Association between objectively measured physical activity and heart rate variability in healthy adults from primary health care

Asociación entre actividad física medida objetivamente y variabilidad de la frecuencia cardíaca en adultos sanos de atención primaria de salud

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Abstract

Objective: To assess the association between daily physical activity and heart rate variability in adults without established cardiovascular disease enrolled in a primary health care unit.
Methods: This cross-sectional study included 197 apparently healthy individuals (mean age 47 ± 13 years; 58% female) enrolled in a primary health care unit. Heart rate variability indices (time domain, frequency domain, and nonlinear indices) were derived from 5-minute resting RR-interval recordings while participants breathed at 12 breaths per minute. Daily physical activity was recorded over 7 consecutive days using accelerometers, with data classified into sedentary time, light, and moderate-to-vigorous physical activity. Correlation and multivariate linear regression analyses examined associations between daily physical activity and heart rate variability indices.
Results: Sedentary time and light physical activity were not associated with any heart rate variability index. Adjusting for age, sex, and resting heart rate, moderate-to-vigorous physical activity was inversely correlated with the low- to high-frequency power ratio (r^2= -0.18). Moderate-to-vigorous physical activity (β= -0.14), along with sex (β= 0.32), waist circumference (β= 0.14), and age (β= 0.05), was an independent predictor of the low-to-high frequency power ratio, explaining 2.3% of its variance.
Conclusion: Although a cardioprotective effect of moderate-to-vigorous physical activity on heart rate variability was not definitively demonstrated, an inverse association was found with the low-to-high frequency power ratio. Sedentary time and light activity, however, were not associated with cardiac autonomic function.

Keywords

Autonomic function; cardiovascular risk factors; hear rate variability; lifestyle; physical activity; primary health care.

Resumen

Objetivo: Evaluar la asociación entre actividad física diaria y variabilidad de la frecuencia cardíaca en adultos sin enfermedad cardiovascular establecida inscritos en una unidad de atención primaria de salud.
Métodos: Estudio transversal en 197 individuos aparentemente sanos (47±13 años; 58% mujeres), inscritos en una unidad de atención primaria. Los índices de variabilidad de frecuencia cardíaca (dominio del tiempo, frecuencia y no lineales) se derivaron de registros de intervalos RR de 5 minutos en reposo. La actividad física diaria se registró durante 7 días consecutivos con acelerómetros, clasificando los datos en tiempo sedentario, actividad ligera y actividad moderada-vigorosa. Se realizaron análisis de correlación y regresión lineal multivariante para examinar asociaciones entre niveles de actividad diaria y variabilidad de frecuencia cardíaca.
Resultados: El tiempo sedentario y la actividad ligera no se asociaron con ningún índice de variabilidad de frecuencia cardíaca. Tras ajustes por edad, sexo y frecuencia cardíaca en reposo, la actividad física moderada-vigorosa se correlacionó inversamente con la razón de potencia de baja-alta frecuencia (r^2= -0.18). La actividad física moderada-vigorosa (β= -0.14), el sexo (β= 0.32), la circunferencia de la cintura (β= 0.14) y la edad (β= 0.05) fueron predictores independientes de esta razón, explicando el 2.3% de su varianza.
Conclusión: Aunque no se demostró claramente un efecto cardioprotector de la actividad física moderada-vigorosa sobre la variabilidad de la frecuencia cardíaca, se observó una asociación inversa con la razón de potencia de baja-alta frecuencia. El tiempo sedentario y la actividad ligera no se asociaron con la función autonómica cardíaca.

Palabras clave

Atención primaria de salud; estilo de vida; factores de riesgo cardiovascular; función autonómica; variabilidad de la frecuencia cardiaca.

