



## Original Article

## Sexual maturation influences cardiac autonomic modulation, anxiety symptoms, and physical fitness in male youth soccer players



Antonio Bernardino Braga Neto <sup>a,\*</sup>, Carlos José Moraes Dias <sup>a</sup>, Emeson Carlos Pimenta Meneses <sup>a</sup>, Manuely Estefanny Vieira Pereira <sup>a</sup>, Michele Brito Correia <sup>a</sup>, Herikson Araújo Costa <sup>b</sup>, Cristiano Teixeira Mostarda <sup>c</sup>, Jefferson Fernando Coelho Rodrigues Junior <sup>a</sup>

<sup>a</sup> Laboratório de Adaptações Cardiorrenais ao Exercício Físico (LACE), Universidade Federal do Maranhão (UFMA), Pinheiro MA, Brazil

<sup>b</sup> Núcleo de Estudos e pesquisas em atividade física (NAFS), Universidade Federal do Maranhão (UFMA), Pinheiro MA, Brazil

<sup>c</sup> Programa de Pós-graduação em Educação Física, Universidade Federal do Maranhão, São Luís MA, Brazil

## ARTICLE INFO

## Keywords:

Anxiety  
Autonomic nervous system  
Heart rate variability (HRV)  
Pubertal maturation

## ABSTRACT

**Objectives::** To analyze cardiac autonomic modulation, anxiety symptoms, and physical fitness in male youth soccer players at different stages of sexual maturation.

**Design::** Cross-sectional study.

**Methods::** A total of 53 male soccer players were evaluated and classified as prepubescent ( $n=22$ ) or pubescent ( $n=31$ ) according to Tanner's self-assessment method. Participants completed the Multidimensional Anxiety Scale for Children (MASC), underwent heart rate variability (HRV) assessment using time, frequency, and nonlinear indices, and performed standardized physical fitness tests following the PROESP-BR protocol. Statistical analyses included Student's  $t$ -test, Mann-Whitney U test, chi-square test, and effect size calculations ( $p \leq 0.05$ ).

**Results::** Pubescent boys showed higher values of vagal autonomic modulation (RMSSD, HF, SD1) and lower sympathetic dominance (LF/HF ratio) compared to prepubescent boys. Significant differences were also observed in stress levels, with 27.3% of prepubescent participants classified as having elevated stress, versus only 5% among pubescent ( $p = 0.009$ ). Physical fitness outcomes indicated superior aerobic capacity, lower limb power, and agility in pubescent players (all  $p < 0.05$ ). Anxiety scores tended to be higher in prepubescent, although the difference did not reach statistical significance ( $p = 0.06$ ).

**Conclusions:** Sexual maturation is associated with improved cardiac autonomic function, reduced stress, and enhanced physical fitness in youth soccer players. Pubertal status should be considered when evaluating physiological and psychological adaptations in young athletes.

## Introduction

Soccer is one of the most widely practiced sports in the world and enjoys broad participation in Brazil,<sup>1</sup> many professional teams invest in identifying and selecting potential new players, fostering the development of youth with the potential to join professional squads<sup>2</sup> in this context, sexual maturation plays a major role in the physical performance of young athletes, particularly in terms of strength and speed. As individuals progress through puberty, muscle development tends to accelerate, contributing to improvements in the body's functional capacities.<sup>2</sup>

In the current landscape, physical fitness is considered a key

determinant of athletic performance, especially in sports with high physiological demands such as soccer. Achieving this critical level of performance requires properly structured and appropriately intense training.<sup>3</sup>

Given the high physical intensity involved in playing soccer, especially among children, it is essential to understand its effects on cardiovascular health. Differences in cardiovascular health can already be present in childhood, with potential long-term consequences. Identifying and monitoring possible risk factors at this stage of life is crucial for the prevention of future cardiovascular diseases.<sup>4</sup>

There are several ways to assess cardiac autonomic modulation, with heart rate variability (HRV) standing out as a non-invasive method used

\* Corresponding author at: Estrada de Pacas, km10 s/n – Pinheiro, MA; Zip code: 65200-000, Brazil.

E-mail address: [antonio.braga@alumni.ufma.br](mailto:antonio.braga@alumni.ufma.br) (A.B. Braga Neto).

