Original Research Article

Talita Dias da Silva-Magalhães*, Íbis Ariana Peña de Moraes, Luiz Carlos Marques Vanderlei, Carlos Bandeira de Mello Monteiro, Marcela Maria Carvalho da Silva, Fernando Pereira, Rodrigo Martins Dias, David Garner, Luis Fernando Grossklauss, Tatiana Dias de Carvalho and Celso Ferreira

Is cardiac autonomic modulation influenced by beta blockers in adolescents with Duchenne Muscular Dystrophy?

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Abstract

Objectives: As the Duchenne Muscular Dystrophy (DMD) is a progressive neuromuscular disorder frequently associated with cardiac dysfunction, this study aimed to evaluate the influence of beta-blocker therapy on cardiac autonomic modulation in adolescents with DMD by analyzing heart rate variability (HRV) indices in patients with and without beta-blockers.

Methods: A cross-sectional study was conducted with 90 participants divided into three groups: (1) participants with DMD receiving beta-blocker therapy (DMDB, n=30), (2) participants with DMD without beta-blocker therapy (GMDM, n=30), and (3) age- and sex-matched typically developing participants (GDT, n=30). HRV was assessed using validated beat-to-beat heart rate monitoring (RS800CX, Polar) under controlled conditions. Linear and non-linear HRV indices (including Detrended Fluctuation Analysis and Symbolic Dynamics) were analysed using Kubios HRV software.

Results: DMD patients exhibited autonomic impairment, characterized by decreased HRV, increased sympathetic dominance, and reduced parasympathetic modulation. Beta-blocker therapy was associated with significantly higher Mean Beat-to-beat interval (RR) and lower Mean Heart Rate (HR) compared to the non-beta-blocker DMD group, with values approaching those observed in typically developing participants. Non-linear indices suggested that DMD patients receiving beta-blockers demonstrated increased HRV complexity and fractal properties compared to those not receiving beta-blockers, although differences remained between the DMD and control groups.

Conclusions: Autonomic dysfunction in DMD is characterized by reduced HRV and altered sympathovagal balance. In our results, beta-blocker therapy was associated with

***Corresponding author: Talita Dias da Silva-Magalhães**, Faculty of Health and Life Sciences, St Lukes Campus, University of Exeter, Exeter, UK; Postgraduate Program in Medicine – Cardiology at Escola Paulista de Medicina, Federal University of São Paulo (EPM/UNIFESP), São Paulo, SP, Brazil; and Postgraduate Program in Bioengeneering, Universidade Brasil, São Paulo, SP, Brazil,
E-mail: ft.talitadias@gmail.com. https://orcid.org/0000-0002-4683-4671
Íbis Ariana Peña de Moraes, Research and Technological Applications in Rehabilitation Group, School of Arts, Science and Humanities, University of São Paulo (PATER-EACH-USP), São Paulo, Brazil; Postgraduate Program in Rehabilitation Sciences, Faculty of Medicine, University of São Paulo (FMUSP), São Paulo, SP, Brazil; and Department of Physiotherapy, Federal University of Juiz de Fora Campus Governador Valadares (UFJF-GV), Governador Valadares, MG, Brazil
Luiz Carlos Marques Vanderlei, Stress Physiology Laboratory, São Paulo State University, UNESP, Presidente Prudentea, SP, Brazil
Carlos Bandeira de Mello Monteiro, Research and Technological Applications in Rehabilitation Group, School of Arts, Science and Humanities, University of São Paulo (PATER-EACH-USP), São Paulo, Brazil; and Postgraduate Program in Rehabilitation Sciences, Faculty of Medicine, University of São Paulo (FMUSP), São Paulo, SP, Brazil
Marcela Maria Carvalho da Silva and Fernando Pereira, Postgraduate Program in Bioengeneering, Universidade Brasil, São Paulo, SP, Brazil
Rodrigo Martins Dias, Postgraduate Program in Medicine – Cardiology at Escola Paulista de Medicina, Federal University of São Paulo (EPM/UNIFESP), São Paulo, SP, Brazil; and Research and Technological Applications in Rehabilitation Group, School of Arts, Science and Humanities, University of São Paulo (PATER-EACH-USP), São Paulo, Brazil
David Garner, Cardiorespiratory Research Group, Department of Biological and Medical Sciences, Faculty of Health and Life Sciences, Oxford Brookes University, Oxford, UK
Luis Fernando Grossklauss and Celso Ferreira, Postgraduate Program in Medicine – Cardiology at Escola Paulista de Medicina, Federal University of São Paulo (EPM/UNIFESP), São Paulo, SP, Brazil
Tatiana Dias de Carvalho, Postgraduate Program in Medicine – Cardiology at Escola Paulista de Medicina, Federal University of São Paulo (EPM/UNIFESP), São Paulo, SP, Brazil; and Departamento de Ciencias de la Salud, Universidad Nacional de La Matanza (UNLaM), San Justo, Buenos Aires, Argentina

improved HRV and enhanced autonomic control. These findings highlight the potential cardioprotective role of beta-blockers in DMD management and emphasize the need for further research into optimizing autonomic function in DMD.

