeated sprints training. A: Girls. B: Women. (bpm): Beats per minute. Data expressed in mean. Groups: Girls (Round-1: n = 20. Round-2: n = 17. Round-3: n = 14) & Women (all round’s: n = 18).

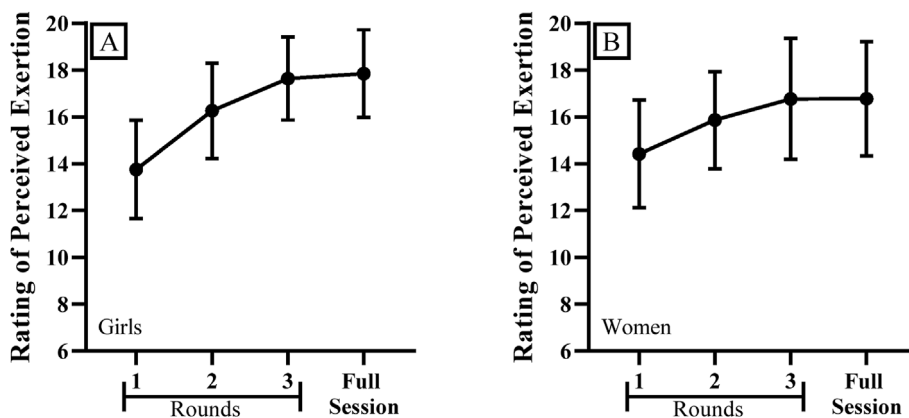


Fig. 3. Behavior of subjective perception of effort during repeated sprint training. A: girls. B: women. Data expressed in mean ± standard deviation. Groups: Girls (Round-1: n = 20. Round-2: n = 17. Round-3: n = 14. Full session: n = 14) & Women (all round's: n = 18).

of RST, no significant differences were observed between the groups. However, after the second (at 60-s, 120-s, 240-s, and 300-s) and third (at 60-s and 300-s) round of RST, the girls' group pointed to better cardiac autonomic recovery compared to the women's group (see Table 2).

Due to the sample loss mentioned in the methods section, it was not possible to perform subgroup analyses considering the PHV stages for RST rounds 2 and 3. Regarding round 1 of RST, the subgroup analyses pointed out that there was a significant effect of time ( $\eta^2 p = 0.48$ ) and biological maturation ( $\eta^2 p: 0.45$ ). Thus, the Late-PHV group showed higher HRR index than the groups in Early- PHV and During-PHV at the fast (60-s:  $\eta^2 p = 0.50, p = 0.003$ ) and intermediate (120-s:  $\eta^2 p = 0.40, p = 0.019, 180-s: \eta^2 p = 0.47, p = 0.004$ , and 240-s:  $\eta^2 p = 0.50, p = 0.004$ ), while at the slow moment it was superior only to the Early-PHV group (300-s:  $\eta^2 p = 0.60, p < 0.001$ ). In addition, the During-PHV group pointed out higher HRR index than the Early-PHV group in the late phase of the intermediate moment (240-s:  $\eta^2 p = 0.45, p = 0.001$ ) and in the slow moment of cardiac autonomic recovery (300-s:  $\eta^2 p = 0.47, p = 0.0001$ ) (Fig. 5).

We point out that for the analyses in Fig. 4 we considered the effect of the biological maturation condition ( $\eta^2 p: 0.45$ ) and with the help of the software G\*Power (Version 3.1, Düsseldorf, Germany), we calculated the

sample power at post-hoc considering an  $\alpha = 0.05$ . This indicated a sample power of 0.85 (critical F: 4.96), confirming that the sample size was adequate.

### 6. Discussion

The objective of the present study was to verify the effect of biological maturation on cardiac autonomic recovery assessed through the heart rate recovery index in female volleyball players. Our initial hypothesis was that cardiac autonomic recovery was dependent on biological maturation, the results confirm such hypothesis. Thus, the present discussion will address the importance of cardiac autonomic recovery for sport training, especially for pediatric sport. It will also address the main points that may justify the effect of biological maturation on cardiac autonomic control.