<https://doi.org/10.1016/j.apunsm.2026.100516>

Received 27 November 2025; Accepted 25 March 2026

Available online 28 April 2026

2666-5069/© 2026 Consell Català de l'Esport. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

to evaluate the autonomic nervous system's modulation at the cardiac sinus node. It describes the oscillations between consecutive RR intervals recorded by the electrocardiogram.<sup>5</sup>

To analyze HRV, parameters can be obtained through linear methods—in both the time and frequency domains—as well as through nonlinear methods.<sup>6</sup> Among the many factors that influence cardiac autonomic modulation, the athlete's emotional state is particularly relevant. An anxious state increases sympathetic modulation, which negatively affects athletic performance in terms of motor skills and decision-making.<sup>7</sup>

Regarding anxiety, it refers to an emotional state of fear or worry.<sup>8</sup> Anxiety rates in children and adolescents increased following the COVID-19 pandemic.<sup>9</sup> However, it is necessary to distinguish between state anxiety and trait anxiety. State anxiety refers to a temporarily elevated level of anxiety, while trait anxiety is considered a personality characteristic or individual profile.<sup>10</sup> Anxiety becomes a key topic in sports due to its direct impact on athletic performance, especially among children and adolescents. When these individuals exhibit high levels of state anxiety, they are unable to reach their full potential in sports, which compromises their physical fitness.<sup>11</sup>

In this context, it is observed that physical fitness among children and adolescents participating in youth academies or soccer schools is progressively developed over their athletic journey toward the professional level. This development is influenced by factors such as training quality, socioeconomic conditions, and the stage of biological and sexual maturation.<sup>12</sup>

Although previous studies have separately investigated cardiac autonomic modulation, anxiety, or physical fitness in young athletes, evidence remains scarce regarding the integrated analysis of these variables while considering different stages of biological maturation.

Based on this, this study aimed to identify cardiac autonomic modulation, anxiety levels, and physical fitness among players at different stages of sexual maturation.

## Materials and methods

### Study design and ethical aspects

This study employed a cross-sectional design with a sample size of 53 youth players, consisting exclusively of male athletes aged between 9 and 12 years, divided into two groups: Pubertal ( $n = 31$ ) with a mean age of  $12,8 \pm 2,71$  and Pre-pubertal ( $n = 22$ ) with a mean age  $9,16 \pm 1,62$ . The athletes participated in structured training three times per week, characterizing regular and systematized sports practice. A minimum of one year of practice was adopted to ensure prior adaptation to training and to reduce the possible influence of beginners on physical fitness and autonomic modulation variables. Exclusion criteria included: (a) lack of parental or guardian consent to participate in the assessments, and (b) failure to complete all stages of the study.

This study is part of the umbrella project entitled "Evaluation of anthropometric, hemodynamic parameters and quality of life in schoolchildren in the city of Pinheiro - MA," which supports and guides various related initiatives. The project was approved by the Research Ethics Committee under protocol number 3.956.296 and complied with all the recommendations of Resolution No 466/2012 of the Brazilian National Health Council. All participants were legally authorized by their parents and/or guardians through the signing of a Free and Informed Consent Form (FICF), in accordance with ethical guidelines.

### Participants and study location

The study was conducted at the Exercise Physiology Laboratory of the Federal University of Maranhão (UFMA), Pinheiro Campus – MA, Brazil. Participants signed the informed consent form and were subsequently guided through an anamnesis questionnaire that collected their name, date of birth, address, phone number, and the length of time they

had been practicing soccer in training schools. For participants unable to provide this information, assistance was provided by their parents or guardians.

### Sexual maturation

For the evaluation of sexual maturation, the criteria proposed by Tanner were adopted. It is a self-assessment method using images, considering the development of the penis and genital hair in boys. Subsequently, the individuals were classified in one of the following five stages. Boys who selected stages P1 and P2 were classified as prepubescent ( $n = 22$ ), while those who selected stages P3 and P4 were classified as pubescent ( $n = 31$ ). In our sample, no participants were classified as stage P5, which represents full sexual maturation.

### Anthropometric measurements

Weight and height were measured using the LS500 electronic scale with an attached stadiometer. Body Mass Index (BMI) was calculated using the formula:  $\text{weight (kg)}/\text{height (m)}^2$ . Nutritional status was classified according to BMI Z-score using the AnthroPlus™ software, version 1.0.4. The BMI percentiles for the study were defined as follows: underweight < 5th percentile; healthy weight = 5th to < 85th percentile; overweight = 85th to < 95th percentile;  $\geq$  95th percentile = obese.<sup>13</sup>

### Anxiety level

Anxiety was assessed using the Multidimensional Anxiety Scale for Children (MASC),<sup>14</sup> which has provided evidence of its validity and reliability for the Brazilian population. The MASC measures different dimensions of anxiety in children and adolescents aged 7 to 19 years and consists of 39 items distributed across four subscales: (1) Physical Symptoms, (2) Social Anxiety, (3) Separation Anxiety, and (4) Generalized Anxiety. Each subscale yields a score, and the total sum reflects the overall anxiety level. Scores  $\leq 56$  indicate a minimal likelihood of anxiety; scores between 55 and 59 represent minimal anxiety; scores between 60 and 64 indicate moderate anxiety; and scores  $\geq 65$  represent severe anxiety.

