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ORIGINAL ARTICLE

Evaluation of cardiac autonomic function and low-grade inflammation in children with obesity living in the Northeast Brazilian region

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KEYWORDS

Children;
Obesity;
Heart rate variability;
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Abstract

Objective: Evaluate autonomic function and low-grade inflammation and characterize the correlation between these variables in schoolchildren with obesity living in the Brazilian northeast region.

Methods: 84 children with obesity and 41 with normal weight were included in this cross-sectional study. Anthropometry, body composition, blood pressure (BP), inflammatory biomarkers, and heart rate variability (HRV) indexes were analyzed in children aged 7 to 11 years.

Results: children with obesity had increased systolic ($p = 0.0017$) and diastolic ($p = 0.0131$) BP and heart rate ($p = 0.0022$). The children with obesity displayed significantly lower SDNN, RMSSD, NN50, HF (ms), HF (nu), SD1, SD2, and higher LF (ms), LF (nu), LF/HF, SD1/SD2, DFA- $\alpha 1$, and DFA- $\alpha 2$, compared to normal weight. A lower and higher capacity for producing IL-10 ($p = 0.039$) and IL-2 ($p = 0.009$), respectively, were found in children with obesity compared to children with normal weight. Although IL-2, IL-4 and IL17A did not correlate with HRV parameters, IL-6 was positively correlated with SDNN, LF (ms) and SD2, TNF- α was positively correlated with LF/HF and SD1/SD2 ratio, and IFN- γ was positively correlated with SDNN, RMSSD, NN50, LF (ms), HF (ms), SD1, and SD2.

Conclusions: The findings suggest that children with obesity have impaired autonomic function and systemic low-grade inflammation compared to children within the normal weight range, the

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inflammatory biomarkers were correlated with HRV parameters in schoolchildren living in the northeastern region of Brazil.