#### 6.1. Sport training & cardiac autonomic recovery

In this study, we used an RST protocol with an unusual distance for volleyball (35 m). We adopted this protocol to promote maximum cardiac autonomic stress, leading the participants to intense zones of

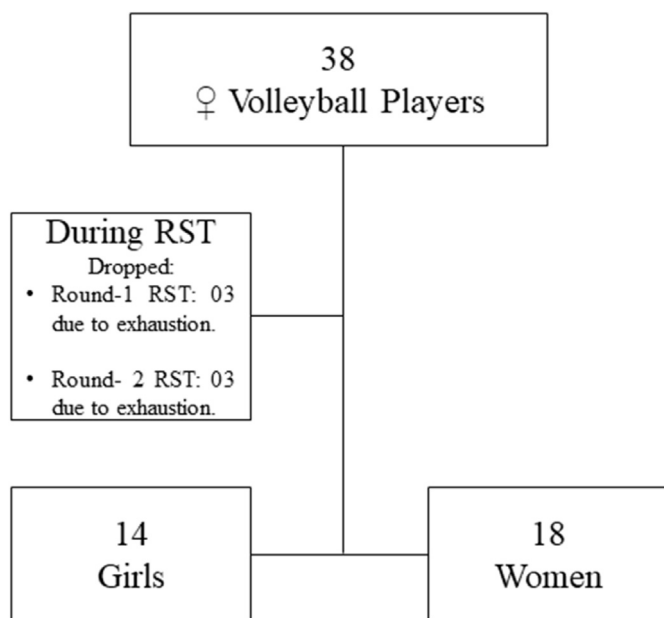


Fig. 4. Number of participants at the beginning and end of the study. ♀: Female. RST: Repeated sprints training.

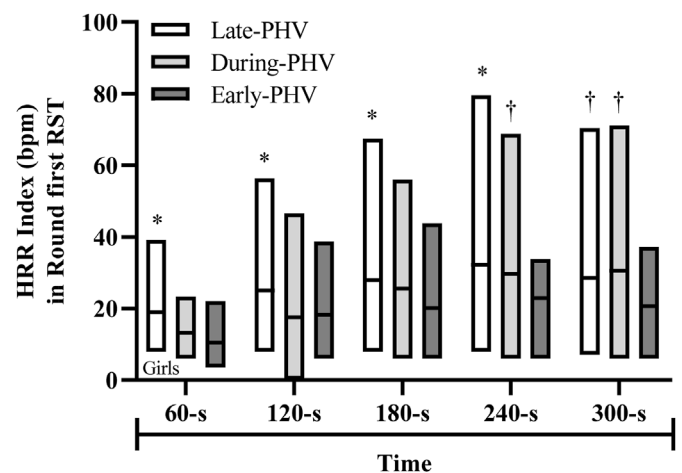


Fig. 5. Comparative analyses considering the subgroups of biological maturation stages in the sample of girls. \* Late-PHV statistically superior to the Early-PHV and During-PHV groups. †: Statistically superior to Early-PHV group. HRR: Heart Rate Recovery. RST: Repeated Sprint Training. (bpm): Beats per minute. (-s): Seconds. Graphical interpretation: Median positioned on the center line of the bars; minimum and maximum values positioned on the bases and tops of the bars, respectively. Groups: Late-PHV (n = 8), during-PHV (n = 6), early-PHV (n = 6).

perceived exertion (RPE). We observed that during the RST, in the first sprint of all the rounds, the girls had a heart rate (HR) approximately 10% lower than that of the women (Round 1: 10.2%. Round 2: 6.9%. Round 3: 5.0%) (Fig. 2). Additionally, the girls reached peak HRs approximately 7.7% higher than the women (Round 1: 6.0%. Round 2: 7.7%. Round 3: 7.6%). However, the resting HR of the girls was around 24% higher than that of the women (Table 1). These data are consistent with the existing literature.<sup>26</sup>

Nevertheless, we analyzed cardiac autonomic recovery using the HRR-Index, which provides a value corrected for the peak HR reached during exercise, enabling a comparison between the groups analyzed. However, the HRR-Index depends on the intensity of the exercise.<sup>10</sup> It should be noted that during the RST, similar categorical RPE ranges were observed between the groups analyzed in this study, suggesting that high intensity was achieved. The reported RPE values ranged from  $14.0 \pm 2.1$  (intense) to  $17.9 \pm 1.8$  (very intense) (Fig. 3). Although the responses varied between the intense and very intense zones, the peak HR values analyzed within each group remained close during the RST rounds (Fig. 2).

In this study, the RPE in the first round of RST was the lowest compared to the others, suggesting that in Round 1, the effort required to induce changes in the HRR-Index had not yet been reached, justifying the lack of difference between the groups. In Round 2, the average RPE approached the high-intensity ranges, which explains the differences between the groups from this round onwards (HRR-Index at 60-s, 120-s, 240-s, and 300-s). In Round 3, the RPE ranges reached very high levels; however, differences were observed only for the HRR-Index at 60-s and 300-s, suggesting a compensation in heart rate recovery between the 120-s and 240-s time points. This may have been due to data loss in the girls' group. Nonetheless, it's important to note that the HRR-Index is defined in intervals of 60-s and 300-s.<sup>10</sup> In our study, we evaluated at 120-s, 180-s and 240-s to analyze the heart rate recovery pattern in relation to the time span between 60-s and 300-s.