Keywords: Muscular Dystrophy; Duchenne; heart rate; autonomic nervous system; adrenergic beta-antagonists

Introduction

Duchenne Muscular Dystrophy (DMD) is the most common recessive neuromuscular disease, and its incidence is 1:3,000–6,000 boy births [1]. It is produced by a mutation in the gene encoding the dystrophin enzyme, located on the short arm of the X chromosome [2], in the Xp21 region [3]. The clinical picture in the early stages is characterized by the presence of myopathic lifting (Gowers sign) and digitigrade gait, with hip swing. The involvement of the scapular girdle occurs between six and seven years of age [1].

As the disease progresses, most patients experience diverse signs and symptoms, including joint contractures and scoliosis aggravated by lumbar lordosis [1, 4]. In general, patients with DMD lose their gait and use a wheelchair at approximately 15 years of age [1]. In addition to motor issues, DMD also promotes changes in the heart, which has been identified as the main cause of death [4, 5].

Neuromuscular diseases are often associated with cardiac dysfunction, as the degeneration and atrophy present in skeletal muscles also occur in the cardiac muscle [5, 6]. Loss of normal dystrophin function in the heart produces four-chamber dilation and reduced left ventricular function that develop after the onset of muscle weakness, in which case atrial and ventricular arrhythmias occur and can be fatal [4]. Currently, for correction of arrhythmias resulting from cardiomyopathy, drugs such as beta-blockers have been widely used to help improve myocardial function [7].

However, unlike musculoskeletal, pulmonary complications [8] and pain [9] from DMD, which are classic and already established in the literature, research studying the effects of the autonomic nervous system on the heart in DMD is still emerging.

Some studies analyzed cardiac autonomic modulation in humans with DMD, through analysis of heart rate variability (HRV), and observed changes characterized by predominance of sympathetic modulation and a reduction in parasympathetic modulation. Inoue et al. [10] found reduced HRV in people with DMD, especially in the SDNN index.

Changes in autonomic modulation, reflected by a reduction in SDANN, SDNN, and PNN50 indices of HRV, have been identified as an indicator of a worse prognosis in cardiovascular diseases [6, 11] and related to the appearance of arrhythmias [12] and sudden death [11]. Besides that, the studies found to date on HRV in DMD have evaluated only linear indices, as can be seen in the time domain, such as RMSSD, SDNN, SDNNi, SDANN, pNN50, and in the frequency domain, such as LF, HF, and LF/HF ratio. SDNN and HF were the indices that showed a reduction in most studies [6, 10, 13], suggesting increased sympathetic activity and/or reduced parasympathetic tone in people with DMD. Nevertheless, none of the reviewed studies incorporated non-linear analysis.

Considering the reasons mentioned above, beta-blockers are widely used in individuals with DMD, primarily to manage tachycardia resulting from cardiomyopathy and to support myocardial function [7]. However, despite their routine use, there is a lack of detailed understanding regarding how beta-blockers influence cardiac autonomic modulation in this population, particularly through advanced methods of HRV analysis.

Linear analyses use simpler mathematical techniques, such as statistical, geometric, and spectral methods, to represent the modulation of sympathetic and parasympathetic components of the autonomic nervous system [14]. In contrast, nonlinear methods – based on chaos theory – have gained increasing attention, as nonlinear behavior predominates in human systems due to their complex and dynamic nature, which cannot be adequately described by linear methods [15]. Thus, this approach provides additional clinical value.

Our focus on adolescents was based on both biological and clinical considerations. Adolescence represents a critical period for the progression of DMD, during which the decline in motor and cardiac function typically accelerates. Furthermore, this age range often marks the initial implementation of beta-blocker therapy as part of cardioprotective interventions [16].

Thus, the present study aims to evaluate the influence of beta-blocker therapy on the autonomic modulation of people with DMD, comparing the HRV indices between participants with DMD receiving and not receiving beta-blockers, as well as a group of typically developed adolescents. By applying heart rate variability analysis – including advanced nonlinear metrics commonly used in exercise physiology – we aim to translate tools traditionally applied in athletic and rehabilitative settings to a clinical, functionally impaired population. This translational approach supports the broader field of exercise biomedicine by identifying autonomic biomarkers and therapeutic

strategies that may enhance cardiovascular health and quality of life in adolescents with neuromuscular disorders such as DMD. The summary of this article is presented in Figure 1.

Materials and methods

The research project is approved by the Research Ethics Committee of the Federal University of São Paulo, under protocol CAAE 09942913.4.0000.5505.

