

# Associations between objectively measured physical activity, sedentary time, and cardiorespiratory fitness with inflammatory and oxidative stress markers and heart rate variability

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## Abstract

**Background:** To assess the associations between physical activity (PA) and sedentary time (SED<sub>T</sub>) with inflammatory and oxidative stress markers, heart rate variability (HRV) and post-exercise recovery (HRR) controlling for cardiorespiratory fitness (CRF) and potential confounders.

**Design and methods:** The following data was collected from 44 participants during 2019 (age = 49.5 ± 6.4 years, 66% women): Plasma levels of C-reactive protein (CRP) and cytokines (IL-1β, INF-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-18, IL-23); catalase (CAT) and glutathione peroxidase (GPX) activities; resting heart (HR) rate for HRV analysis, anthropometric measures, a submaximal cycling test to evaluate CRF with active recovery to assess and HRR (absolute and ΔHR), and 7-day accelerometry.

**Results:** Women spent significantly more SED<sub>T</sub> ( $p=0.035$ ), had higher inflammatory markers (IL-6 and TNF) and lower HRV indices [SDNN, LF/HF, SD2 ( $p>0.05$ )]. Significant associations were found between SED<sub>T</sub> and markers of inflammation [CRP,  $B=0.006$ ,  $p=0.001$ ; MCP-1,  $B=0.003$ ,  $p=0.038$ ]. HRV indices were significantly associated with inflammatory/oxidative stress markers [IL-10 ( $p=0.04$ ), GPX ( $p=0.014$ ), ln-IL 23 ( $p=0.036$ ), CAT ( $p=0.026$ )] while HRR was positively associated with light PA [ $\Delta 3$  ( $B=0.051$ ,  $p=0.043$ ),  $\Delta 4$  ( $B=0.062$ ,  $p=0.021$ )] and inversely related to catalase [ $\Delta 3$  ( $B=-54.7$ ,  $p=0.042$ ),  $\Delta 4$  ( $B=-54.1$ ,  $p=0.021$ )] and CRP [ $\Delta 5$  ( $B=-19.8$ ,  $p=0.033$ )]. Higher CRF showed lower values for TNF-α ( $p=0.02$ ) and IL-10 ( $p=0.003$ ) and better HRV/HRR indices [RMSSD, PNS, SampEn, SD1 ( $p<0.05$ )].

**Conclusions:** SED<sub>T</sub> had a higher impact on inflammation and autonomic balance, independently of PA levels with differences by sex and CRF. PA appears to be more important for a better HRR. Lower HRV and HRR could be indicative of inflammatory status.

## Keywords

Sedentary lifestyles, cytokines, autonomic control, cardiorespiratory fitness, cardiometabolic risk

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## Introduction

Non-communicable diseases (NCDs) and particularly cardiometabolic diseases (CMDs), such as type 2 diabetes (T2DM), obesity, and hypertension, are at the genesis of cardiovascular diseases, being the primary cause of death<sup>1</sup> and representing one of the biggest issues in global public health.

The combination of prolonged exposure to a non-healthy diet, low levels of physical activity (PA), and/or sedentary behavior (SED), induce the activation of the stress axis and the subsequent inflammatory response.<sup>2</sup> It is well established that low-grade chronic inflammation is a prospective risk factor linking several physio-pathological processes in the etiology of NCDs.<sup>3</sup> Potential mediators of the systemic inflammation include reactive oxygen species and a variety of inflammatory cytokines as a part of the complex interplay of immunometabolism<sup>4</sup> that amplify inflammation through stimulation of pro-inflammatory mediators, such as C-reactive protein (CRP).<sup>5</sup> In addition, an association between autonomic dysfunction and metabolic deregulation has been suggested<sup>6,7</sup> with sympathovagal imbalance being an independent risk factor for CVDs,<sup>8</sup> whereas a higher vagal activity is related to reduced expression of inflammatory cytokines.<sup>9,10</sup> Alterations in autonomic modulation within different cardiometabolic conditions can be identified through heart rate variability (HRV).<sup>11</sup> Additionally, post-exercise heart rate recovery (HRR) has been utilized as a simple measure of cardiac autonomic control and is considered a morbidity and mortality predictor in patients with CVDs<sup>12</sup> with both HRV and HRR being inversely related to inflammatory markers.<sup>13,14</sup>