According to White & Raven,<sup>35</sup> dynamic exercise is associated with reduced parasympathetic activity and increased sympathetic activity of the heart. Michael, Graham & Davis,<sup>36</sup> point out that both processes are reversed during recovery with partial or complete stabilization of basal homeostasis. In addition, Špenko et al.<sup>37</sup> discuss that full recovery post submaximal exercise can take days. Thus, when conducting sport training sessions on a daily basis, it is likely that a successive session will occur before full recovery from the previous session.

Previous studies have pointed out that recovery after training is dependent on the time it takes HR and HR variability to return or approach pre-exercise values.<sup>38,39</sup> Thus, HRR behavior in the first seconds after exercise (i.e., 60-s to 300-s) can yield valuable information about the recovery pattern of the subject assessed.<sup>10,40</sup> Thus, it is of utmost importance to understand the ways to optimize cardiac autonomic recovery during the stimuli provided in a training session, especially for the pediatric individuals who possess physiological particularities due to BM stages. Given this, considering the autonomic recovery pattern of BM stages may optimize training outcomes in recreational sports players and pediatric athletes.

## 6.2. Biological maturation & cardiac autonomic recovery

The present study found as a primary endpoint that cardiac autonomic recovery was greater in girls than in women, particularly in the more biologically immature girls (Late-PHV), suggesting that sympathovagal balance depends on biological maturation. In a pioneering research, Palmer et al.<sup>41</sup> performed stimulation of sympathetic activities in groups of different chronological age groups (10–20 years & 42–63 years). The authors' findings pointed out that during rest the sympathetic nervous system activity was lower in younger subjects compared to older ones. Ohuchi et al.,<sup>42</sup> suggested that compared to adults' pediatric subjects have greater parasympathetic modulation, favoring HRR.

According to Baraldi et al.,<sup>43</sup> this can be explained by the fact that

during rest, younger subjects have lower circulating concentrations of norepinephrine, which is a neurotransmitter of the sympathetic nervous system. This neurotransmitter exerts an  $\alpha$  adrenergic-II agonist effect that antagonizes the  $\alpha$  adrenergic-I receptor; this mechanism promotes increased systemic resistance due to the occurrence of vasoconstriction of the vascular system, which consequently generates sympathetic cardiovascular responses (e.g., increased blood pressure and heart rate).<sup>44–46</sup>

According to Rang et al.<sup>46</sup> noradrenaline is metabolized in oxidation and methylation inactivity, being catabolized by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), respectively. MAO is predominant on the outer surface of mitochondria and in nerve endings that secrete noradrenaline (e.g., sympathetic postganglionic, adrenal medulla cells).<sup>47</sup> COMT is distributed among nerve endings of the heart and spleen.<sup>46</sup> In a review study, Ratel & Blazevich<sup>23</sup> point out that children and adolescents indicated similar mitochondrial density to that of adult athletes, apparently late BM stages are favored over higher mitochondrial density.

Converging, Kaczor et al.<sup>48</sup> identified that immature subjects point to higher concentrations of aerobic enzymes (e.g., carnitine palmitoyl-transferase & 2-oxoglutarate dehydrogenase) compared to their mature counterparts. This may favor cardiorespiratory conditioning to the extent that prepubertal pediatrics indicate cardiorespiratory conditioning similar to that of endurance athletes.<sup>49,50</sup> We emphasize that cardiorespiratory conditioning is associated with cardiac autonomic recovery, favoring the most conditioned.<sup>10,18</sup>

Among the factors favoring cardiac autonomic recovery, body surface area (BSA) is identified as a determining factor. Washigton et al.,<sup>51</sup> highlighted that the smaller the body surface area, the greater the cardiac autonomic recovery analyzed by HRR. In the present study, pediatric women indicated lower BSA compared to adult women, this may also justify our findings. Ratel & Brlazevich<sup>23</sup> points out that the smaller the overall body size, the greater the efficiency of aerobic metabolism. The authors' justification is based on the premise that vascular connections between the heart and lungs travel over a smaller area, favoring gas exchange (i.e., between  $O_2$  &  $CO_2$ ) in the cardiorespiratory system and collaborating to increased mitochondrial density.

According to Nováková et al.,<sup>52</sup> body size is also associated with the baroreflex mechanism, being more efficient in subjects with smaller body size. The baroreflex is the main physiological mechanism acting in the control of heart rate and blood pressure, it acts as a regulator of HR and vascular sympathetic tone moment-to-moment.<sup>53</sup> Previous studies have identified that advancing chronological age is associated with reduced efficiency of the baroreflex mechanism.<sup>54–56</sup> According to Lenard et al.<sup>57</sup> BM is especially associated with the cardiovascular baroreflex.