Participants

A cross-sectional study was carried out, in which 90 participants aged between 11 and 18 years were evaluated, divided into three groups: (1) 30 people with a medical diagnosis of DMD undergoing treatment with beta-blocker (DMDB); (2) 30 people diagnosed with DMD who were not currently using beta-blockers and had never used them previously (GDMD) and (3) 30 people with typical development (TD), matched for age and sex to DMD groups. The recruitment of the DMD

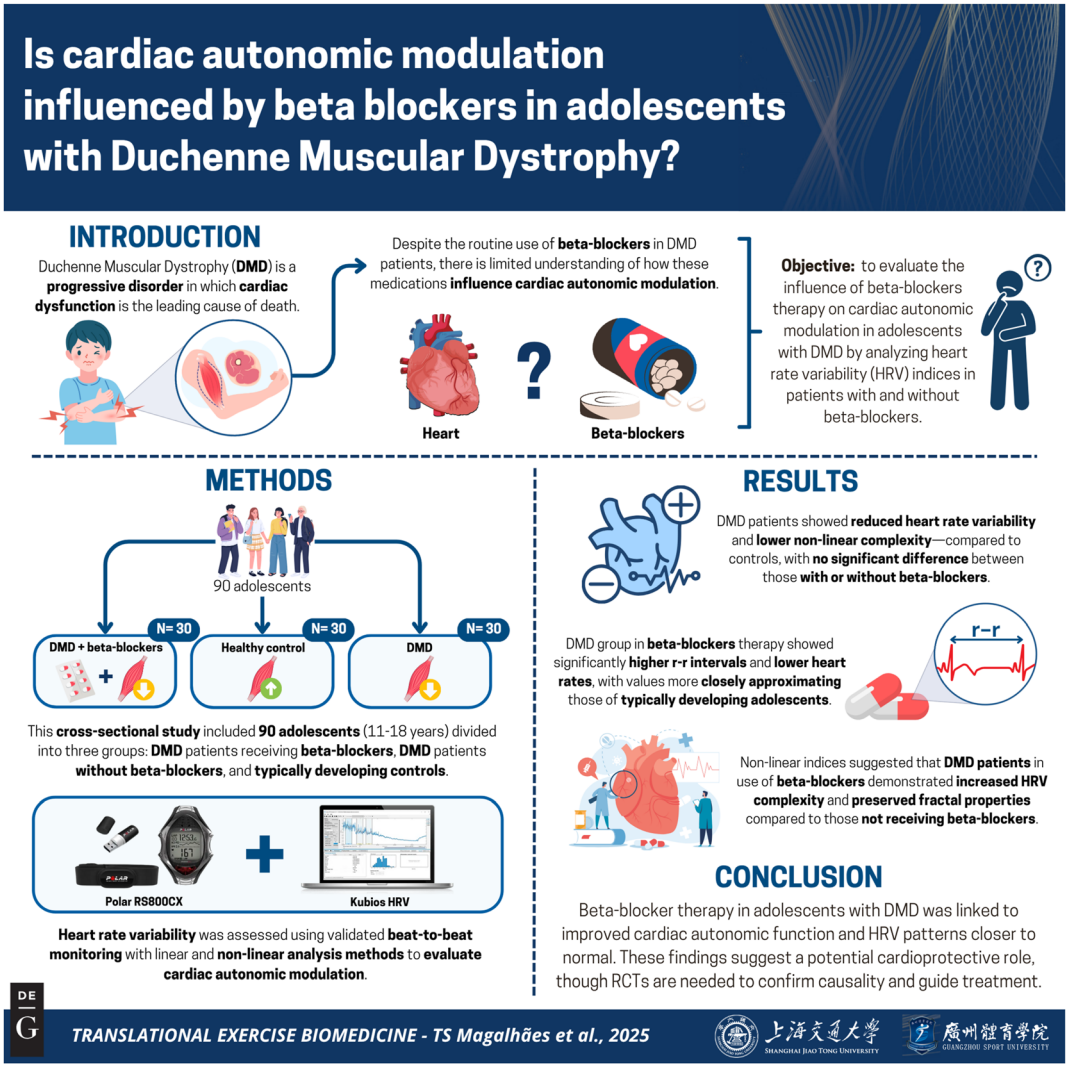

Figure 1: Graphical representation of this study. Key points: (1) The preservation of autonomic complexity in beta-blocker-treated patients may have implications for exercise prescription and physical therapy programs, as better autonomic regulation could indicate improved exercise tolerance and cardiovascular stability during physical activities. (2) Beta-blocker therapy was evaluated in 90 adolescents with DMD (ages 11–18), comparing cardiac autonomic function between treated and untreated patients vs. healthy controls using comprehensive heart rate variability analysis. (3) Beta-blocker therapy was associated with improved autonomic regulation and cardioprotective effects, suggesting HRV monitoring could serve as a clinical biomarker for treatment response and cardiovascular risk assessment in DMD patients, although longitudinal studies are crucial to establish causal relationships and optimize therapeutic strategies for cardiac autonomic dysfunction in DMD. Figure created with BioRender.

groups was carried out at the Brazilian Association of Muscular Dystrophy (ABDIM) and at the Neuromuscular Diseases Outpatient Clinic of the Federal University of São Paulo (UNIFESP). The protocol was applied during the assessment carried out in the follow-up outpatient visits.