Introduction

Cardiovascular diseases (CVD) remain the primary cause of global mortality and a significant factor in the onset of disability (Roth et al., 2020). Heart rate variability (HRV) is an indicator of intrinsic cardiac autonomic function (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). HRV reflect the modulation of both the sympathetic and parasympathetic branches of the autonomic nervous system on the sinus atrial node (Shaffer et al., 2014; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Impaired autonomic function is a sign of cardiac electrical instability that augments the risk of cardiovascular events (Tsuji et al., 1996). In this context, reductions in resting HRV have been associated with a 32% to 45% increased risk of a first cardiovascular event (Hillebrand et al., 2013) and up to a 27% higher risk of sudden death in adults (Maheshwari et al., 2016). It has been observed that low HRV indicates a shift toward sympathetic predominance and parasympathetic reduction (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Engaging in regular physical activity (PA) is highly recommended for the prevention and treatment of cardiovascular disease (Strath et al., 2013). Appropriate amounts of moderate-to-vigorous PA (MVPA) are related to cardiovascular health benefits (Nagata et al., 2022). Conversely, elevated levels of physical inactivity and sedentary behavior have been associated with a higher incidence of CVD and an increased risk of mortality across all causes (Bakrania et al., 2016; Ekelund et al., 2019; Katzmarzyk et al., 2019). Nonetheless, high levels of moderate-to-vigorous PA (MVPA) seem to have the capacity to mitigate or even counteract this deleterious effect, particularly in individuals who are less active (del Pozo-Cruz et al., 2018; Knaeps et al., 2018; Stamatakis et al., 2019).

There is an abundance of literature on PA and its effects on HRV, both in terms of exercise interventions (Amekran & El Hangouche, 2024; El-Malahi et al., 2024) and large-scale cohort studies (Felber et al., 2008; Soares-Miranda et al., 2014; Soares-Miranda et al., 2012). Generally, it is established that appropriate levels of MVPA or aerobic exercise training are associated with improved cardiac autonomic modulation (Alansare et al., 2022; Felber et al., 2008; Soares-Miranda, Sandercock, et al., 2012). Despite that, the impact of lower-intensity PA, such as sedentary time and light PA, on cardiac autonomic function is not clearly established. Furthermore, most studies assessed PA through self-reporting questionnaires (Felber et al., 2008; Garet et al., 2005; Soares-Miranda et al., 2014), which have been reported to overestimate total PA and time spent at different levels of intensity, and therefore represent a methodological constraint (Dyrstad et al., 2014). Few studies have utilized objective methods, such as accelerometers, to assess PA. The findings from these investigations have shown heterogeneity in their results related to HRV (Hallman et al., 2019; Niemelä et al., 2019; Spina et al., 2019). The implementation of these objective assessment methods could enhance result accuracy (Buchheit et al., 2005; Soares-Mirandaet al., 2012).

Clinical trials have demonstrated that low-grade inflammation (Sloan et al., 2007; Soares-Miranda, Negrao, et al., 2012) and metabolic risk factors (Koskinen et al., 2009; Soares-Miranda, Sandercock, et al., 2012) are independent predictors of a less favorable HRV profile, which justify the consideration of those variables as possible confounders in the relationship between PA and HRV. Furthermore, PA can also modulate the association between “inflammation and HRV” (Soares-Miranda, Negrao, et al., 2012) and “metabolic risk factors and HRV,” (Soares-Miranda, Sandercock, et al., 2012) making itself a confounder and not an independent predictor.

Based on the background, the main purpose of this study was to investigate the extent to which different intensities of daily PA are associated with HRV in healthy adults.

Method

Study Design

This cross-sectional study was conducted in a Portuguese primary health care center (Porto, Portugal). The initial appointment included information on socio-demographics; pre-existing clinical conditions

and medications; determination of smoking status; and measurements of anthropometrics, blood pressure and HRV; as well as delivery of accelerometers. Within 7 days, participants underwent a second appointment after a fasting overnight for blood collection and to return the accelerometers.

Participants

Participants, both men and women, were recruited from a database of 8000 individuals. Inclusion criterion was age ≥18 and ≤65 years old. Exclusion criteria were an established diagnosis of cardiovascular disease or cognitive disorders, neurological and orthopedic impairments, arrhythmias, severe hypertension (systolic blood pressure higher than 180 mmHg or diastolic blood pressure higher than 100 mmHg), acute coronary syndromes and peripheral arterial disease, thyroid disorders, severe pulmonary and renal disorders, or infectious and chronic immunological diseases. First, an age filter was applied to the clinical file database, leaving 4,600 individuals (57.5%) aged between 18 and 65 years. From this filtered group, a simple random sampling technique was used to select 1,200 individuals, divided into six sets of 200 participants each. These individuals were then invited to participate via phone calls. The study was approved by the local ethical commission (Administração Regional de Saúde do Norte (25/2010). All procedures were conducted according to the declaration of Helsinki, and participants signed informed consent to participate.