### Heart rate variability

Heart rate variability (HRV) was analyzed using a 12-lead electrocardiogram (Micromed Biotecnologia, Wincardio), with participants in the supine position for 10 min. Data collection was followed by time and frequency domain analysis using Kubios HRV software, version 4.1.0 (Kubios, Finland).

HRV was analyzed using the following time and frequency domain indices, respectively: (a) Time domain: mean RR intervals, standard deviation of RR intervals (SDNN), root mean square of the successive differences between adjacent RR intervals (RMSSD), (b) Frequency domain: high frequency (HF), and sympathovagal balance (LF/HF); standard deviation of instantaneous beat-to-beat variability (SD1), long-term standard deviation of continuous RR intervals (SD2), and the SD2/SD1 ratio, which is the ratio between short-term (SD1) and long-term (SD2) standard deviations of RR intervals on the electrocardiogram. HRV measures in the frequency domain were characterized using the Fast Fourier Transform.

### Stress index

The Baevsky Stress Index (SI) was calculated from RR interval distribution as an indicator of sympathetic predominance and autonomic regulatory tension. To normalize its distribution, square root transformation was applied. Baevsky's stress index classification was performed as follows: 50–100 (normal), 100–900 (high), and >900 (very

high).<sup>15</sup>

**Physical fitness classification**

To determine physical fitness related to sports performance, tests were applied following the guidelines of the PROESP – Projeto Esporte Brasil manual, 2016<sup>16</sup>, which aims to assess the physical fitness level of Brazilian children and adolescents. The tests used in this study were: cardiorespiratory fitness (6-minute run/walk test); lower limb power (standing long jump); agility (square test); speed (20-meter sprint test).

**Statistical analysis**

After testing for normality using the Shapiro-Wilk test, data were presented as mean ± standard deviation or as median with interquartile ranges, as appropriate. To analyze potential statistical differences in parametric variables, the Student’s t-test was used, preceded by verification of homogeneity using Levene’s test. For non-parametric variables, the Mann-Whitney test was applied. The chi-square test and Fisher’s exact test were used for associations. Results were considered statistically significant at  $p \leq 0.05$ . The GraphPad Prism 9.0 and Jamovi 2.3.28 software were used for data handling and analysis. For interpretation of effect size, Cohen’s classification (1992) was used: ( $r = 0.10$  small;  $r = 0.30$  medium;  $r = 0.50$  large).

**Results**

The findings presented in Table 1 showed statistically significant differences between the groups in anthropometric and physical performance variables. Prepubescent boys had significantly lower weight and height compared to pubescent boys ( $p = 0.01$  for both variables). In the aerobic capacity test, the prepubescent group covered a shorter distance than the pubescent group ( $p < 0.01$ ), indicating lower cardiorespiratory performance. Similar differences were observed in the standing long jump ( $p = 0.01$ ) and square test ( $p = 0.04$ ), with poorer performance among prepubescent participants. On the other hand, no significant differences were found in BMI percentile ( $p = 0.39$ ) or the 20-meter sprint test ( $p = 0.55$ ).

Regarding cardiac autonomic modulation (Table 2), heart rate variability analyses showed that prepubescent boys exhibited reduced values in time-domain indices such as SDNN ( $p = 0.007$ ) and RMSSD ( $p = 0.003$ ), indicating lower parasympathetic activity. In the frequency domain, significant differences were found between groups in both absolute and normalized HF values ( $p = 0.003$  and  $p = 0.001$ ,

**Table 1**  
Anthropometric characteristics and physical performance variables.

Variables	Prepubertal (n = 22)	Pubertal (n = 31)	P	ES
Age (years)	9,36 ± 1,62	12,8 ± 2,71	0,01	1,49
Weight (kg)	26,9 (24,3 – 35,4)	41,5 (32,0 – 53,5)	0,01	0,90
Height (cm)	133,0 (123,3 – 137,6)	149,5 (140,8 – 167,8)	0,01	1,16
BMI Percentile	52,29 ± 35,41	44,44 ± 31,40	0,39	-0,23
Aerobic Capacity (m)	759,95 ± 64,58	957,62 ± 121,30	0,01	1,94
Standing Long Jump (m)	130,0 (122,8 – 158,5)	175,0 (156,0–187,5)	0,01	0,84
Square Test (s)	7,73 ± 6,83	6,83 ± 0,99	0,04	-0,83
20-meter Sprint (s)	4,53 ± 0,85	4,37 ± 1,02	0,55	-0,16
<b>Physical Fitness Classification</b>				
Risk Zone	17 (77,3%)	16 (51,6%)	0,08	
Healthy Zone	5 (22,7%)	15 (48,4%)		