Physical activity has a pivotal role in health promotion and disease prevention. Appropriate levels of PA are simple and low-cost interventions for the improvement of cardiorespiratory fitness (CRF), a factor strongly linked to the incidence and risk of most cardiometabolic diseases<sup>15</sup> and one of the main determinants of long term survival.<sup>16</sup> Conversely, there is evidence suggesting that physical inactivity is the fourth risk factor related to worldwide mortality.<sup>17</sup> Previous reports showed that sedentary time (SED) is related with increased levels of inflammatory mediators<sup>18,19</sup> independently of sex and PA. Moreover, other evidence suggests that SED is associated with worse health outcomes independent of participation in moderate to vigorous PA.<sup>20</sup> On the other hand, the evidence suggests that regular exercise and PA reduce the levels of inflammatory and oxidative stress markers while increasing anti-inflammatory factors in young and older adults,<sup>21,22</sup> while the sedentary breaks seem to have a positive effect on cardiometabolic risk markers.

The objectives of the present study are to (1) analyze the associations between PA, SED, CRF, and the levels of inflammatory and oxidative stress markers; (2) analyze the association between PA, SED, CRF and HRV/HRR; and

(3) determine the associations between HRV/HRR indices and inflammatory and oxidative stress markers in volunteers not engaged in any structured exercise regime. Moreover, we propose to assess if PA, SED, inflammatory/oxidative stress markers or HRV/HRR indices are related to a higher cardiometabolic risk. Our main hypothesis was that individuals with higher SED will have an increased inflammatory/oxidative stress markers and lower autonomic control independently of PA levels but dependent on CRF.

## Design and methods

### Participants

A sample of 44 individuals (66% women) between 39 and 70 years from a larger cohort belonging to the Lifestyles and Oxidative Stress Markers in Cardiometabolic Risk Study (SECYT, UNC, Res. No. 455-18). To be included participants should not be engaged in any structured exercise regime at the time of the study. Also, participants should not undertake any special dietary regime nor possess clinically significant acute or chronic inflammatory process, physical disability or using any medication interfering with the measurement of inflammatory markers. Before their participation, all individuals were informed about the risks and benefits of the study and signed an informed consent form. The protocol of the study was approved by the HNC ethics committee (No. 193/14), and all methods employed are in accordance with the Declaration of Helsinki for research on human subjects.

### Procedures

Once selected to be included in the study, participants attended to the HNC with all procedures performed between 8.00 and 10.30 A.M. Immediately after arrival to the HNC, a blood sample was drawn for biochemical analysis. Then the participants rested quietly for 15 min in seated position, allowing for a stabilization, after which resting heart rate (HR) data were collected for 10 min. Subsequently, blood pressure was measured and categorized according to the guidelines of the American Hypertension Association<sup>23</sup> using a standardized automatic device (Omron M7 Intelli IT, model HEM-7322T-E, Japan). Then anthropometric measures [weight, height, and waist circumference (WC)] were obtained. Waist circumference was classified according to the standards of the WHO in normal (<70 cm for women and <93 cm for men), high risk (80–87 cm for women, 94–101 for men), very high risk (>88 cm for women, >102 cm for men). Finally, participants completed a submaximal exercise test to assess cardiorespiratory fitness (CRF) with dynamic recovery, during which HR data was collected for HRR analysis.

### Physical activity monitoring

PA and sedentary time (SED) were objectively measured by triaxial accelerometry (ActiGraph GT9X Link, USA) for 7 consecutive days. Participants were equipped with the accelerometers at the end of the testing day and wore the devices on their right hip. Data were recorded continuously at 30 Hz and stored at 60 s epochs,<sup>24</sup> except during bathing and sleeping hours. Upon returning the devices back, accelerometry data were downloaded and analyzed using the ActiLife Software (ActiGraph, Pensacola, FL, USA). Time validation was performed according to a software built-in algorithm with a minimum wearing time of  $\geq 10$  h per wearing day.<sup>24</sup> In the present study, the well-established<sup>25</sup> cut-off limits were chosen to determine sedentary time (SED  $< 150$  counts  $\text{min}^{-1}$ ), low (LPA,  $< 150$ – $2689$  counts  $\text{min}^{-1}$ ), moderate (MPA,  $2690$ – $6166$  counts  $\text{min}^{-1}$ ), moderate to high (MVPA,  $\geq 2689$  counts  $\text{min}^{-1}$ ) and vigorous (VPA,  $\geq 6167$  counts  $\text{min}^{-1}$ ) PA categories. Both PA and SED are expressed in minutes per day.