## 6.3. Suggestions of practical applicability

Based on an understanding of the factors involved with cardiac autonomic recovery patterns, coaches and trainers can optimize the prescription of recovery during sports training, particularly high-intensity training such as RST. To this end, we suggest that in pediatric subjects the recovery periods between stimuli provided during a high-intensity training session be altered taking into account the stage of biological maturation, thus providing a longer period of time for more mature subjects. Our suggestions are based on the results of the present study, where women (mature organism) recovered less heart rate than girls (maturing organism) during and after RST. The recovery time values shown in Table 3 were determined based on Fig. 5 of the results of the present study.

## 6.4. Limitations

The present study has as main limitations: (i) Not having analyzed other components of cardiac autonomic control (e.g., HR and blood pressure variability), analyzing more components could bring a more concise answer regarding the influence of BM on the cardiac autonomic

**Table 3**

Suggestions for considering the stages of biological maturation to determine cardiac autonomic recovery time (minimum and maximum) between stimuli provided during a high-intensity training session.

Biological maturation stage	Indicated recovery period	
	Minimum	Maximum
Early - PHV	300-s	–
During - PHV	240-s	300-s
Late - PHV	60-s	300-s

PHV: Peak Height Velocity. (-s): Second's.

recovery pattern. (ii) While the participants' sleep patterns were not analyzed, it is known that sleep patterns can influence cardiac autonomic recovery.<sup>26</sup> Additionally, BM is associated with variations in the sleep patterns of pediatric patients, particularly during adolescence.<sup>58–60</sup> Sleep analysis could have broadened our understanding of cardiac autonomic recovery in relation to BM. (iii) Not having analyzed indicators of puberty in girls (e.g., secondary sexual characteristics), which would have made the interpretation of the results more holistic.

### 6.5. Suggestions for further studies

Recently Špenko et al.,<sup>37</sup> found that exercise intensity can affect parasympathetic reorganization up to ~24 h after exercise. As well, it may affect the sleep pattern of pediatric athletes,<sup>61</sup> which may influence cardiac autonomic recovery (CAR) during sports training. Based on this information and the findings of the present study, we suggest that future studies: (i) verify if recovery intervals provided during training have an impact on CAR between consecutive training sessions and/or sports competitions. (ii) verify if recovery intervals provided during training are associated with sleep pattern pre- and post-training session, analyzing if subjects that indicate better CAR during and after training also indicate better sleep pattern (iii) analyze markers of puberty (e.g., hormone levels and secondary sexual characteristics). associated with markers of biological maturation (e.g., bone age and PHV). This may contribute to improve the physical conditioning of pediatric sports practitioners, helping to avoid chronic fatigue and possible cases of overreaching and/or overtraining.

## 7. Conclusion

We conclude that biological maturation has a significant effect on cardiac autonomic recovery, apparently on restabilization of sympathovagal balance. Thus, girls show better heartbeat recovery than women; and internally among girls, the late-PHV and during-PHV biological maturation stages are favored compared to the early PHV stage.

### Authors' contributions

Paulo Almeida-Neto & Fernanda de Oliveira: Conception of the initial idea, elaboration of the study protocols, interpretation of results, writing and final validation of the manuscript. Matheus Rocha: Recruitment of the sample, responsible for data collection regarding cardiac autonomic recovery, writing and final validation of the manuscript. Marcondes Júnior & Júlio Alves: Assisting in data collection, writing and final validation of the manuscript. Iago Medeiros & Felipe Rocha: Application of the repeated sprints protocol, responsible for operating the photocell kit during data collection, writing and final validation of the manuscript. Paulo Dantas: Project supervision, data analysis/interpretation, and drafting of the article. Breno Cabral: Concept/design, project supervision, data collection, drafting of the article, and critical revision of the article.

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### Ethical approval statement

This study has the approval of the Ethical Committee of the Federal University of Rio Grande do Norte (#5.792.835/2022) in Rio Grande do Norte state, Brazil. All participants and their respective guardians (in the case of minors) were introduced to all research procedures and those who agreed to participate in the research signed the informed consent form (assent - in the case of children and adolescents). The protocol of the present study was registered a priori and is publicly available on the Open Science Framework Registries platform (DOI: 10.17605/OSF.IO/53PBV).

### Data availability

The database for this study is publicly available at: <https://figshare.com>, under the <https://doi.org/10.6084/m9.figshare.22155359>.

### Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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