Values are expressed as mean ± standard deviation or median and interquartile range. Kg, kilograms; Cm, centimeters; BMI, body Mass Index; M, meters; S, seconds; ES, Effect Size.  $p < 0.05$  is considered statistically significant.

**Table 2**

Heart rate variability in the time and frequency domain divided into prepubertal and pubertal.

Variáveis	Prepubertal (n= 22)	Pubertal (n = 31)	P	ES
HR(bpm)	110,5 ± 22,41	81,9 ± 9,83	0,01	1,56
<b>Time Domain</b>				
RR (ms)	717,1 ± 92,28	854,1 ± 124,05	0,01	1,20
SDNN (ms)	49,9 ± 18,18	63,6 ± 16,97	0,007	0,78
RMSSD (ms)	55, 8 ± 26,19	75,4 ± 19,01	0,003	0,88
<b>Frequency Domain</b>				
LF (ms <sup>2</sup> )	702,0 (455,2 – 1360)	795(610 – 1169)	0,52	0,10
HF (ms <sup>2</sup> )	971,5 (574,5 – 1931)	2543(1737 – 3405)	0,01	0,52
LF (nu)	41,7 ± 14,2	28,4 ± 13,7	0,001	-0,95
HF (nu)	58,1 ± 14,2	70,5 ± 14,5	0,003	0,85
LF/HF	0,66 (0,44 – 1,05)	0,34 (0,21 – 0,61)	0,002	0,51
<b>Nonlinear</b>				
SD1(ms)	35,9 (24,8 – 51,4)	48,9 (44,5 – 73,5)	0,01	0,55
SD2(ms)	60,8 (41,7–74,8)	65,4(58,6 –81,7)	0,05	0,31
SD2/SD1	1,48(1,24–1,93)	1,20(1,02 –1,40)	0,002	0,51

Data are expressed as mean ± standard deviation and median with interquartile range. HR, heart rate; RR, mean RR intervals; SDNN, standard deviation of the intervals; RMSSD, root mean square of successive differences of RR intervals; LF (ms<sup>2</sup>), low-frequency spectrum in milliseconds squared; HF (ms<sup>2</sup>), high-frequency spectrum in milliseconds squared; HF (nu), high-frequency spectrum in normalized units; LF (nu), low-frequency spectrum in normalized units; LF/HF, sympathovagal balance; SD1, standard deviation of instantaneous beat-to-beat variability; SD2, long-term standard deviation of continuous RR intervals; SD2/SD1, ratio between the short-term (SD1) and long-term (SD2) standard deviations of RR intervals on the electrocardiogram; ES, effect size.

respectively), with lower values among the prepubescent group. Additionally, a higher LF/HF ratio was observed ( $p = 0.002$ ), suggesting greater sympathetic dominance in this group. Nonlinear indices also reflected lower autonomic modulation in prepubescent participants, with reduced values of SD1 ( $p = 0.01$ ), SD2 ( $p = 0.005$ ), and the SD2/SD1 ratio ( $p = 0.002$ ). These findings support the hypothesis that puberty is associated with changes in cardiac autonomic balance, with greater parasympathetic involvement observed in pubescent boys.

The analysis of anxiety classification based on the MASC scale scores (Table 3) revealed a trend toward a difference between the groups, with a p-value of 0.06. It was observed that 96.8 % of pubertal boys presented minimal levels of anxiety (score ≤ 54), while among prepubertal boys this percentage was 77.3 %. Additionally, only the prepubertal group presented cases with higher levels of anxiety: 13.6 % were classified as having mild anxiety (55–59), 4.5 % with moderate anxiety (60–64), and 4.5 % with severe anxiety (≥ 65), whereas no pubertal participants fell into these categories.