Instrument and measurements

Anthropometry

For anthropometrics, participants wore light clothing without shoes. Height (m) was assessed using a standard wall-mounted stadiometer and weight (kg) using a scale (Tanita, Inner Scan BC-522, Tokyo, Japan). Body mass index (kg/m2) was thereafter calculated. Waist circumference (cm) was measured at the midpoint between the lowest rib and the iliac crest, with the participant standing and arms hanging freely, following the expert recommendations of the World Health Organization (World Health Organization, 2011).

Heart rate variability and blood pressure

For HRV assessment, participants were instructed to refrain from strenuous exercise and to avoid consuming caffeine-containing products or alcohol for at least 24 hours before evaluation. The HRV assessment was made at rest in a quiet, semi-dark room with an average temperature of 21º C. Recording of R-R interval data was performed using the Polar RS800CX (Polar Electro OY, Kempele, Finland) with a temporal resolution of 1ms (Nunan et al., 2009). The HRV assessment methodology was conducted according to the procedures described by Oliveira et al. (2014). In brief, assessments were performed in the supine position, controlling the breathing rate by matching it to a metronome-paced frequency of 12 breaths/min. After 20 min of recording, R-R interval data were downloaded into Polar Precision Performance Software SW (Polar Electro OY, Kempele, Finland). Using the Kubious Software 2.0 for Windows (The Biomedical Signal Analysis Group, University of Kuopio, Finland) ectopic beats or arrhythmias were excluded and R-R data were de-trended (Tarvainen et al., 2002) and resampled at 4Hz. Finally, the last 5 minutes of recording were selected and used for calculating HRV indices. Time domain indices included standard deviation of the R-R interval (SDNN) and the square root of the mean of the squares of successive R-R interval differences (RMSSD). SDNN reflects global variability and RMSSD is liked to vagal activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Frequency domain indices were determined using the non-parametric method (Fast Fourier transform) and encompassed very low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0.04-0.15 Hz), high frequency (HF, 0.15-0.4 Hz). Absolute LF (ms2), HF (ms2) and the ratio LF/HF were used as frequency domain variables. LF is a doubtful measurement of sympathetic activation of the heart , while HF is related with vagal activity (Shaffer et al., 2014; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). It has been described that the LF/HF ratio might indicate sympatho-vagal interaction. An increase in the ratio could signify a shift toward sympathetic dominance, while a decrease might suggest parasympathetic dominance (Lombardi & Stein, 2011; Shaffer et al., 2014).
Nonlinear HRV indices were (i) short-term fractal-scaling exponent (DFA1) and (ii) the Poincare ratio (SD12), which is the ratio between the short (SD1) and the long (SD2) diameter ellipses. Nonlinear indices represent complexity measures of biological signals and seem to encompass both short-term

and long-term variability (Golińska, 2013; Lombardi & Stein, 2011; Shaffer et al., 2014). The mean resting heart rate was also calculated as part of this evaluation.
Three blood pressure measurements were made in the left arm using a Colin model BP 8800 monitor (Critikron, Inc., Tampa, USA) following the 20 minutes of HRV assessment. Additional readings were performed when differences between readings exceed 5 mmHg. Systolic, diastolic and mean blood pressures were computed as the average of the 3 measurements with 1-minute intervals in between.

Physical activity

Daily PA was assessed using accelerometers (Actigraph GT1M, Actigraph LLC, Pensacola, USA) worn over the right hip for 7 consecutive days. Accelerometers were worn during waking hours except while bathing and during water-based activities. ActiLife software version 6.9 (Actigraph, Pensacola, USA) was used to reduce the raw activity data into daily PA. The average number of minutes/day spent at sedentary, light and MVPA was determined according to cut points relating counts/min to PA intensity (Troiano et al., 2008). To be considered as valid data, individuals must had a minimum of 4 days recorded with at least 8 wear-time hours per day. The average minutes/day spent at different categories of PA intensity was determined according to cut points that relate counts/min to PA intensity levels: sedentary (Sed) time (≤ 99 counts/min), light PA (LPA) (100 - 2019 counts/min) and MVPA (≥ 2020 counts/min) (Troiano et al., 2008).