Inclusion and exclusion criteria

The inclusion criteria for DMD groups required participants to have: (1) a confirmed diagnosis of Duchenne muscular dystrophy established through molecular methods and/or skeletal muscle protein expression analysis; (2) age between 11 and 19 years; (3) absence of cardiac arrhythmias and atrioventricular block; (4) no congenital anomalies including congenital heart disease or pulmonary malformations; and (5) no current use of antidiabetic medications. The inclusion criteria of the participants in the healthy control group we consider adolescents aged between 11 and 19 years, matched by age and sex to the DMD participants. They were required to have no history of neuromuscular, cardiovascular, pulmonary, or metabolic disorders, including diabetes mellitus assessed by an anamnese done by the experimenter. All participants had to be free from cardiac arrhythmias and congenital anomalies, and not taking any medications known to influence autonomic or cardiovascular function. Written informed consent was obtained from all participants' parents or legal guardians. As exclusion criteria, we consider errors in the analysis of HRV above 5 %.

Study design and data acquisition

For data collection, a form was used and filled out from the patient's medical record to obtain information about medications and age. For the assessment of patients' motor function, the Vignos scale was applied [17]; and to classify and assess the functionality of each patient, the Egen Klassification (EK) [18, 19] and Motor Function Measure (MFM) [20] scales were used.

The control of ambient temperature (temperature between 21 °C and 23 °C), humidity (humidity between 40 and 60 %) and the preparation of the equipment used were carried out before the arrival of the participants at the place destined for the tests. A minimum number of people was allowed to circulate around the room during the collections in order to reduce the anxiety of these participants.

The participants were oriented not to practice high physical effort, drink coffee or eat before the measurements. The capture strap was placed on the volunteers' chest and, in their wrist, the heart rate receiver (RS800CX, Polar), previously validated equipment for beat-to-beat heart rate capture and the use of their data for HRV analysis [21]. After placement of the strap and monitor, the participants were placed in dorsal decubitus and remained at rest breathing spontaneously for 25 min.

For data collection of HRV data in the dorsal decubitus position, the 15-min period with the greatest signal stability was selected. To verify signal stability was evaluated by calculating the percentage difference in the mean and standard deviation between consecutive segments of the time series, allowing to identify the most homogeneous portion for analysis [22].

The HRV acquisition protocol used was selected based on prior validation studies that demonstrated improved reliability and stability of nonlinear indices with longer recordings. While short-term recordings (5 min) are common for time and frequency domain metrics, nonlinear analyses – such as Detrended Fluctuation Analysis (DFA) and Symbolic Dynamics (SD) – require longer (25 min), artifact-free RR series for robust interpretation [23]. Additionally, the supine posture was chosen for its ability to minimize sympathetic activation and promote parasympathetic tone, thereby optimizing the detection of autonomic changes in a clinical population with limited mobility [24].

R-R interval data were collected using heart rate monitors (Polar), which apply a proprietary filtering process – specifically, the standard moderate filter with a 6-beat protection zone [22]. However, as this filtering may not fully remove ectopic beats, artifacts, or noise, visual inspection and manual correction were performed using Microsoft Excel to ensure data quality. In this manual process, artifacts and ectopic beats were eliminated without replacement [22]. Only series with more than 95 % sinus beats and containing 1,000 consecutive RR intervals were included in the analysis [19]. Kubios HRV software was also used for additional filtering – applying a medium threshold, as recommended for younger populations – and to calculate HRV indices [19].

HRV analysis was conducted using linear methods (time and frequency domains) and non-linear methods (chaos domain).

Heart rate variability

The linear indices of time and frequency domain were calculated using the Kubios software (v. 2.2, Kuopio, Finland). Time-domain analysis was performed by RMSSD which is Square root of the mean of squared differences between successive beat intervals (RR), PNN50 that correspond to the percentage of adjacent RR intervals that differ by more than 50 ms, SDNN that corresponds to the standard

deviation of all normal RR intervals, and also Mean RR and Mean HR [25–29].

In the frequency domain, the analysis included the spectral components of low frequency (LF: 0.04–0.15 Hz) and high frequency (HF: 0.15–0.40 Hz), measured in both milliseconds squared (ms^2) and normalized units (nu). The spectral assessment was conducted using the Fast Fourier Transform algorithm [25].

Non-linear methods were employed for HRV analysis, including Detrended Fluctuation Analysis (DFA) and Symbolic Dynamics (SD).

DFA quantifies the presence or absence of fractal correlation properties in RR intervals and has been validated using time series data. It calculates the root mean square fluctuation of the integrated and detrended time series, allowing for the detection of intrinsic self-similarity within non-stationary signals [30]. The DFA plot is not strictly linear; rather, it consists of two distinct regions separated by a transition point. These represent two scaling exponents: a short-term fractal exponent (α1), typically reflecting fluctuations over 4–11 beats (or sometimes 4–13), and a long-term exponent (α2) for fluctuations beyond 11 beats [31].

The SD Analysis estimates the complexity of sympathetic and parasympathetic modulation on cardiac autonomic control and is well-suited for analyzing short RR interval sequences. The RR intervals are grouped into symbolic patterns based on their sequential variation, classified into four categories [32]: 0V%: No variation (e.g., 4–4–4), 1V%: One variation (e.g., 4–4–2), 2LV%: Two like variations in the same direction (e.g., 1–2–4), 2UV%: Two unlike variations forming a peak or valley (e.g., 2–4–2). In addition, Shannon Entropy (SE) quantifies the complexity of these symbolic patterns [33, 34].