### Cardiorespiratory fitness

Cardiorespiratory fitness was assessed utilizing a submaximal test with the Åstrand-Rhyming protocol<sup>26</sup> using an electrically braked cycle ergometer (CycleErgo Lite, CardioCom, Buenos Aires, Argentina) which uses exercise HR to estimate maximal oxygen uptake ( $\text{VO}_{2\text{max}}$ ). The test consisted of a 6 min exercise bout on a cycle ergometer with a power output of 75–125 W for females and 100–150 W for males. Participants were instructed to maintain a pedal frequency of 50 rpm. Heart rate during exercise and 5 min post-exercise was recorded continuously using a telemetric device (Polar V800, Polar Electro Oy, Finland). Absolute  $\text{VO}_{2\text{max}}$  ( $\text{Lmin}^{-1}$ ) and relative  $\text{VO}_{2\text{max}}$  ( $\text{mLkg}^{-1}\text{min}^{-1}$ ) were calculated using the Åstrand and Rhyming nomogram.

### Heart rate variability and post-exercise heart rate recovery

Resting HR was continuously monitored beat-by-beat with a telemetric device (V800, Polar Electro Oy, Finland) for 10 min with the participants in a seated position while post-exercise HR was recorded for 5 min at the end of the exercise test. Recorded HR data were downloaded to CSV files from the Polar Flow website. HRR assessment included both raw and relative parameters as well as HRR kinetics as previously described.<sup>27</sup> Raw HR was defined as the HR value at a given point of the recovery period (i.e. from 30 s to 5 min), and relative HR recovery was defined as the difference between the HR registered at the end of exercise and after each of the measured intervals (i. e.  $\Delta 30$ ,  $\Delta 1$ ,  $\Delta 2$ ,  $\Delta 3$ ,  $\Delta 4$ , and  $\Delta 5$ ). For the assessment of HRR kinetics, individual data were modeled with a monoexponential fit

(Sigmaplot 12; SPSS Science, Chicago, IL) using the following equation:  $\text{HR}(t) = \text{HR}_0 + \text{HRamp} \times e^{(-t/\tau)}$ , where,  $\text{HR}(t)$  is the HR at a given time;  $\text{HR}_0$  is the asymptotic value of HR (bpm);  $\text{HRamp}$  is the amplitude of HR decrement from  $\text{HREnd}$  to  $\text{HR}_0$  for  $t = \infty$  (bpm); and  $\tau$  is the time constant.

Short-term HRV indices were calculated for the last 5 min of the 10-min record period. Time-domain indices included the standard deviation of the R-R series (SDNN) and the square root of the mean of the sum of the squares of differences between adjacent R-R intervals (RMSSD). Spectral analysis included the low frequency (LF) band, the high frequency (HF) band and the ratio LF/HF. Non-linear analyses of HRV included: the long (SD1) and the short (SD2) axes from Poincaré Plots reflecting the short- and the long-term modulation, respectively; HR complexity analysis via sample entropy (SampEn); and the detrended fluctuations of short- ( $\alpha 1$ ) and long-term ( $\alpha 2$ ) fractal scaling as determined from a double log graph that assesses the correlation within the signal. Also, we included the parasympathetic nervous activity [ $\text{PNS}_{\text{INDEX}} = \text{Mean RR, RMSS, and SD1}(\%)$ ] and sympathetic nervous activity indices [ $\text{SNS}_{\text{INDEX}} = \text{mean HR, Stress Index and SD2}(\%)$ ], where stress index is the square root of Baevisky Stress Index. All HRV indices were derived using the Kubios software (University of Kuopio, Kuopio, Finland) following standard procedures with default values of the software.