The analysis of the stress index by sexual maturation (Table 4) revealed a statistically significant difference between the prepubertal

**Table 3**  
Distribution of anxiety classifications (MASC) by sexual maturation.

	Prepubertal (n = 22)		Pubertal (n = 31)		P	ES
MASC Categories	N	%	N	%		
Minimal (≤54)	17	77,3	31	96,8		
Mild (55–59)	3	13,6	1	3,2	0,06	0,31
Moderate (60–64)	1	4,5	0	0,0		
Severe (≥ 65)	1	4,5	0	0,0		

Results are presented by frequency distribution and relative percentage; chi-square test. ES, effect size.

and pubertal groups ( $p = 0.009$ ). Pubertal children showed a higher prevalence of low (35.5 %) and normal (64.5 %) stress levels, whereas in the prepubertal group, these values were 18.2 % and 50 %, respectively. On the other hand, 27.3 % of prepubertal individuals presented elevated stress levels (score between 13 and 22), compared to only 5 % in the pubertal group. Additionally, only the prepubertal group had one case of high stress (4.5 %), and no child in either group was classified as having very high stress (score  $>30$ ). These data indicate that children in the prepubertal phase tend to exhibit higher levels of stress, suggesting possible emotional vulnerability prior to the onset of sexual maturation.

## Discussion

This study aimed to analyze cardiac autonomic modulation, anxiety levels, and physical fitness in players at different stages of sexual maturation. The main results of the present study indicate that pubertal boys exhibit greater vagal autonomic modulation and lower sympathetic cardiac autonomic modulation. Sexual maturation is associated with changes in cardiac autonomic modulation and stress levels, corroborating the study by Leonard et al.<sup>17</sup>, which showed improved vagal autonomic indices in adolescents, reinforcing that sexual maturation influences cardiac autonomic function.

Additionally, it is extremely important to consider other factors that modulate HRV, such as physical activity level. According to Sharma et al.,<sup>18</sup> regular physical exercise positively influences heart rate variability by increasing parasympathetic activity. Furthermore, global variability indices were higher in this group, indicating a better adaptive capacity of the cardiac autonomic nervous system (ANS), similar to the findings of Chen 2022.,<sup>19</sup> who reported improved global HRV indices in adolescents.

These findings are consistent with other studies that have demonstrated differences in vagal HRV in pubertal individuals. As observed by Chen et al.,<sup>20</sup> sexual maturation, combined with a high level of physical activity, influences cardiac autonomic control. Similarly, Oliveira et al.<sup>21</sup> reported greater parasympathetic predominance in pubertal boys. These findings reinforce the hypothesis that sexual maturation promotes more efficient autonomic modulation. In the same logic, according to Sisakova,<sup>22</sup> although children already have cardiac autonomic regulation, this balance only becomes truly effective as they grow, providing greater maturation of the cardiac ANS in this population.

In this sense, these results can be explained from a physiological standpoint. In a study by Dogru et al.<sup>23</sup> conducted on men, a positive correlation between total testosterone and parasympathetic activity and a negative correlation with sympathetic activity was evidenced. Testosterone plays important roles in neural maturation and functioning. Similarly, Oskui et al.<sup>24</sup> state that testosterone causes vasodilation in coronary arteries and can reduce inflammatory markers. Therefore, normal testosterone levels play an important role in maintaining cardiovascular health. Furthermore, Butler et al.<sup>25</sup> confirmed that testosterone increases as puberty progresses, with differences across

all levels of sexual maturation. Thus, the hypothesis raised regarding our results is that pubertal boys have higher testosterone levels compared to prepubertal boys, suggesting better cardiovascular adaptation, as well as a significant influence on performance tests.

Moreover, this study identified that pubertal boys showed superior performance in aerobic capacity, lower limb power, and agility tests compared to prepubertal boys. Manzano et al.<sup>26</sup> found similar results, stating that the transition process to the pubertal phase leads to body improvement and development of physical capacities.

Our study compared the sexual maturation of boys; however, as shown by Mandigout et al.,<sup>27</sup> who studied the effects of a training program in preadolescents classified in stage 1 of the Tanner scale, their study found increased global HRV variables in healthy prepubertal children. Nevertheless, as analyzed in our study, when compared to boys in the pubertal phase, there are differences in HRV indices when compared to pubertal boys.

Beyond the difference observed in cardiac autonomic modulation, we found that prepubertal individuals show higher cardiac stress indices according to Baevsky's stress index, as well as higher anxiety scores. These findings align with Dieleman et al.<sup>28</sup> who highlight the influence of anxiety on an individual's physiological stress, which can serve as an indicator of chronic stress in children. Similarly, Sharma et al.<sup>18</sup> showed that children classified as anxious had parasympathetic reactivity outside the normal range. These findings reinforce the importance of maturational stage and emotional aspects, such as anxiety, in interpreting HRV indices in pediatric populations, suggesting that prepubertal boys have poor cardiac autonomic nervous system (ANS) adaptation, indicating that the onset of puberty may represent a phase of greater psychophysiological vulnerability.