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1	Introduction	
2	Childhood obesity is a global health issue associated with	
3	infancy and lifelong adverse health consequences, including	
4	an increased risk of suffering or developing cardio-metabolic	
5	diseases. ¹ In Brazil, the prevalence of childhood obesity in	
6	the last three decades was 8.2%, increasing with age,	
7	decade, and in more developed regions. ²	
8	Traditional factors, such as insulin resistance, hypertension,	
9	and reduced high-density lipoprotein cholesterol (HDL-C) are	
10	linked to cardiovascular disease. However, the detection of the	
11	early stages of cardiac autonomic dysfunction and related	
12	causes could be a useful strategy to identify the presence of	
13	cardiovascular risk in childhood obesity, and heart rate variabil-	
14	ity (HRV) could be used as a warning sign of cardiac diseases. ³	
15	Autonomic nervous system (ANS) control of the heart is a	
16	dynamic process modulated by parasympathetic and sympa-	
17	thetic innervation. ⁴ RR-variation or HRV obtained from an elec-	
18	trocardiogram, Holter monitor or chest strap has been a	
19	commonly used and validated test to assess cardiac autonomic	
20	function through time, frequency, and non-linear domains indi-	
21	ces that quantify parasympathetic (RMSSD, NN50, HF, and	
22	SD1), and sympathetic (LF, LF/HF and SD2) modulation. ⁵	
23	Although an early study carried out with German children	
24	has not found significant differences in HRV parameters	
25	between children with normal weight and obesity, ⁶ cardiac	
26	autonomic dysfunction and reduced HRV has been reported	
27	in children with obesity. ^{7,8} Concomitantly, chronic low-grade	
28	inflammation and obesity have been reported in children. ⁹ In	
29	this condition, excess adipose tissue dysregulates the	
30	immune system, affecting the balance and levels of cyto-	
31	kines and increasing the levels of pro-inflammatory interleu-	
32	kin-6 (IL-6), tumor necrosis factor-alpha (TNF α), and C-	
33	reactive protein (CRP). ^{10,11}	
34	Regarding the association of autonomic impairment with	
35	chronic low-grade inflammation in children with obesity, The	
36	authors found only one cross-sectional study with a reduced	
37	sample size demonstrating that impaired HRV and low-grade	
38	chronic inflammation coexist in childhood obesity in the United	
39	States of America, but no significant relationships were found	
40	between any of the measured HRV variables and cytokines lev-	
41	els. ⁹ Considering that inflammation and autonomic function	
42	may be modulated by the environment and lifestyle and may	
43	have ethnic-specific implications, and the scarcity of studies	
44	linking inflammation and autonomic function in children, this	
45	study aimed to evaluate autonomic function and low-grade	
46	inflammation and to characterize the correlation between	
47	these variables in schoolchildren with obesity living in the	
48	Northeastern region of Brazil. The hypotheses tested are that	
49	children with obesity have lower HRV and higher low-grade	
50	inflammation compared to normal-weight children and that	
51	there is a positive association between autonomic dysfunction	
52	and chronic low-grade inflammation in children living in the	
53	Northeast region of Brazil.	
	Methods	54
	Ethical aspects	55
	This cross-sectional study was conducted in agreement with	56
	the declaration of Helsinki. The study was approved by a	57
	Human Research Ethics Committee (Health Sciences Center,	58
	Federal University of Paraiba – João Pessoa – PB – Brazil;	59
	Reference number protocol n° 4.676.103) and all procedures	60
	were conducted in agreement with Resolution 466/2012 of	61
	the National Health Council. All parents received informa-	62
	tion about the study and gave written informed consent	63
	before data collection.	64
	Participants and study design	65
	According to data from the Department of Education and	66
	Culture of the Municipality of João Pessoa (SEDEC), in 2020	67
	there were 23.861 students regularly enrolled in 97 primary	68
	public schools in the municipality of João Pessoa (PB, Brazil),	69
	distributed in the four geographic regions of the municipal-	70
	ity (north, south, east and west). To calculate the sample	71
	size, the following parameters were considered: the size of	72
	the reference population equal to 23.861 children from the	73
	1st to the 5th year of primary public schools, the prevalence	74
	of obesity outcome equal to 13.3%, ¹² 95% confidence inter-	75
	val, test power of 80%, and design effect (deff) equal to 1.	76
	Based on these parameters, the minimum size of the original	77
	sample was established at 76 children, a number that was	78
	plus 20% to compensate for losses and refusals, resulting in a	79
	final sample of 125 children.	80
	Children between 7 and 11 years of age, of both sexes,	81
	enrolled in public primary schools were included in the	82
	study, and recruitment and data collection were conducted	83
	in schools. Children were excluded if they presented any of	84
	the following: physical inability to perform the anthropo-	85
	metric measures, psychological or behavioral disturbances,	86
	use of any medication, or having a disease compromising any	87
	analysis.	88
	In the study, 125 children were allocated to two groups:	89
	84 children in the obesity (OB) group and 41 in the normal	90
	weight (NW) group. Children were evaluated for anthropo-	91
	metry, body composition, and cardiovascular parameters,	92
	evaluating blood pressure, HRV, and cytokines levels. All col-	93
	lections were performed at the school.	94
	Anthropometry and body composition	95
	The body weight was measured with an electronic scale	96
	(Omron [®] , HBF-514C) and height was assessed using a stadi-	97
	ometer (alturaexata [®]). Nutritional status, using body mass	98
	index for age (BMI/A) and sex according to WHO standards	99
	was determined using Anthon plus (version 1.0.4; WHO)	100

101 Children were classified in normal weight: ≥ -2 z-score \leq
 102 $+1$, overweight: $> +1$ z-score $\leq +2$, and obesity z-score $>$
 103 $+2$.

104 To measure the waist circumference, a flexible steel
 105 measuring tape with a scale of 0–200 cm and precision of
 106 0.1 mm (Sanny®) was used. Skinfolts were measured three
 107 times always on the right side of the body, using a scientific
 108 adipometer with a precision of 0.1 mm (Sanny®). The aver-
 109 age values were calculated and subsequently used to esti-
 110 mate the body fat percentage.