Blood Sampling

Twelve-hour fasting blood samples were collected by venipuncture of the antecubital vein into a serum separator and EDTA coated tubes. Serum glucose, high-density lipoprotein (HDL) cholesterol and triglycerides were measured in an automated clinical chemistry Olympus AU5400 (Beckman-Coulter, Brea, USA). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. Serum high-sensitivity C-reactive protein (hs-CRP) was determined using a particle-enhanced immunonephelometric assay in a Dimension Vista 1500 nephelometer (Siemens, Erlangen, Germany). To determine plasma levels of leptin, a high sensitive Milliplex map kit (Millipore, Darmstadt, Germany) was used and assayed in a Luminex 200™ analyzer (Luminex Corporation, Austin, USA). Adiponectin plasma levels were determined using a commercial enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden). Leptin-to-adiponectin ratio was calculated by dividing leptin per adiponectin levels. All assays were performed in duplicate according to the manufacturers' instructions.

Metabolic risk score

The risk factors hypercholesterolemia, hypertension and diabetes were recorded as previously described (Mancia et al., 2014). A metabolic risk score was computed as the sum of z-scores derived from continuous values of waist circumference, triglycerides, glucose level, and systolic blood pressure, minus the HDH z-score, and then divided by 5 in order to obtain the mean metabolic risk score.

Data analysis

Normal data distribution was verified by the Kolmogorov-Smirnov test. Variables not normally distributed were transformed to their natural logarithm or square root for subsequent analysis and then transformed back to the original scale for the purpose of clarity. Data are expressed as mean ± standard deviation. T-test and chi-square were used to compare differences between sexes.

Bivariate and partial correlations, controlling for age, sex and resting heart rate, were used between PA variables and HRV indices.

Multivariate linear regression was applied on the determination of the association between HRV indices, PA variables that showed significant correlation, and potential confounders. The "enter" method was elected for variables selection in order to ensure that age, sex and resting heart rate were constantly present. Age dependency of HRV could be caused by a structural modification linked to a loss of sinoatrial pacemaker cells and a reduction of arterial distensibility (Voss et al., 2015). Sex differences in the younger ages are probably caused by the different hormonal situation that tends to reduce and even disappear with age (Voss et al., 2015). Adjustment for heart rate has been recommended due to the strong association between HRV and the heart rate at which they were measured (Monfredi et al., 2014).

In order to sort out the contribution of potentials confounders we performed a clustered selection of variables (Briet et al., 2006). Clusters were established as follows: morphometric (waist circumference

and body mass index), medications (antidepressants, antihypertensive- including beta-blockers, lipid lowering and oral anti diabetics), metabolic (triglycerides, HDL cholesterol, LDL cholesterol, total cholesterol, fasting plasma glucose and mean metabolic risk score), inflammation (leptin, adiponectin, leptin-to-adiponectin ratio and hs-CRP) and blood pressure (systolic, diastolic and mean blood pressure). Within each cluster, variables were included with age, sex and resting heart rate in a competitive manner in multivariate models. If covariance was too high within clusters, the variable with the highest univariate significance level with HRV indices was kept.

Statistical analysis was performed using IBM SPSS 20 software (SPSS, Chicago, USA). The significance level was set at 95%. Power analysis was calculated post hoc, and it was higher than 0.8 for both partial correlation and multiple regression analysis.

Results

From the random sampling of 1,200 individuals, 318 did not answer phone calls, 244 declined to participate, 348 met the exclusion criteria, and 33 missed their appointments. A total of 257 individuals attended the first appointment, of whom 197 had valid data for HRV and PA measurements, constituting the final study sample. Of these participants, 116 (58.9%) were women, with a mean age of 47.4 ± 12.9 years and a BMI of 26.8 ± 4.3.

Table 1 describes the participants' clinical characteristics along with comparisons between sexes.