Data analysis

The dependent variables (RR Mean, HR Mean, SDNN, RMSSD, pNN50, LF, HF, LF/HF, alfa 1, alfa 2, alfa 1/alfa 2, Sham Entropy, 0V%, 1V%, 2LV% and 2ULV%) were submitted to a 3 (groups: DMDB, GDMD, TD group) factor Multiple Analysis of Variance (MANOVA). The Least Significant Difference (LSD) was used as a post-hoc test. The partial eta-squared (η_p^2) was reported to measure the effect size and interpreted as small (effect size>0.01), medium (effect size>0.06), or large (effect size>0.14) [35].

For the comparison between the groups of the characterization variables (Table 1), such as age, body mass, body height and BMI, one-way ANOVA was used. Student's *t*-test was used to compare the scales. Values of p<0.050 were considered significant. The software used was SPSS (Statistical Product and Service Solutions), 20.0 (Chicago, Illinois, USA).

Table 1: Characterization of the participants of the sample according to age, anthropometric variables and heart rate in the three groups (mean ± standard deviation), as well as motor function scales (mean ± standard deviation), cardiac medicines and use of corticoid in relative and absolute numbers in the DMD groups.

Variable	DMD with betablocker	DMD with no betablocker	Typical development
n	30	30	30
Age, years	15.0 ± 1.9	14.9 ± 2.0	14.8 ± 1.9
Height, m	1.53 ± 0.16	1.58 ± 0.12	1.67 ± 0.11*#
Weight, kg	54.69 ± 15.5	53.93 ± 16.9	55.74 ± 13.9
BMI ($kg\ m^{-1}$)	23.11 ± 4.51	21.41 ± 5.06	19.78 ± 3.21*
Motor scales	**DMD with betablocker**	**DMD with no betablocker**	
EK	9.3 ± 4.6[a]	10.9 ± 5.1[b]	
MFM-D1	16.1 ± 24.1	13.5 ± 24.1	
MFM-D2	72.3 ± 21.8	70.9 ± 25.2	
MFM-D3	80.6 ± 14.4	83.5 ± 14.4	
MFM-total	51.1 ± 19.1	50.3 ± 19.5	
Medicines	**n (%)**	**n (%)**	
Betablocker			
Carvedilol	23	0	
Metropolol	7	0	
None	0	30	
Total	30	30	
Ace inhibitors			
Enalapril	17	5	
Captopril	1	2	
None	12	23	
Total	30	30	
Corticoids			
Deflazacort	26	11	
Prednisolona	4	9	
None	0	10	
Total	30	30	
No medicines	0	10	

DMD, Duchenne Muscular Dystrophy; BMI, Body mass Index; kg, kilograms; $kg\ m^{-1}$, kilogram per meter; EK, Egen Klassifikation scale; MFM, motor function measurement scale, D1–D3, first to third domain of MFM, scale; n, number of patients. *p<0.05 typical developed group vs. DMD, group with betablocker; #p<0.05 typical developed group vs. DMD, group with no betablocker; [a]7 wandering participants and 23 wheelchair users; [b]8 wandering participants and 22 wheelchair users; ACE, inhibitors, angiotensin converting enzyme inhibitor.

Results

Participants characteristics

Initially 78 patients with DMD were recruited according to the inclusion criteria and 18 were excluded from the analysis for presenting an error above 5 % on Kubios software. The characterization of the sample is shown in Tables 1 and 2, in which the participants are matched by age between groups.

Table 2: Distribution of participants with DMD on the Vignos scale.

	Vignos scale	n (%)
1	Detectable change in posture or gait; climbs stairs without the aid of a handrail.	4 (7)
2	Walks, but climbs stairs only with the help of the handrail.	3 (5)
3	Walks, but climbs eight steps with assistance for more than 25 s.	2 (3)
4	Walks, but doesn't climb stairs.	4 (7)
5	Walks without external assistance, but does not climb stairs or get out of a chair.	2 (3)
6	Walks only with external assist (use of orthoses).	0 (0)
7	In a wheelchair. Sits upright, can touch the chair and is able to perform activities of daily living in bed or chair.	28 (47)
8	In a wheelchair. Sit upright, unable to perform activities in bed or chair without assistance.	16 (27)
9	In a wheelchair. Sit upright only with support. Can only perform minimal activities of daily living.	1 (2)
10	In bed, cannot perform activities of daily living without assistance.	0 (0)

n: number of patients in each item on the scale.

The weight was not statistically significant, however the height and BMI showed significant differences (TD group is taller than the participants of the two DMD groups, and TD group presented BMI significantly lower than those in the DMDB). There were no significant differences in motor scales.

Heart rate variability indices

Time and frequency domains

Considering the time domain indices, only the Mean RR was significantly higher in the TD group, in relation to the two DMD groups (Table 3). In the frequency domain, no significant differences were detected in any of the HRV indices between the three groups evaluated (Table 3).