111 Blood pressure measurement

112 Blood pressure was measured with a digital sphygmomanom-
 113 eter (Omron healthcare® HBP-1100) validated for use in chil-
 114 dren by the Brazilian Society of Cardiology. After an initial
 115 stabilization period (5 min), three consecutive measure-
 116 ments were taken for each child at 1 min intervals, and the
 117 average values were used in the analysis. The measurement
 118 was performed with the child in a seated position and with
 119 an adequate cuff. In addition, the arm was free and posi-
 120 tioned at heart level, resting on a surface and with the palm
 121 facing up.

122 Heart rate variability analysis

123 It was recommended that children do not engage in vigorous
 124 physical activity or drink caffeinated beverages for at least
 125 24 h prior to HRV analysis. Recordings were made in the
 126 morning in a quiet room. A short-term recording was per-
 127 formed with the children at rest, in the supine position, and
 128 breathing normally at tidal volume. HRV analysis was per-
 129 formed in 10 min record, according to previous studies.¹³⁻¹⁵

130 A smartphone (Iphone12) was used to record the RR intervals
 131 on an APP (Elite HRV LLC, Asheville, NC, USA, and Release
 132 4.0.2, 2018). An elastic belt of adjustable size was posi-
 133 tioned comfortably around the participant's chest and a
 134 transmission device (Polar model H10, Polar Electro, Fin-
 135 land; 5 kHz transmission system coded with Owncodes, Polar
 136 Electro) was attached on the front at the level of the xiphoid
 137 process. The raw data recorded was exported as a text file
 138 and analyzed by Kubios HRV Standard software (version
 139 3.2.0; Biosignal Analysis and Medical Image Group, Depart-
 140 ment of Physics, University of Kuopio, Kuopio, Finland). The
 141 interpolation of the series was performed by cubic spline,
 142 and frequency values of 4 Hz were set. The R-wave time
 143 instants were automatically detected by applying a built-in
 144 QRS detection algorithm of Kubios software and when
 145 ectopic beats exceeded 5% of the total analyzed, the mea-
 146 surement was repeated. Early studies have demonstrated
 147 that smartphone apps and chest strap (Polar) provide excel-
 148 lent electrocardiogram compliance for all variables in the
 149 time domain, frequency domain, and nonlinear indexes.^{16,17}

150 The following measures of HRV analysis were determined; 1)
 151 time-domain parameters: the standard deviation between
 152 the duration of NN intervals (SDNN), the square root of the
 153 mean of the sum of the squares of the successive differences
 154 between adjacent normal-to-normal beats (RMSSD), adja-
 155 cent NN with differences in duration greater than 50 (NN50)
 156 low-frequency band (LF), high-frequency band (HF), stan-
 157 dard deviation of the instantaneous variability of continuous
 158 NN intervals in the Poincaré graph (SD1), standard deviation

of long-term continuous NN intervals (SD2) and detrended
 fluctuation analysis (DFA). 159 160

Blood samples and cytokines measurements 161

162 Blood samples were collected by a qualified nurse after 12 h
 163 fasting and without strenuous exercise in the last 24 h.
 164 Serum samples were used for the measurement of cytokines
 165 through the Cytometric Bead Array (CBA) technique. Th1/
 166 Th2/Th17 CBA kits from Becton Dickinson Biosciences were
 167 used to quantify the cytokines IL-2, IL-4, IL-6, IL-10, IL-17a,
 168 IFN- γ , and TNF- α . The Becton Dickinson (BD)™ CBA uses a
 169 series of particles (microspheres or beads) with different
 170 fluorescence intensities to simultaneously detect soluble
 171 cytokines through a capture surface. Each bead is conju-
 172 gated to a specific antibody and cytokines are detected by
 173 the fluorochrome phycoerythrin. The fluorescence intensity
 174 of each complex reveals the cytokine concentration (pg/
 175 mL). An Accuri C6 BD® flow cytometer was used, and analysis
 176 of CBA data was performed using FCPA 1.0.1 software.