Pubertal maturation is characterized by significant biological and neuroendocrine changes that can influence both emotional regulation and the functioning of the autonomic nervous system. These developmental changes have been associated with increased emotional sensitivity and a higher risk of anxiety symptoms during early adolescence.<sup>29</sup> Additionally, puberty is also associated with modifications in cardiovascular autonomic regulation. Lower heart rate variability (HRV) values have been linked to reduced parasympathetic activity and impaired emotional regulation, which may increase vulnerability to symptoms of anxiety and stress.<sup>30</sup> Some studies have already demonstrated associations in which adolescents presenting anxiety symptoms show alterations in HRV indices compared with healthy peers, suggesting dysregulation of cardiac autonomic control.<sup>31</sup>

Although some studies suggest that anxiety symptoms increase during adolescence, other longitudinal studies have reported a decrease in anxiety symptoms during the early stages of adolescence.<sup>32</sup> These findings suggest that the developmental trajectory of anxiety during the transition from childhood to adolescence is heterogeneous and influenced by multiple biological, psychological, and environmental factors. However, changes in body perception during adolescence may also influence psychological outcomes, including anxiety and depressive symptoms.<sup>9</sup> Therefore, differences in maturational stage may partially explain variations in anxiety levels observed among young athletes.

The differences in physical fitness observed between pre-pubertal and pubertal players may also be influenced by differences in chronological age. In the present study, pubertal athletes were older, which may reflect greater exposure to training, motor learning, and accumulated practice time. Therefore, the observed differences may not be exclusively related to biological maturation but also to age-related adaptations and training experience. Latorre-Román, et al.,<sup>33</sup> found that developmental changes occurring during childhood may influence physical fitness and autonomic cardiac modulation, indicating that age-related factors may also contribute to the differences observed between groups.

The present study has some limitations that should be mentioned. First, we used a cross-sectional design, which does not allow the establishment of causal relationships between the investigated

**Table 4**

– Stress index classified by sexual maturation.

	Prepubertal (n = 22)		Pubertal (n = 31)		p	ES
	N	%	N	%		
Stress Index						
Low (<7)	4	18,2	11	35,5		
Normal (7–12)	11	50,0	20	64,5		
Elevated (13–22)	6	27,3	2	5,0	0009	0,47
High (23–30)	1	4,5	2	5,0		
Very High (>30)	0	0	0	0		

Results are presented by frequency distribution and relative percentage; chi-square test. ES, effect size.

variables. Therefore, the findings should be interpreted as associations between sexual maturation, cardiac autonomic modulation, anxiety levels, and physical fitness in young soccer players. Second, although biological maturation provides important information about developmental status, differences in chronological age within the same maturation stage may influence physiological and psychological variables such as cardiac autonomic modulation, anxiety levels, and physical fitness. Consequently, chronological age should be considered a potential confounding factor when interpreting the results.

Third, sociodemographic variables such as socioeconomic status, family environment, and educational background were not assessed in the present study. These factors may influence psychological outcomes, particularly anxiety levels, represent a potential source of uncontrolled variability. Fourth, although Tanner staging is widely used to assess sexual maturation, some limitations should be acknowledged. Previous studies have reported potential misclassification, particularly when self-assessment methods are used, as children and adolescents may overestimate or underestimate their pubertal stage.<sup>34</sup>

Fifth, the relatively small sample size ( $n = 53$ ) may have reduced the study's statistical power and does not allow the generalization of the findings. Sixth, another sample limitation was the inclusion of only boys, limiting the findings to a single gender. Seventh, in the present study, five participants classified as Tanner stage P2 were included in the prepubertal group. Although Tanner stage P2 is often considered an early stage of pubertal development, it represents the transition from prepuberty to puberty and may already reflect initial hormonal changes.

This transitional characteristic may introduce some variability in physiological and psychological responses, potentially influencing variables such as autonomic cardiac modulation, anxiety levels, and physical fitness. Therefore, the inclusion of participants at this stage may represent a potential source of classification bias and should be considered when interpreting the findings.