### Biochemical determinations

Participants were required to fast for at least 12 h before blood sample collection. All blood samples (5 mL) were obtained from the antecubital vein by professionals of the HNC laboratory using standardized techniques. Samples were centrifuged at 700–1000g and the supernatant (plasma) was removed and stored at  $-20^\circ\text{C}$  until analyses. The remaining blood was treated with HPLC-grade water and centrifuged at 10,000g for 15 min. The supernatant (erythrocyte lysate) was collected and stored at  $-80^\circ\text{C}$  for the analysis of oxidative stress markers.

Determination of C-reactive protein (CRP), fibrinogen, lipidic profile [total cholesterol (TC), total triglycerides (TG), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C)], and glycemia was performed in the HNC laboratory as a part of the routine biochemical determinations. Plasma cytokines were quantified using a multiplex bead-based assay (#740118, Legend Plex™, Biolegend Inc.) and flow cytometry. The assay allows quantification of human inflammatory cytokines/chemokines, including interleukin (IL)-1 $\beta$ , Interferon  $\gamma$  (IFN- $\gamma$ ), Tumor Necrosis Factor (TNF- $\alpha$ ), Monocyte Chemoattractant Protein [MCP-1 (CCL2)], IL-6, IL-8 (CXCL8), IL-10, IL-18, and IL-23. The staining protocol was performed according to the manufacturer's instructions, using a BD FACS Canto II

(Becton Dickinson Biosciences, NJ, USA) flow cytometer. The data files were analyzed using the Legend Plex Data Analysis Software. Oxidative stress markers, catalase (CAT), and glutathione peroxidase (GPX) were measured using spectrophotometric methods according to manufacturer's instructions (Cayman Chemical, #707002 & #703102), using an absorbance microplate reader (Spectro Star Nano, BMG Labtech, Freiburg, Germany).

### Cardiometabolic risk

To assess the cardiometabolic risk and its relationship with PA, SEDT, HRV, and inflammatory/oxidative stress markers a cardiometabolic cluster was created from five biomarkers as previously recommended.<sup>28</sup> Briefly, a point was assigned whether a standard clinical risk cut point was exceeded: WC > 102 cm (men) or 88 cm (women); blood glucose > 110 mg dL<sup>-1</sup>, HDL-C < 40 mg dL<sup>-1</sup> (men) or < 50 mg dL<sup>-1</sup> (women), SBP ≥ 130 mmHg, and DBP ≥ 85 mmHg. Those with ≥ 3 risk points were classified as a high risk while those with < 3 points were classified as low risk. This clustering was further used for the logistic regressions (i.e. < 3 points = 0 and ≥ 3 points = 1).

### Statistical analysis

Descriptive statistics were used to compute means and standard deviations (SD) as well frequencies and percentages. The normal distribution of the variables was assessed using the Shapiro-Wilk test and visual inspection of Q-Q plots. For those variables showing a non-normal distribution, a natural logarithmic transform was applied while those variables showing a non-normal distribution despite transformation were assessed using non-parametric techniques.

For clustering CRF, PA levels (z-scores of LPA and MVPA) and age we used k-means cluster analysis with a maximum of 10 iterations. This procedure allowed to identify three clusters of CRF [Low (VO<sub>2max</sub> = 19.6 ± 2.2 mL kg<sup>-1</sup> min<sup>-1</sup>, n = 18), Moderate (VO<sub>2max</sub> = 28.3 ± 3.4 mL kg<sup>-1</sup> min<sup>-1</sup>, n = 18) and High (VO<sub>2max</sub> = 43.5 ± 4.6 mL kg<sup>-1</sup> min<sup>-1</sup>, n = 8)], three clusters of PA [High MVPA and Low LPA (n = 10), High LPA Low MVPA (n = 14), Low LPA and Low MVPA (n = 19)] and three age clusters [Younger = 43.4 ± 2.1 years, n = 17; Middle-Age = 49.6 ± 1.9 years, n = 16; Older = 58.8 ± 3.2 years, n = 11] To assess the associations between inflammatory/oxidative stress markers, PA/SEDT and HRV/HRR univariate general linear models were performed controlling for potential confounding factors as covariates (i.e. sex, age, CRF, WC, smoking and socioeconomic status). The influence of significant covariates was further explored using *t*-tests or one-way ANOVA (with Bonferroni *post hoc* comparisons) for normally distributed variables or Mann-Whitney U Test and Kruskal-Wallis Test for non-normal distributed variables. Variance homogeneity was assessed by the Levene test. Also, the