Statistical analysis 177

178 The data normality was assessed by the Kolmogorov-Smirnov
 179 test. Descriptive statistics are presented as mean (standard
 180 deviation) or median (min-max). Comparison intergroup
 181 (NW and OB) was performed using Student's t-test in the
 182 case of the parametric data or the Mann-Whitney test for
 183 non-parametric data. The relationships between variables
 184 were analyzed using Pearson or Spearman correlation test
 185 according to data distribution. The statistical analysis was
 186 performed using GraphPad Prism® (version 6.01) and the sig-
 187 nificance was maintained at $p < 0.05$. An a posteriori power
 188 analysis was performed to assess differences between chil-
 189 dren with obesity and normal weight. Power was calculated
 190 using a two-sided 95% confidence interval, a prevalence of
 191 63.51% of children with obesity having a heart rate above
 192 the mean, a prevalence of 35.48% of children with normal
 193 weight having a heart rate above the mean, a prevalence
 194 ratio of 1.8; and a prevalence difference of 28.03. The sta-
 195 tistical power was performed using OpenEpi software (ver-
 196 sion 3.01) and the value obtained was 75.75%. Lastly, the
 197 effect size between-groups differences were calculated
 198 using Cohen's d test and classified as small ($d \geq 0.20$ and $<$
 199 0.50), medium ($d \geq 0.50$ and < 0.80), and large ($d \geq 0.80$).

Results 200

201 A total of 1.957 children were evaluated and classified
 202 according to BMI, with 1.009 males and 948 females. Of the
 203 total number of children assessed, 79 (4.04%) were classified
 204 as underweight, 1.249 (63.82%) as normal weight, 328
 205 (16.76%) as overweight, and 301 (15.38%) as obese. One hun-
 206 dred and twenty-five of these children were included in this
 207 study, 41 with NW and 81 with obesity.

208 The anthropometric and cardiovascular characteristics of
 209 children included in the study are presented in Table 1. The
 210 groups were similar in terms of sex ($p = 0.702$) and age
 211 ($p = 0.205$). The obesity group had higher BMI/A
 212 ($p < 0.0001$), waist-hip ratio (WHR) ($p < 0.0001$), body fat
 213 (BF) ($p < 0.0001$), SBP ($p = 0.0017$), DBP ($p = 0.0131$), and

Table 1 Anthropometric and cardiovascular characteristics of children with normal weight (NW) and obesity (OB).

Variables	NW (n = 41)	OB (n = 84)	p-value	Effect size Cohen's d
Male – n (%)	17 (41.5)	39 (46.4)	0.702	
Female – n (%)	24 (58.5)	45 (53.6)		-
Age (years)	9.6 (1.2)	9.2 (1.3)	0.205	-0.31
BMI/A (z-score) †	-0.18 (-1.53–1.00)	2.70 (2.01–5.66)	< 0.000	4.13
WHR	0.82 (0.04)	0.88 (0.06)	< 0.000	1.10
BF (%) †	21.30 (13.70–34.50)	36.60 (22.75–68.20)	< 0.000	2.30
SBP (mmHg)	103.0 (10.44)	110.7 (13.05)	0.001	0.62
DBP (mmHg) †	58.50 (43.00–85.00)	62.50 (40.00–94.00)	0.013	0.50
HR (bpm)	87.0 (65.0–111.00)	95.0 (63.00–121.00)	0.002	0.67

Values are expressed as n (%), mean (SD) or median (min-max), and applied chi-square, T test or † Mann–Whitney, respectively.

BMI/A, Body mass index for age; WHR, waist hip ratio; BF, body fat; SBP, Systolic blood pressure; DPB, Diastolic blood pressure; HR, heart rate.

214 heart rate (HR) ($p=0.0022$) when compared to the NW
215 group, with the medium effect size for SBP, DBP and HR.