Despite these limitations, a cross-sectional study was conducted on a subject rarely explored in the literature. The analysis of cardiac autonomic modulation, anxiety levels, and physical fitness in active and healthy children is rarely studied. Future studies should adopt a longitudinal research methodology to observe the evolution of performance, emotional, and physiological variables, providing a better understanding of the effects of sexual maturation on these variables. Additionally, hormone testing should be considered to determine total testosterone levels and understand the effects of this hormone in the maturation process of children and adolescents who play soccer. Therefore, this research becomes an important reference for future studies, providing relevant parameters in this field.

The findings of this study have important practical implications for professionals working with youth athletes. Understanding the relationship between pubertal maturation, cardiac autonomic modulation, anxiety levels, and physical fitness may help coaches, physical trainers, and health professionals better monitor the physiological and psychological development of young soccer players. In addition, these results may contribute to the development of training and monitoring strategies that consider maturational status, promoting athlete well-being and optimizing performance during adolescence.

## Conclusion

We conclude that both vagal and global autonomic modulation are greater in pubescent athletes compared to prepubescent athletes. Additionally, we observed higher performance variable values and lower cardiac stress levels in this group. Regarding anxiety, the results indicated differences between maturation stages, highlighting the importance of considering sexual maturation when analyzing psychological and physiological variables in young athletes. These findings suggest that the pubertal stage promotes greater maturation of the cardiac autonomic nervous system and enhanced development of physical capacities.

## Conflicts of interest

The authors declare no conflicts of interest.

## References

- Coelho E, Machado JM, Schutz EDSF. Fatores motivacionais para a prática de futsal e futebol por crianças e adolescentes: uma revisão sistemática. *RBFF - Rev Bras Futsal Futeb.* 2022;13(55):604–614.
- Rodrigues Júnior JFC, Dias-Filho CAA, Oliveira Júnior MSD, et al. Effects of soccer training during preseason on physical and physiological variables in youth soccer players. *Motriz.* 2021;27.
- Sammoud S, Negra Y, Bouguezzi R, et al. Effects of plyometric jump training on measures of physical fitness and lower-limb asymmetries in prepubertal male soccer players: a randomized controlled trial. *BMC Sports Sci Med Rehabil.* 2024;16(1):37.
- Bongers-Karmaoui MN, Jaddoe VVV, Roest AAW, Gaillard R. The cardiovascular stress response as early life marker of cardiovascular health: applications in population-based pediatric studies—a narrative review. *Pediatr Cardiol.* 2020;41(8):1739–1755.
- Turcu AM, Ilie AC, Ștefăniu R, et al. The impact of heart rate variability monitoring on preventing severe cardiovascular events. *Diagnostics.* 2023;13(14).
- Lins-Filho OL, Andrade-Lima A, Torres AD, et al. Association between sleep quality and cardiac autonomic modulation in adolescents: a cross sectional study. *Sleep Sci.* 2023;16(4):e462–e467.
- Ayuso-Moreno R, Fuentes-García JP, Collado-Mateo D, Villafaina S. Heart rate variability and pre-competitive anxiety according to the demanding level of the match in female soccer athletes. *Physiol Behav.* 2020;222, 112926.
- Zhao C, Wang K, Li D, et al. Relationship between state anxiety, heart rate variability, and shooting performance in adolescent shooters. *BMC Psychol.* 2024;12(1):736.
- Racine N, McArthur BA, Cooke JE, Eirich R, Zhu J, Madigan S. Global prevalence of depressive and anxiety symptoms in children and adolescents during COVID-19: a meta-analysis. *JAMA Pediatr.* 2021;175(11):1142–1150.
- Martínez-Rodríguez A, Peñaranda-Moraga M, Vicente-Martínez M, et al. Relationship between anthropometric measures and anxiety perception in soccer players. *Int J Env Res Public Health.* 2022;19(15).
- Wilczyńska DM, Abrahamson F, Popławska A, Aschenbrenner P, Dornowski M. Level of anxiety and results of psychomotor tests in young soccer players of different performance levels. *Biol Sport.* 2022;39(3):571–577.
- Campos CG, Carlos FDM, Muniz LA, et al. Atividade física na adolescência e maturidade sexual: uma revisão sistemática. *Ciênc Saúde Colet.* 2021;26.
- Albano RD, Souza SBD. Estado nutricional de adolescentes: "risco de sobrepeso" e "sobrepeso" em uma escola pública do município de São Paulo. *Cad Saúde Pública.* 2001;17.
- Nunes MM, Asbahr FR, Lage Castillo ARG, Litvov J, Lotufo Neto F. Validity and reliability of the multidimensional anxiety scale for children (MASC) %. *J Bol - Acad Paul Psicol.* 2021;41:236–244.
- Dias CJ, Barroso R, Dias-Filho CAA, et al. Possible influences of vitamin D levels on sleep quality, depression, anxiety and physiological stress in patients with chronic obstructive pulmonary disease: a case control study. *Sleep Sci.* 2022;15(Spec 2):369–374.
- Gaya AR, Gaya ACA, Pedretti A, Mello JB. *Projeto Esporte Brasil, PROESP-Br: Manual de Medidas, Testes e Avaliações.* Porto Alegre: UFRGS/ESEFID; 2021:2021.
- Lenard Z, Studinger P, Mersich B, Kocsis L, Kollai M. Maturation of cardiovascular autonomic function from childhood to young adult age. *Circulation.* 2004;110(16):2307–2312.
- Sharma RK, Balhara YP, Sagar R, Deepak KK, Mehta M. Heart rate variability study of childhood anxiety disorders. *J Cardiovasc Dis Res.* 2011;2(2):115–122.
- Chen H, Xu J, Xie H, Huang Y, Shen X, Xu F. Effects of physical activity on heart rate variability in children and adolescents: a systematic review and meta-analysis. *Ciênc Saúde Colet.* 2022;27.
- Chen H, Xu J, Xie H, Huang Y, Shen X, Xu F. Effects of physical activity on heart rate variability in children and adolescents: a systematic review and meta-analysis. *Cien Saude Colet.* 2022;27(5):1827–1842.
- Oliveira RS, Leicht AS, Bishop D, Barbero-Álvarez JC, Nakamura FY. Seasonal changes in physical performance and heart rate variability in high level futsal players. *Int J Sports Med.* 2013;34(5):424–430.
- Šisáková M, Helánová K, Hnatkova K, Andršová I, Novotný T, Malik M. Intra-individual relationship between heart rate variability and the underlying heart rate in children and adolescents. *J Clin Med.* 2024;13(10).
- Doğru MT, Başar MM, Yuvaç E, Simşek V, Sahin O. The relationship between serum sex steroid levels and heart rate variability parameters in males and the effect of age. *Türk Kardiyol Dern Ars.* 2010;38(7):459–465.
- Oskui PM, French WJ, Herring MJ, Mayeda GS, Burstein S, Kloner RA. Testosterone and the cardiovascular system: a comprehensive review of the clinical literature. *J Am Heart Assoc.* 2013;2(6), e000272.
- Butler GE, Walker RF, Walker RV, Teague P, Riad-Fahmy D, Ratcliffe SG. Salivary testosterone levels and the progress of puberty in the normal boy. *Clin Endocrinol (Oxf).* 1989;30(5):587–596.
- Manzano-Carrasco S, García-Unanue J, Lopez-Fernandez J, et al. Differences in body composition and physical fitness parameters among prepubertal and pubertal children engaged in extracurricular sports: the active health study. *Eur J Public Health.* 2022;32(Supplement\_1):i67–i72.