Brown-Forsythe robust test of equality of means was included in case the homogeneity of variance could not be assumed. To identify the variables associated with the cardiometabolic risk cluster, a logistic regression adjusted by sex and age was used. Correlations were computed between different variables using the Pearson correlation or Spearman Rho coefficients. Effects sizes (ES) were assessed through Cohen's *d* or eta squared ( $\eta^2$ ). Statistical power (1- $\beta$ ) was calculated *a posteriori* and presented for each analysis. All statistics were performed using the IBM SPSS Statistics for Windows® (Version 20.0; Armonk, NY). The statistical significance was set at an alpha level of 0.05.

### Results

Clinical, anthropometrical, and biochemical characteristics of the study participants are shown in Table 1. Only one individual was excluded from the PA and SEDT analysis after the accelerometer wear time validation, since he did not meet the ≥ 10 h day<sup>-1</sup> threshold. In general, individuals spent 435.6 ± 86.8 min day<sup>-1</sup> (60.7%) in sedentary, 257.8 ± 74.4 min day<sup>-1</sup> (35.6%) in LPA, and 25.7 ± 16.4 min day<sup>-1</sup> (3.7%) in MVPA. There were differences in SEDT between men and women (397.9 ± 70.9 min day<sup>-1</sup> vs 455.8 ± 88.8 min day<sup>-1</sup>, *p* = 0.035, 95% CI = 4.20 to 111.64, ES = 0.72) as well as in daily length of sedentary brakes (1000.7 ± 69.7 vs 930.7 ± 77.9 s, *p* = 0.014, 95% CI = -124.7 to -15.2, ES = 0.94). No other differences were observed between men and women regarding PA or SEDT indices.

### Cardiometabolic risk markers

No adjusted models were found between PA or SEDT and cardiometabolic risk markers. Similarly, no differences were found among the PA clusters. However, the logistic regressions showed an association between ln CRP and the cardiometabolic risk cluster (OR = 7.66, 95% CI = 1.66 to 35.45, *p* = 0.009). No other association was identified with the logistic regressions. In the low-risk cluster ln TNF- $\alpha$  and ln IL-6 were inversely correlated with MVPA (*r* = -0.52 and -0.54, *p* = 0.01, respectively) and relative VO<sub>2max</sub> (*r* = -0.54 and -0.47, *p* = 0.006 and 0.03, respectively) while ln IL-10 was correlated with relative VO<sub>2max</sub> (*r* = -0.75, *p* < 0.001). On the other hand, in the high-risk cluster only VO<sub>2max</sub> and TNF- $\alpha$  were inversely related (*r* = -0.81, *p* = 0.001).

### Physical activity and sedentary time versus inflammatory and oxidative stress markers

There was an adjusted association between SEDT and ln-CRP and ln-MCP-1 (Table 2). Moreover, these relationships were maintained after controlling for LPA (*p* = 0.024 for both), and MVPA (*p* = 0.039 for ln-CRP and (*p* = 0.009