Table 1. Clinical characteristics along with comparisons between sexes

	Total (n = 197)	Female (n = 116)	Male (n = 81)	*p*
Age, years	47.4 ± 12.9	47.3 ± 12.3	47.4 ± 11.6	0.93
Anthropometrics				
Waist circumference, cm	92.2 ± 11.7	90.5 ± 11.7	94.5 ± 11.3	0.01
BMI, kg/m^2	26.8 ± 4.3	26.6 ± 4.6	27.1 ± 3.9	0.26
Risk factors				
Smoking, %	29.4	21.9	39.8	0.005
Dyslipidemia, %	71.1	69.3	73.5	0.31
Hypertension, %	43.1	39.5	48.2	0.14
Diabetes, %	9.2	6.3	13.3	0.07
Medications				
Lipid-lowering, %	22.3	19.3	26.5	0.15
Antihypertensive, %	33.0	28.9	38.6	0.10
Oral anti-diabetic, %	8.1	6.1	10.8	0.17
Antidepressants, %	17.3	21.9	10.8	0.03
Blood pressure				
SBP, mmHg	126.8 ± 16.3	126.2 ± 17.7	127.6 ± 14.2	0.38
DBP, mmHg	74.7 ± 10.9	73.3 ± 11.1	76.5 ± 10.3	0.03
MBP, mmHg	94.5 ± 13.2	93.9 ± 14.0	95.0 ± 11.9	0.55
Metabolic biomarkers				
Triglycerides, mg/dL	111.4 ± 55.9	103.8 ± 48.6	121.6 ± 63.3	0.02
HDL cholesterol, mg/dL	55.9 ± 15.2	61.7 ± 15.3	48.0 ± 10.9	< 0.001
LDL cholesterol, mg/dL	118.9 ± 35.9	120.0 ± 36.6	117.4 ± 35.1	0.63
Total cholesterol, mg/dL	197.2 ± 38.6	203.1 ± 38.5	189.2 ± 37.5	0.01
Fasting glucose, mg/dL	96.2 ± 29.6	94.7 ± 35.7	98.1 ± 18.2	0.06
Mean metabolic risk score, a.u.	-0.0 ± 0.6	-0.1 ± 0.6	0.2 ± 0.5	< 0.001
Inflammatory biomarkers				
Leptin, mg/mL	13.6 ± 12.0	17.8 ± 11.8	8.0 ± 9.8	< 0.001
Adiponectin, mg/mL	10.8 ± 5.2	12.1 ± 4.1	8.9 ± 5.9	< 0.001
Leptin-to-adiponectin ratio	1.4 ± 1.4	1.6 ± 1.4	1.0 ± 1.1	< 0.001
hs-CRP, mg/dL	0.3 ± 0.6	0.3 ± 0.4	0.3 ± 0.8	0.04

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; a.u.: arbitrary units; HDL: high-density lipoprotein; LDL: low-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein.

Table 2 shows descriptive statistics for PA and HRV indices and differences between sexes. On average, the number of days recorded with accelerometers was 6.4 ± 1.1 days. Accelerometers mean wear time for participants with valid data was 13.3 ± 1.5 hours/day, ranging from 8.7 to 17.5 hours/day. Compared to males, females exhibited significantly less sedentary (P = 0.001) and MVPA (P = 0.04), but higher light PA (P = 0.01). For heart rate-related variables, males exhibited a lower heart rate (P = 0.003) and higher

mean R-R intervals (P = 0.008), LF (P = 0.01), LF/HF (P < 0.001), and DFA1 (P = 0.001) than females, who in turn had significantly higher SD12 (P = 0.03).

Table 2. Mean values (mean ± SD) of daily physical activity and heart rate variability and between sex comparisons

	Total	Female	Male	*p*
Physical activity				
Sedentary, min/day	460.4 ± 92.8	442.5 ± 90.3	485.0 ± 91.1	0.001
LPA, min/day	301.3 ± 99.2	315.1 ± 97.4	282.3 ± 99.1	0.01
MVPA, min/day	36.3 ± 26.2	33.1 ± 23.4	40.6 ± 29.2	0.04
Heart rate variability				
Resting heart rate, bpm	63.6 ± 9.4	65.2 ± 8.6	61.3 ± 10.1	0.003
Time domain				
Mean RR interval, ms	917.6 ± 130.3	896.3 ± 120.3	946.9 ± 138.4	0.008
SDNN, ms	41.5 ± 25.8	40.9 ± 26.4	42.4 ± 25.0	0.55
rMSSD, ms	43.2 ± 31.1	43.9 ± 33.1	42.4 ± 28.3	0.91
Frequency domain				
LF, ms²	734.1 ± 1252.9	573.7 ± 822.8	954.4 ± 1653.6	0.01
HF, ms²	1277.1 ± 1839.8	1412.7 ± 1963.1	1090.7 ± 1649.0	0.35
LF/HF	1.0 ± 1.4	0.7 ± 0.9	1.4 ± 1.8	< 0.001
Non-Linear				
DFA1	0.9 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	< 0.001
SD12	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.03