216 As reported in [Table 2](#), children with obesity had
217 increased IL-2 levels ($p=0.009$) and decreased IL-10 levels
218 ($p=0.039$) when compared to the NW group ([Table 2](#)), with a
219 medium effect size for the IL-2 and small effect size for the
220 IL-10. Serum levels of IL-4, IL-6, IL-17A, TNF α , and IFN- γ
221 were similar between groups ([Table 2](#), $p > 0.05$).

222 Regarding the HRV analysis, children with obesity had a
223 predominance of sympathetic ANS in the detriment of para-
224 sympathetic. In the time domain, children with obesity had
225 decreased SDNN ($p=0.002$), RMSSD ($p=0.001$), and NN50
226 ($p=0.008$) compared to the NW children, with a larger
227 effect size for SDNN and RMSSD ([Table 3](#)). In time-frequency,
228 children with obesity had increased LFnu band ($p=0.001$)
229 and LF/HF ratio ($p=0.003$) and decreased HFnu band
230 ($p=0.004$) compared to NW children, with the medium
231 effect size for the three variables ([Table 3](#)). In non-linear
232 indices, children with obesity had decreased SD1 ($p < 0.000$)
233 and SD2 ($p=0.004$), and increased SD2/SD1 ratio ($p=0.001$),
234 DFA- α 1 ($p=0.002$), and DFA- α 2 when compared to NW chil-
235 dren, with medium effect size for SD2/SD1 and DFA- α 1 and
236 larger effect size for DFA- α 2 ([Table 3](#)).

237 Correlations between HRV and inflammatory variables are
238 shown in [Table 4](#). Although IL-2, IL-4, and IL17A did not cor-
239 relate with HRV parameters, IL-6 showed a positive

240 correlation with SDNN, LF, and SD2, and IL-10 with LF (ms).
241 TNF- α was positively associated with LF/HF ratio and SD1/
242 SD2 ratio. IFN- γ showed a positive correlation with SDNN,
243 RMSSD, NN50, LF, HF, SD1, and SD2.

Discussion

244
245 This study identified that children with obesity had higher
246 levels of SBP, DBP, HR, and higher serum levels of IL-2 and a
247 lower serum level of IL-10 than NW children. The children
248 with obesity also had autonomic dysfunction, as observed in
249 linear and non-linear indices. The authors also found a posi-
250 tive correlation between HRV indices and inflammatory bio-
251 markers, suggesting that autonomic and inflammatory
252 dysfunction may occur simultaneously in children.

253 Some important variables related to obesity and cardio-
254 vascular disease were also different in these children, such
255 as the increase in SBP, DBP, and HR. There is strong evidence
256 of greater sympathetic activation in obesity, including
257 changes in resting BP and HR, which is consistent with the
258 results found in this study in children with obesity. It is
259 important to highlight that, despite the existing differences
260 according to nutritional status, the values of BP and HR were
261 within the normal limits proposed by the Brazilian Guide-
262 lines of Hypertension and III Guidelines of the Brazilian

Table 2 Comparison of the values of cytokines between children with normal weight (NW) and obesity (OB).

Variables	NW (n = 41)	OB (n = 84)	p-value	Effect size Cohen's d
IL-2 (pg/mL) †	7.15 (0.78–13.95)	8.34 (0.50–39.59)	0.009	0.63
IL-4 (pg/mL) †	3.41 (0.43–11.90)	3.73 (0.33–28.92)	0.734	0.17
IL-6 (pg/mL) †	10.14 (0.05–80.32)	9.82 (0.21–95.43)	0.794	0.16
IL-10 (pg/mL) †	5.22 (0.82–14.94)	2.70 (0.17–14.80)	0.039	-0.41
IL-17A (pg/mL) †	52.69 (2.32–111.6)	50.12 (2.97–157.70)	0.808	0.02
TNF- α (pg/mL) †	4.14 (0.15–10.64)	4.62 (0.11–29.31)	0.591	0.15
IFN- γ (pg/mL) †	9.74 (1.59–15.38)	7.40 (0.23–34.18)	0.101	-0.08

Values are expressed as median (min-max). Mann-Whitney test.

IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; IL-10, interleukin-10; IL-17A, interleukin-17A; TNF- α , tumor necrosis factor- α ;
IFN- γ , interferon- γ .

Table 3 Assessment of heart rate variability in children with normal weight (NW) and obesity (OB).

Variables	NW (n = 41)	OB (n = 84)	p-value	Effect size Cohen's d
Time domain				
SDNN (ms) †	47.9 (20.8–119.8)	37.9 (12.7–84.9)	0.002	–0.82
RMSSD (ms) †	42.5 (15.3–140.6)	30.1 (7.2–92.5)	0.001	–0.89
NN50 (beats) †	151.0 (4.0–459.0)	76 (0.0–357.0)	0.008	–0.69
Frequency domain				
LF (ms ²) †	1000 (58.0–4435)	650.0 (100.0–3555)	0.0053	–0.67
HF (ms ²) †	942.0 (107.0–7470)	383.0 (18.0–2833)	0.0005	–0.89
LF (nu)	50.9 (15.7)	60.53 (13.8)	0.001	0.67
HF (nu)	47.6 (13.6)	39.4 (13.8)	0.004	–0.59
LF/HF †	1.105 (0.417–7.762)	1.509 (0.295–5.908)	0.003	0.68
Non-linear measurements				
SD1 (ms) †	30.1 (10.8–99.5)	21.3 (5.1–65.5)	< 0.000	–0.90
SD2 (ms) †	57.2 (25.5–137.3)	48.3 (17.3–100.2)	0.004	–0.77
SD2/SD1 (ms) †	1.77 (1.03–3.55)	2.15 (1.22–3.57)	0.001	0.61
DFA- α 1	1.02 (0.21)	1.15 (0.20)	0.002	0.65
DFA- α 2	0.45 (0.14)	0.57 (0.13)	< 0.000	0.90

Values are expressed as mean (SD) or median (min-max); T test or † Mann–Whitney, respectively.

SDNN, standard deviation between NN intervals; RMSSD, square root of the mean of the square of the differences between consecutive NN intervals; NN50, adjacent NN with differences in duration greater than 50; LF, low frequency; HF, high frequency; SD1, standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph; SD2, standard deviation of long-term continuous NN intervals; DFA, detrended fluctuation analysis; ms: milliseconds; nu: normalized units.

263 Society of Cardiology on Analysis and Issue of Electrocar-
264 diographic Reports, respectively.¹⁸⁻²⁰

265 Inflammation in obesity occurs due to functional changes
266 in adipose tissue, resulting in the secretion of pro-inflamma-
267 tory cytokines produced by activated macrophages residing
268 in adipose tissue, and decreased secretion of protective adi-
269 pokines, such as adiponectin.²¹ The mechanisms underlying
270 this dysfunction occur in response to the structural changes
271 in adipose tissue and may involve endoplasmic reticulum
272 stress, hypoxia, and cellular senescence.²¹ Chronic low-
273 grade inflammation results from a persistent failure to orga-
274 nize the inflammatory response. The interaction of

275 macrophages and monocytes and other inflammatory media-
276 tors has been studied in the pathogenesis of obesity.²²

277 This study was the first to evaluate a broad cytokine pro-
278 file in children and showed that although IL-4, IL-6, IL17A,
279 TNF- α and IFN- γ were similar between groups, children with
280 obesity had increased serum levels of IL-2 and decreased
281 serum levels of IL-10 compared to NW children, suggesting
282 that children with obesity may have a loss of ability to regu-
283 late the monocyte-mediated immune response to produce
284 cytokines.²³ Physical inactivity can, among other things,
285 lead to obesity, impair immune system function, and
286 increase the risk of low-grade chronic inflammation. A

Table 4 Linear correlation coefficients between heart rate variability measures and inflammatory variables.