Detrended fluctuations analysis (DFA) and symbolic dynamics (DS)

Considering DFA results, there was a significant difference between the TD group and GDMD, with the α2 ratio being higher (closer to 1) in the TD group when compared to the GDMD, while the α1/α2 ratio was higher in the GDMD. No differences were found in relation to the DMDB (Table 3).

For DS, all indices showed differences from the TD group to DMD, except for 1V%. SE values were significantly higher in the TD group when compared to the DMDB, but there was no difference when compared to the GDMD, the same was found in the 2LV% index.

The 0V% index was lower in the TD group both in relation to the DMDB and the GDMD, while in the 2UV% index the TD group presented higher values when compared to the two DMD groups. There were no significant differences between the DMD groups with and without beta-blockers.

Discussion

Our hypothesis was partially supported, as our main findings indicate that adolescents with DMD exhibit impaired cardiac autonomic modulation, reflected by reduced HRV and decreased complexity in nonlinear dynamics. Notably, beta-blocker therapy was associated with partial improvements in HRV parameters, with values in the DMDB group more closely approximating those observed in typically developing peers, which did not occur in the GDMD group. However, differences between the DMD groups (with and without beta-blockers) were not statistically significant. These findings suggest a potential modulatory effect of beta-blockers on autonomic function, though causal relationships cannot be established from this cross-sectional analysis.

Comparison between DMDB and GDMD

The comparison between DMD patients with and without beta-blockers (DMDB vs. GDMD) revealed significant differences in Mean RR and mean HR values, with the DMDB group showing higher Mean RR and lower mean HR. These findings are consistent with the known pharmacological effects of beta-adrenergic blockade, which typically results in heart rate slowing and longer RR intervals. This observed difference in autonomic parameters, although not leading to full normalization, aligns with previous studies demonstrating the effects of heart rate reduction [36].

Individuals with DMD experience marked dysfunction in cardiac autonomic regulation, characterized by reduced parasympathetic activity and heightened sympathetic dominance, which tends to worsen with advancing age and disease progression [32, 37]. In a review of patients with heart failure, Borovac et al. [33] highlighted that sympathetic nervous system overactivity, commonly seen in certain pathological conditions, is associated with impaired baroreceptor and chemoreceptor reflexes, increased neuronal and circulating catecholamine levels, diminished parasympathetic tone, and elevated sympathetic output to the heart, kidneys, and skeletal muscles. When these sympathoexcitatory effects persist over time, they contribute to the development and exacerbation of heart failure by

Table 3: Values of mean, standard error and confidence interval of Time Domain indices, Frequency Domain indices and non-linear indices (of the DFA and Symbolic Dynamic) of Heart Rate Variability on the three groups.

Indices	Group (1): DMD	Group (2): DMDB	Group (3): Control	Effect for groups (ANOVA)	POST-HOC – least significance difference (LSD)		
	M ± SE [CI]	M ± SE [CI]	M ± SE [CI]	F; p-Value; η_p^2 (DF=2, 84)	(DMD) × (DMDB)	(DMD) × (C)	(DMDB) × (C)
Mean RR, ms	638.3 ± 18.1 [602.3–674.3]	697.9 ± 17.8 [662.5–733.3]	768.1 ± 17.5 [733.3–802.9]	13.4; <0.001; 0.24	0.021	<0.001	0.006
Mean HR, bpm	95.4 ± 2.1 [91.2–99.6]	87.9 ± 2.1 [83.7–92.0]	80.5 ± 2.1 [76.4–84.6]	12.7; <0.001; 0.23	0.013	<0.001	0.014
SDNN, ms	43.8 ± 4.1 [35.6–52.0]	51.0 ± 4.1 [42.9–59.1]	59.9 ± 4.0 [52.0–67.9]	3.93; 0.023; 0.09	–	0.006	–
RMSSD, ms	28.7 ± 3.4 [21.8–35.5]	34.2 ± 3.4 [27.5–40.9]	42.8 ± 3.3 [36.2–49.4]	4.44; 0.015; 0.10	–	0.004	0.073*
pNN50, %	11.4 ± 2.9 [5.7–17.2]	15.1 ± 2.8 [9.5–20.8]	21.7 ± 2.8 [16.1–27.3]	3.38; 0.039; 0.07	–	0.012	–
LF ms^2	743.6 ± 167.2 [411.0–1,076.3]	939.9 ± 164.4 [613.0–1,266.8]	1,152.7 ± 161.6 [831.4–1,474.1]	–	–	0.082*	–
LF n.u.	66.8 ± 2.3 [62.3–71.3]	64.8 ± 2.2 [60.4–69.2]	59.3 ± 2.2 [55.0–63.6]	3.10; 0.050; 0.07	–	0.019	0.081*
HF ms^2	420.3 ± 120.5 [180.7–659.8]	531.8 ± 118.4 [296.4–767.2]	813.5 ± 116.4 [582.1–1,045.0]	2.96; 0.057*; 0.07	–	0.021	
HF n.u.	33.0 ± 2.2 [28.6–37.5]	35.1 ± 2.2 [30.7–39.4]	40.7 ± 2.2 [36.4–45.0]	3.26; 0.043; 0.07	–	0.016	0.073*
LF/HF	2.311 ± 0.216 [1.882–2.740]	2.149 ± 0.212 [1.727–2.570]	1.825 ± 0.208 [1.410–2.239]	–	–	–	–
A1	0.989 ± 0.031 [0.927–1.051]	1.022 ± 0.031 [0.961–1.083]	0.960 ± 0.031 [0.899–1.021]	–	–	–	–
A2	0.799 ± 0.018 [0.763–0.835]	0.828 ± 0.018 [0.793–0.864]	0.862 ± 0.018 [0.826–0.898]	3.06; 0.052; 0.07*	–	0.016	–
A1/A2	1.252 ± 0.043 [1.166–1.338]	1.252 ± 0.043 [1.167–1.337]	1.121 ± 0.043 [1.036–1.206]	3.10; 0.050; 0.07	–	0.035	0.033
SHAN ENTR	3.623 ± 0.070 [3.485–3.761]	3.764 ± 0.068 [3.628–3.900]	3.802 ± 0.068 [3.666–3.938]	–	–	0.070*	–
0V%	30.2 ± 2.0 [26.2–34.2]	26.5 ± 2.0 [22.6–30.4]	21.8 ± 2.0 [17.8–25.7]	4.48; 0.014; 0.10	–	0.004	–
1V%	46.5 ± 0.8 [44.8–48.1]	46.7 ± 0.8 [45.1–48.3]	45.6 ± 0.8 [44.0–47.2]	–	–	–	–
2LV%	9,1 ± 0.8 [7.4–10.7]	9.9 ± 0.8 [8.3–11.5]	12.2 ± 0.8 [10.5–13.8]	3.98; 0.022; 0.09	–	0.008	0.047
2ULV%	14.3 ± 1.6 [11.0–17.5]	16.9 ± 1.6 [13.7–20.1]	21.2 ± 1.6 [18.0–24.4]	4.78; 0.011; 0.10	–	0.003	0.058*