27. Mandigout S, Melin A, Fauchier L, N'Guyen LD, Courteix D, Obert P. Physical training increases heart rate variability in healthy prepubertal children. *Eur J Clin Invest*. 2002;32(7):479–487.
28. Dieleman GC, Huizink AC, Tulen JH, Utens EM, Tiemeier H. Stress reactivity predicts symptom improvement in children with anxiety disorders. *J Affect Disord*. 2016;196:190–199.
29. Pfeifer JH, Allen NB. Puberty initiates cascading relationships between neurodevelopmental, social, and internalizing processes across adolescence. *Biol Psychiatry*. 2021;89(2):99–108.
30. Beauchaine TP, Thayer JF. Heart rate variability as a transdiagnostic biomarker of psychopathology. *Int J Psychophysiol*. 2015;98(2 Pt 2):338–350.
31. Chalmers JA, Quintana DS, Abbott MJ, Kemp AH. Anxiety disorders are associated with reduced heart rate variability: a meta-analysis. *Front Psychiatry*. 2014;5:80. <https://doi.org/10.3389/fpsy.2014.00080>.
32. McLaughlin KA, King K. Developmental trajectories of anxiety and depression in early adolescence. *J Abnorm Child Psychol*. 2015;43(2):311–323.
33. Latorre-Román PÁ, de la Casa Pérez A, Pancorbo-Serrano D, et al. Influence of physical fitness and weight status on autonomic cardiac modulation in children. *Pediatr Res*. 2023;94(5):1754–1763.
34. Rasmussen AR, Wohlfahrt-Veje C, Tefre de Renzy-Martin K, et al. Validity of self-assessment of pubertal maturation. *Pediatrics*. 2015;135(1):86–93.