Variables	IL-2	IL-4	IL-6	IL-10	IL-17A	TNF- α	IFN- γ
SDNN (ms) †	0.161	0.071	0.246*	0.206	0.033	0.134	0.401*
RMSSD (ms) †	0.148	0.086	0.161	0.125	–0.044	0.015	0.299*
NN50 (ms) †	0.116	0.118	0.115	0.071	–0.043	0.010	0.229*
LF (ms) †	0.0856	0.0853	0.2924*	0.2317*	0.1037	0.1957	0.4713*
HF (ms) †	0.1147	0.0600	0.1798	0.1528	–0.0111	0.0061	0.3163*
LF (nu)	–0.012	0.024	0.088	0.048	0.155	0.166	0.113
HF (nu)	0.006	–0.026	–0.089	–0.041	–0.149	–0.167	–0.106
LF/HF †	–0.023	–0.010	0.034	0.023	0.115	0.239*	0.024
SD1 (ms) †	0.134	0.083	0.161	0.136	–0.041	0.010	0.308*
SD2 (ms) †	0.151	0.062	0.256*	0.217	0.052	0.167	0.425*
SD1/SD2 (ms) †	–0.071	–0.034	0.029	0.019	0.173	0.219*	–0.024
DFA- α 1	–0.030	0.036	0.118	0.077	0.162	0.196	0.139
DFA- α 2	0.033	–0.037	–0.068	–0.093	0.010	–0.006	–0.177

SDNN, standard deviation between NN intervals; RMSSD, square root of the mean of the square of the differences between consecutive NN intervals; NN50, adjacent NN with differences in duration greater than 50; LF, low frequency; HF, high frequency; SD1, standard deviation of the instantaneous variability of continuous NN intervals in the Poincaré graph; SD2, standard deviation of long-term continuous NN intervals; DFA, detrended fluctuation analysis; ms: milliseconds; nu: normalized units.

† = nonparametric data.

287 higher concentration of IL-2-producing cells and a lower
288 capacity to produce IL-10 have previously been reported in
289 sedentary children compared to active children.²⁴ It has
290 been demonstrated that obesity alters immune function and
291 that the imbalance between pro- and anti-inflammatory
292 mediators in obesity may induce the establishment or pro-
293 gression of cardiovascular complications and
294 dysautonomia.^{10,25}

295 HRV is a measure of the natural changes occurring
296 between beats in a row in heart rate, which represents the
297 number of heart beats per minute.²⁶ The HRV measurement
298 must be carried out on normal cardiac cycles (N), without
299 artifacts and ectopic beats. For this reason, The authors
300 used the denomination N-N interval and not the R-R interval
301 in this study.⁵ An optimal HRV level reflects adequate func-
302 tioning, characterized by an inherent capacity for self-regu-
303 lation, adaptability, or resilience. In contrast, a very small
304 variation indicates age-related system depletion, chronic
305 stress, pathology, or improper functioning at various levels
306 of self-regulatory control systems.²⁶

307 HRV analysis using linear and non-linear methods has been
308 used to better understand complex and dynamic heart
309 rhythms. In 2010, cross-sectional studies conducted in Bra-
310 zilian children with obesity aged between eight and 12 years
311 showed a reduction in non-linear HRV measures and
312 decreased LF and HF indices, suggesting a reduction in both
313 sympathetic and parasympathetic activities.^{27,28} Another
314 study conducted in Brazilian children showed increased LFnu
315 band and LF/HF ratio in children with obesity when com-
316 pared to children within the normal weight range but with-
317 out changes in non-linear HRV measures.⁸