*Marginally significant results. M ± SE [CI], mean ± standard error [confidence interval]; TD, time domain; DMD, DMD, group with no betablocker; DMDB, DMD, group with betablocker; ANOVA, analysis of variance; RR, intervals, intervals between heart beats; HR, heart rate; bpm, beats per minute; SDNN, standard deviation of normal to normal RR, interval; RMSSD, the square root of the mean squared differences of successive normal to normal intervals; pNN50, the proportion derived by dividing the number of interval differences of successive NN, intervals greater than 50 ms by the total number of NN, intervals; FD, frequency domain; LF, low-frequency component, ranging between 0.04 and 0.15 Hz; HF, High-frequency component, ranging from 0.15 to 0.4 Hz; LF/HF, a ratio that reflects the absolute and relative changes between the sympathetic and parasympathetic components; nu, normalized units. α1, Short term component; α2, Long term component; α1/α2, Ratio between short- and long-term component; Shan Entr, degree of complexity of the distribution of samples of a signal; 0V%, percentual of no variations; 1V%, percentual of one variation; 2LV%, percentual of two equal variations; 2UV%, percentual of two different variations.

promoting cardiomyocyte apoptosis, maladaptive remodeling of the myocardium and vasculature, the occurrence of arrhythmias, and ultimately, a poorer prognosis [38].

Based on these mechanisms, we propose that beta-blocker therapy in patients with DMD may be associated with more favourable cardiovascular outcomes by

potentially mitigating autonomic dysfunction and its associated risks.

Although no statistically significant differences were observed in other HRV indices between the DMD groups, the DMDB group consistently demonstrated values that aligned more closely with those of typically developing adolescents compared to the GDMD group, which showed significant differences from the TD reference group (Table 3). While this cross-sectional observation cannot establish causation, it suggests that beta-blocker therapy may be associated with attenuated autonomic dysfunction in DMD, warranting further investigation through longitudinal studies.

Comparison with typically developing controls

When examining both DMD groups relative to TD participants as a reference for normal autonomic function, distinct patterns emerged. Participants with DMD, regardless of beta-blocker use, showed lower HRV and reduced complexity in nonlinear dynamics compared to healthy controls, consistent with previous literature on autonomic impairment in neuromuscular diseases.

Importantly, using the TD group as a reference point for normal physiological values revealed that the DMDB group exhibited HRV patterns that more closely approximated normal ranges compared to the GDMD group. In the linear analysis, the DMDB group demonstrated parasympathetic-related indices – such as RMSSD, pNN50, HF, and LF/HF ratio – as well as global HRV measures like SDNN and LF, with values that did not differ significantly from those observed in the TD reference group. This observation is clinically relevant given the established association between higher HRV and better cardiovascular prognosis.

Regarding non-linear analysis, DFA results indicated a loss of fractal properties (α2) in the long-term range for the GDMD group when compared to TD controls, whereas the DMDB group showed α2 values that did not differ significantly from the TD reference group. Since a reduction in α2 is associated with increased cardiac risk [32], the observation that DMDB values remained within the normal range suggests a potential protective association with beta-blocker therapy, though longitudinal studies are needed to confirm this relationship.