LPA: light physical activity; MVPA: moderate to vigorous physical activity; SDNN: standard deviation of the normal-to-normal R-R intervals; RMSSD: square root of the mean of the squared differences between successive NN intervals differences; LF: low frequency power; HF: high frequency power; DFA1: short-term fractal scaling exponent; SD12: Poincare ratio; bpm: beats per minute.

Bivariate correlation between HRV indices and daily PA showed that MVPA was positively correlated with HF (r = 0.15, P = 0.02) and negatively correlated with LF/HF (r = -0.13, P = 0.05). However, in the partial correlation, only MVPA and LF/HF remains significantly associated (r = -0.18, P = 0.01). Correlations between sedentary and light PA with HRV indices were not significant (Table 3).

Table 3. Bivariate correlations between daily physical activity and heart rate variability indices

	Sedentary time (min/day)	LPA (min/day)	MVPA (min/day)
SDNN, ms	0.08 (0.91)	0.04 (0.51)	0.12 (0.08)
rMSSD, ms	0.03 (0.67)	0.02 (0.77)	0.08 (0.07)
LF, ms²	0.08 (0.25)	0.02 (0.72)	0.05 (0.44)
HF, ms²	0.00 (0.99)	0.03 (0.62)	0.15 (0.02) *
LF/HF	0.10 (0.15)	-0.15 (0.84)	-0.13 (0.05) *
DFA1	-0.02 (0.78)	0.07 (0.32)	-0.06 (0.40)
SD12	0.02 (0.70)	-0.03 (0.63)	0.03 (0.62)

Note: Values are r (p).
LPA: light physical activity; MVPA: moderate to vigorous physical activity; SDNN: standard deviation of the normal-to-normal R-R intervals; rMSSD: square root of the mean of the squared differences between successive NN intervals LF: low frequency power, HF: high frequency power, LF/HF: ratio between low frequency power and high frequency power; DFA1: short-term fractal-scaling exponent; SD12: Poincare ratio.
* p < 0.05.

Table 4 presents multivariate analyses for HRV variables. Time domain indices SDNN and RMSSD, were independently predicted by age, resting heart rate, and metabolic syndrome score. Models demonstrated 34% and 33% of the variance of SDNN and RMSSD, respectively. Regarding frequency domain indices, MVPA was an independent predictor of LF/HF (β = -0.14, P = 0.03), controlling for sex, waist circumference, resting heart rate, and age. The best model demonstrated 16% of the total variance of LF/HF, with the contribution of MVPA being 2.3%.

Sex, waist circumference, resting heart rate, and anti-hypertensive medication were independent predictors as related to non-linear indices DFA1 and SD12 (Table 4).

Table 4. Predictors of frequency domain indices of heart rate variability

	R^2 increment (%)	β	*p*
Time domain indices			
Ln SDNN (R^2: 0.34)			
Age	22.2	-0.40	< 0.001
Ln Resting heart rate	9.9	-0.26	< 0.001
Mean metabolic risk score	1.8	-0.19	0.03
Sex	0.0	0.05	0.38
Ln rMSSD (R^2: 0.33)			