318 The present study showed that children with obesity had
319 decreased HRV measures in the time domain, increased LFnu
320 and LF/HF ratio in the frequency domain, and increased
321 SD2/SD1 and DFA- α 1 and DFA- α 2 in non-linear measures. The
322 results may indicate unresolved cardiac stress characterized
323 by parasympathetic withdrawal and sympathetic hyperacti-
324 vation in obese children.²⁹ Although it is not possible to
325 determine the underlying causes involved in the fluctuations
326 in HRV variables in children with obesity, it is reasonable to
327 suggest that lifestyle changes over the last decade, particu-
328 larly evidenced by the increase in consumption of ultra-
329 processed and hypercaloric foods, associated with reduced
330 physical activity, may have played a key role in altering HRV
331 indices. This reaffirms the importance of the present study,
332 which is essential to understand and identify the potential
333 risk of autonomic dysfunction in children, as well as to pro-
334 pose effective intervention strategies to prevent cardiovas-
335 cular disease later in life.

336 Regarding the contribution of hormonal, reflex, meta-
337 bolic, endothelial, and inflammatory mechanisms in cardio-
338 vascular homeostasis mediated by the autonomic nervous
339 system,¹⁸ the main mechanisms to explain alterations of the
340 ANS in obesity are sympathetic overactivity, dysfunction of
341 arterial baroreceptor properties, a key mechanism involved
342 in the control of sympathetic and vagal tonus,¹⁸ and
343 impairment of the respiratory-cardiovascular coupling,
344 which may result in sympathetic activity and autonomic
345 dysfunction.¹⁸

346 The positive correlations found between HVR indices in
347 the time, frequency, and non-linear domains with bio-
348 markers of inflammation are consistent with available

evidence regarding the association between inflammation 349
and ANS activity.^{18,30} An early study found a positive associa- 350
tion between HRV and pro-inflammatory markers, although a 351
meta-analysis had shown that a negative correlation 352
between HRV and pro-inflammatory markers is more com- 353
mon.³⁰ In this sense, an important point to highlight is the 354
fact that vagal hyperactivity is positively correlated with 355
first-response inflammatory mediators, such as TNF- α and 356
IFN- γ , through the elimination of the parasympathetic 357
nervous system via acetylcholine, which may partially explain 358
the positive correlation of RMSSD, NN50, HF and SD1 with 359
IFN- γ . On the other hand, the positive correlation of LF and 360
SD2 with IL-6 indicates a slower action, characteristic of the 361
sympathetic nervous system via epinephrine and norepi- 362
nephrine. Furthermore, parameters that reflect total HRV 363
(e.g., SDNN) are influenced by both mechanisms, which 364
explains the positive correlation with IL-6 and IFN- γ .³⁰ 365

366 This study contributes to a better understanding of dysau- 366
tonomia and low-grade chronic inflammation in children, 367
demonstrating the coexistence of these conditions in chil- 368
dren with obesity from the Northeast region of Brazil. The 369
positive correlation between HRV indices and inflammation 370
provides valuable information and encourages mechanistic 371
studies to better elucidate this issue. In addition, this study 372
may help to establish reference values for HRV indices in 373
children, which could be an essential screening tool for car- 374
diovascular risk in health outpatient clinics. 375

376 This study has some limitations. First, the cross-sectional 376
design allows only the study of associations between varia- 377
bles, not causal relationships. Second, HRV and inflamma- 378
tory biomarkers may differ by sex, and stratification by sex 379
could be examined in further studies; and third, a broad 380
assessment of metabolic variables panel could help to better 381
understand the cardiometabolic risk in children with obesity 382
in a post-pandemic scenario. These limitations will be 383
addressed in future studies. 384

385 Conclusion

386 The results of this study show several cardiovascular risk fac- 386
tors in obese children compared to children in the normal 387
weight range. At such a young age, obesity is already associ- 388
ated with lower HRV and low-grade chronic inflammation, 389
which reflects and reinforces the need to develop effective 390
strategies to prevent and combat childhood obesity. Further- 391
more, the correlations between inflammatory markers and 392
HRV indices underscore the importance of elucidating the 393
mechanisms by which such interactions may occur. 394

395 Conflicts of interest

396 The authors declare no conflicts of interest. 396

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