Shannon entropy values were higher (although not significantly) in the DMDB group, indicating greater complexity and variability of heart rate patterns. Given that higher entropy is associated with healthier autonomic regulation [34], this pattern suggests a possible beneficial association with beta-blocker therapy.

Symbolic dynamics analysis revealed lower sympathetic modulation (0V%) in the DMDB group, with values comparable to those observed in the TD reference group. However, the parasympathetic-related indices (2LV% and 2ULV%) differed significantly among all three groups, with the highest values recorded in the TD group and the lowest in the GDMD group.

Previous studies have shown that the symbolic dynamics indices 0V% and 2LV% may reflect shifts in the dynamic balance between sympathetic and vagal modulation in response to stress in healthy individuals [39, 40]. Therefore, this method may serve as a promising non-invasive tool for evaluating cardiac beta-adrenergic regulation, given its sensitivity to heart rate fluctuations mediated by the sympathetic nervous system [39–42].

Nevertheless, the interpretation of these results requires caution due to the cross-sectional nature of our study. While the DMDB group consistently showed HRV indices that approximated normal values more closely than the GDMD group, direct comparisons between DMD groups did not reach statistical significance. The pattern observed – where DMDB values frequently fell between GDMD and TD values – provides valuable clinical context but cannot establish causal relationships. We acknowledge that future studies with larger sample sizes and longitudinal designs are essential to better elucidate the true impact of beta-blockers on autonomic modulation in adolescents with DMD.

Clinical relevance

The clinical significance of these findings extends beyond statistical comparisons to practical implications for patient care. Our results suggest that beta-blocker therapy in adolescents with DMD may be associated with autonomic parameters that more closely approximate normal physiological ranges, potentially indicating improved cardiac autonomic regulation.

Specific Clinical Implications:

(1) Treatment Monitoring: The observed patterns suggest that HRV analysis, particularly nonlinear indices like DFA α2 and symbolic dynamics, could serve as non-invasive biomarkers for monitoring autonomic function in DMD patients receiving beta-blocker therapy. This could help clinicians assess treatment response beyond traditional echocardiographic parameters.
(2) Risk Stratification: Given that reduced HRV and altered fractal dynamics are associated with increased cardiovascular risk, the observation that beta-blocker-treated patients showed values closer to normal ranges may

indicate lower cardiovascular risk profiles. This information could inform clinical decision-making regarding the timing and intensity of cardiovascular interventions.

(3) Therapeutic Decision-Making: While our cross-sectional design cannot establish causation, the consistent pattern of improved autonomic parameters in the beta-blocker group provides preliminary evidence supporting current clinical practice guidelines that recommend beta-blocker therapy for DMD patients with cardiac involvement.

(4) Exercise and Rehabilitation Programs: The preservation of autonomic complexity in beta-blocker-treated patients may have implications for exercise prescription and physical therapy programs, as better autonomic regulation could indicate improved exercise tolerance and cardiovascular stability during physical activities.

This aligns with recent research by Oliveira et al. [43] and Dias et al. [32], showing that different pharmacological therapies, including corticosteroids, exert varying levels of cardiac protection. The application of nonlinear HRV analysis tools, traditionally used in exercise physiology, highlights the translational potential of these methods for monitoring disease progression and therapeutic responses in clinical populations with reduced mobility.

Future research directions

Longitudinal studies evaluating HRV changes before and after the initiation of beta-blocker therapy are crucial to establish causal relationships and optimize therapeutic strategies for cardiac autonomic dysfunction in DMD. Such studies would strengthen the evidence base for clinical decision-making and potentially establish HRV monitoring as a standard component of cardiovascular care in DMD patients.

Limitations

This study has several limitations. First, participants were taking additional medications such as ACE inhibitors and corticosteroids, which may have influenced HRV. Second, detailed information on beta-blocker dosage and duration was unavailable, limiting dose-response analysis. Third, while pubertal status can affect HRV, we minimized variability by selecting an age-matched sample (11–18 years) but did not directly assess Tanner stages. Fourth, although our artifact correction threshold (≤5 %) aligns with standard HRV guidelines, stricter thresholds (<3 %) could further improve signal quality. Fifth, although none of the participants were engaged in structured exercise programs, DMD participants routinely attended physiotherapy sessions (1–3 times/week), which could subtly affect autonomic outcomes despite no significant differences in motor function between groups. Finally, the cross-sectional design limits causal inference, and future longitudinal studies assessing HRV before and after beta-blocker initiation are needed to better understand therapeutic effects.

Conclusions

This study demonstrates that adolescents with Duchenne Muscular Dystrophy exhibit significant impairment in cardiac autonomic modulation, reflected by reduced heart rate variability and decreased nonlinear complexity. Although no statistically significant differences were found between DMD groups treated or not with beta-blockers, the group receiving beta-blocker therapy showed HRV patterns more similar to those of typically developing peers, suggesting a potential modulatory and protective effect on the autonomic nervous system.

Research ethics: The research project is approved by the Research Ethics Committee of the Federal University of São Paulo, under protocol CAAE 09942913.4.0000.5505 and registered at ClinicalTrials.Gov under number NCT04740554.
Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.
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