Age	16.5	-0.34	< 0.001
Ln Resting heart rate	13.9	-0.33	< 0.001
Mean metabolic risk score	2.6	-0.16	0.03
Sex	0.0	0.02	0.74
Frequency domain indices			
Ln LF ms² (R^2: 0.27)			
Ln Resting heart rate	18.5	-0.22	< 0.001
Age	4.9	-0.44	< 0.001
Sex	3.3	0.13	0.03
HF ms² (R^2: 0.36)			
Age	26.0	-0.43	< 0.001
Ln Resting heart rate	7.6	-0.25	< 0.001
Mean Metabolic syndrome score	2.8	-0.17	0.02
Sex	0.0	-0.06	0.32
Ln LF/HF (R^2: 0.16)			
Sex	9.2	0.32	< 0.001
Waist circumference	4.1	0.14	0.04
Sqrt MVPA	2.3	-0.14	0.03
Ln Resting heart rate	0.05	0.07	0.30
Age	0.02	0.05	0.45
Non-linear indices			
DFA 1 (R^2: 0.20)			
Ln Resting heart rate	9.1	0.008	< 0.001
Sex	5.4	0.151	< 0.001
Anti-hypertensive treatment	3.4	-0.13	0.003
Waist circumference	1.8	0.004	0.01
Age	0.04	0.002	0.30
Sqrt SD12 (R^2: 0.15)			
Sex	5.0	-0.20	0.003
Ln Resting heart rate	4.5	-0.24	< 0.001
Anti-hypertensive treatment	3.0	0.28	0.001
Waist circumference	2.3	-0.15	0.04
Age	0.02	-0.05	0.42

Ln SDNN: natural logarithm of standard deviation of the normal-to-normal R-R intervals; Ln RMSSD: natural logarithm of the square root of the mean of the squared differences between successive NN intervals; Ln LF: natural logarithm of the low frequency power; Ln HF: natural logarithm of the high frequency power; Ln LF/HF: natural logarithm of the ratio between low frequency power and high frequency power; DFA1: short-term fractal-scaling exponent; Sqrt SD12: squared root of the Poincare ratio; Ln: natural logarithm; Sqrt: squared root.

Discussion

The main finding of this study is that in adults without established cardiovascular disease, MVPA was negatively associated with LF/HF, explaining 2.3% of its variance. The observed association was independent of sex, waist circumference, and age. Conversely, sedentary time and light PA were not associated with any HRV indices.

Our result possibly highlights the positive effect of MVPA on cardiac autonomic modulation. In our study, the LF/HF might mainly reflect the vagal control of the heart due to recordings based in short-term measures and obtained under laboratory conditions, where participants were supine and under paced breathing (Shaffer et al., 2014; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In this context, higher MVPA levels were associated with lower LF/HF ratios, suggesting a potential shift toward parasympathetic dominance, a characteristic of healthier cardiac autonomic regulation (Buchheit et al., 2004; Felber et al., 2008). Supporting this, we observed a weak but statistically significant positive correlation between HF and MVPA.

However, our results must be interpreted with caution. MVPA was not an independent predictor of any single frequency domain index (LF ms2 and HF ms2), but was only a predictor when both were combined.

Furthermore, the utility of the LF/HF ratio in representing the balance of autonomic function is questionable, especially due to the ambiguity surrounding the origin of the LF index (which may reflect vagal, sympathetic, and baroreflex activity) and the potential non-linear (and non-reciprocal) relationship between sympathetic and parasympathetic nervous activity (Billman, 2013; Shaffer et al., 2014). Finally, cardiac autonomic deregulation is a risk factor for adverse cardiovascular events (La Rovere et al., 1998), but the LF/HF had an inferior capacity to predict deaths from cardiovascular disease when compared to others index of HRV, such as LF and HF (Bigger et al., 1993). Therefore, clinical meaning of the observed association between MVPA and LF/HF must be ascertained.

Physiological mechanisms by which MVPA could influences cardiac autonomic function are (i) the reduction of sympathetic neural outflow to the sinoatrial node and the attenuation of the heart rate in response to myocardial stretch (Smith et al., 1989); (ii) the reduction of GABAergic neurotransmissions in the nucleus tractus solitary, involved in heart rate control, and therefore increasing vagal influences on cardiac pace maker activity (Mueller & Hasser, 2006); (iii) the increment of central 5-hydroxytryptamine synthesis, which has been associated with increases in vagal modulation (Ngampramuan et al., 2008); and (iv) the improvement of cardiomyoctyes contractility (Wisløff et al., 2009) and enhanced cardiac electrical stability (Billman, 2009).

Our sample was selected from a general population encompassing a wide age range and diverse health conditions (such as hypertension, dyslipidemia, and diabetes) and habitual medication. This fact makes it difficult to compare the study with other studies that were mainly performed with a restricted age range (adults (Sandercock et al., 2008; Soares-Miranda, Sandercock, et al., 2012), middle-aged adults (Buchheit et al., 2005) or elderly (Soares-Miranda et al., 2014) and with homogeneous health conditions (Sandercock et al., 2008; Soares-Miranda, Negrao, et al., 2012; Soares-Miranda, Sandercock, et al., 2012), which might limit the clinical utility of results for the general population as users of primary health care centers.

Contrary to what was previously described (Sandercock et al., 2008; Soares-Miranda et al., 2014; Soares-Miranda, Sandercock, et al., 2012), we did not find a significant association between MVPA and time domain indices that reflect circadian rhythms (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Possible methodological issues might explain these differences. We assessed HRV in short recordings and the accurate assessment of global cardiac autonomic function requires long-term R-R intervals recordings, lengthened to at least 18 hours, including day and night time (Min et al., 2008). This reasoning can also be used to justify the lack of significant associations of MVPA with non-linear indices. In fact, the measuring of HRV by short-term electrocardiogram recording (20 minutes) seems to be an acceptable method to assess the frequency domain indices of HRV (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), but it can be questioned if it is as sensitive to non-linear indices as the 24-h recordings (Min et al., 2008; Voss et al., 2015).

In the present study, sedentary time was not found to be associated with HRV indices. These results align with recent findings from the meta-analysis conducted by Alansare et al. (2021) who also did not observe an association between sedentary time and HRV. Additionally, no association was identified between light PA and HRV in our study. However, it has been previously reported that in the elderly population, higher levels of total leisure-time activity, as assessed through questionnaires, were prospectively linked to more favorable HRV indices (Soares-Miranda et al., 2014). This suggests that any level of PA, regardless of its intensity, may have a positive impact on HRV.

Considering the generality of results from a public health point of view, it is important to fulfill the international guidelines for MVPA together with a reduction of time spent in sedentary activities and replaced by light PA intensity.

Obesity is characterized by a marked sympathetic activation and, thus, autonomic nervous system impairment (Imai et al., 2006). Cardiac autonomic function seems to be impaired in the presence of obesity, with an up-regulation of the sympathetic branch. Our results corroborate this statement because waist circumference was an independent predictor of LF/HF, SD12, and DFA. In the same direction, our results showed that the mean metabolic risk score represents an independent predictor of time-domain indices and HF, and this is also already described (Soares-Miranda, Sandercock, et al., 2012).

Reference values for HRV indices are still not established, and huge discrepancies between studies due to methodological approaches make comparisons difficult (Nunan et al., 2010). Despite that, a value close to 1 for the non-linear index DFA is representative of a “healthy” area (Voss et al., 2015), and our mean for both sex were close to it.

The present study presents several strengths. According to our knowledge, this is the first time that short-term HRV, objective measurement of daily PA, and traditional risk factors were simultaneously assessed in a primary health care setting. Furthermore, in this study we have considered in the analysis multiple potential confounders of the relationship between HRV and PA, including the adjustments for

resting heart rate, which have been strongly suggested (Monfredi et al., 2014). Furthermore, we assessed daily PA through accelerometers, which overcome the expected overestimation of total PA and time spent in each category of intensity (Dyrstad et al., 2014) and might contribute to a better understanding of the association between exposure (daily PA) and outcome (HVR indices) (Soares-Miranda et al., 2014).

Limitations of the present study should be outlined. First, HRV reflects long-term lifestyle habits that might not have been brought out by the7- day actigraphy. Second, we adjusted all models for multiple confounders, but the influence of residual confounding cannot be excluded. Third, it should be recognized that short-term electrocardiogram recordings did not present the same stability and reproducibility as 24-h recordings, especially for the assessment of time domain and non-linear indices of HRV (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Since we have included participants with a wide age range and general health conditions, generalization of the results should be made with caution because of the study sample size.

Conclusions

The results of this study did not clearly demonstrate the cardioprotective effect of MVPA on HRV indices, although there was an inverse association with one frequency-domain index (LF/HF). Conversely, sedentary time and light PA were not associated with cardiac autonomic function.

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