**Table 1.** Participant's characteristics. Data are expressed as means  $\pm$  SD or median (interquartile range).

	Women (n=29)	Men (n=15)	All (n=44)
Age (years)	50.4 $\pm$ 6.6	47.8 $\pm$ 6.2	49.5 $\pm$ 6.4
Height (cm)	160.8 $\pm$ 7.6	173.5 $\pm$ 7.6*	165.1 $\pm$ 9.7
Body mass (kg)	67.9 $\pm$ 12.3	90.0 (11.0)*	77.2 $\pm$ 20.5
BMI (kg·m <sup>-2</sup> )	24.8 (6.9)	28.7 (4.5)*	28.1 $\pm$ 6.2
WC (cm)	81.6 $\pm$ 9.6	94.0 (11.0)*	87.7 $\pm$ 14.5
WC risk [n (%)]			
Normal	15 (51.7%)	3 (20.0%)	18 (40.9%)
High risk	5 (17.2%)	2 (13.3%)	7 (15%)
Very high risk	9 (31.0%)	10 (66.7%)	19 (43.2%)
T2DM [n (%)]	1 (3.4%)	1 (6.7%)	2 (4.5%)
HBP [n (%)]	9 (31.0%)	3 (20.0%)	12 (27.3%)
Smoking [n (%)]	4 (13.8%)	1 (6.7%)	5 (11.4%)
Socioeconomic status [(n)%]			
High	10 (40.0%)	8 (53.3%)	18 (45.0%)
Middle	6 (24.0%)	4 (26.7%)	10 (25.0%)
Low	9 (36.0%)	3 (20.0%)	12 (30.0%)
VO <sub>2max</sub> (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	22.1 (8.8)	32.2 (16.6)*	27.52 $\pm$ 9.16
VO <sub>2max</sub> (L·min <sup>-1</sup> )	1.95 (0.73)	4.22 (2.12)*	2.79 $\pm$ 1.27

BMI: body mass index; WC: waist circumference; T2DM: type 2 diabetes mellitus; HBP: high blood pressure; VO<sub>2max</sub>: maximal oxygen uptake.

\*Significantly different than women ( $p < 0.05$ ).

**Table 2.** General linear models between physical activity, sedentary time, inflammatory and oxidative stress markers and heart rate variability and heart rate recovery indices.

	B	95% CI		p-Value	$\eta^2$	1- $\beta$
		Lower	Upper			
SED <sub>T</sub> vs Ln CRP	0.006	0.003	0.009	0.001	0.34	0.84
SED <sub>T</sub> vs Ln MCP-1	0.003	0.002	0.006	0.03	0.15	0.55
LPA vs $\Delta 3$	0.051	0.002	0.100	0.043	0.13	0.52
LPA vs $\Delta 4$	0.062	0.010	0.114	0.021	0.20	0.72
PNS <sub>INDEX</sub> vs Ln IL-10	0.046	0.006	0.091	0.04	0.25	0.52
Ln LF vs GPX	-0.47	-0.83	-0.11	0.014	0.29	0.73
Ln HF vs IL-23	0.39	0.03	0.75	0.036	0.21	0.57
Ln HF vs CAT	-8.90	-16.45	-1.32	0.026	0.44	0.66
HRR <sub>ramp</sub> vs IL-23	5.85	0.74	10.97	0.027	0.22	0.62
HRR <sub>5</sub> vs GPX	-3.43	-5.95	-0.90	0.011	0.36	0.77
$\Delta 3$ vs CAT	-54.7	-106.8	-2.69	0.042	0.42	0.57
$\Delta 4$ vs CAT	-54.1	-97.4	-10.7	0.021	0.51	0.71
$\Delta 5$ vs CRP	-19.8	-32.1	-1.52	0.033	0.24	0.59

SED<sub>T</sub>: sedentary time; LPA: light physical activity; CRP: C-reactive protein; MCP-1: monocyte chemoattractant protein 1; GPX: glutathione peroxidase; CAT: catalase;  $\Delta 3$ ,  $\Delta 4$ , and  $\Delta 5$ : difference between heart rate at the 3, 4, and 5 min of post-exercise recovery and the final heart rate during the exercise test; HRR<sub>ramp</sub>: amplitude of the exponential model for heart rate recovery.

for Ln MCP-1. Significant influence of covariates was observed for CRP, TNF- $\alpha$ , IL-6, IL-10. For Ln-CRP there was a difference between those individuals with normal risk assessed by WC and those with very high risk ( $p=0.013$ , ES=1.21). Also, when models for CRP were performed by sex, women showed a positive association between CRP and WC [ $B=0.06$ ,  $p=0.0001$ ] and SED<sub>T</sub> [ $B=0.003$ ,  $p=0.021$ ], while men only showed and

association between CRP and SED<sub>T</sub> [ $B=0.012$ ,  $p=0.029$ ]. Correlations were observed between MVPA and Ln-IL-6 ( $r=-0.44$ ,  $p=0.015$ ) in the whole sample, and between SED<sub>T</sub> versus CRP ( $r=0.72$ ,  $p=0.029$ ) and between daily length of sedentary breaks versus GPX ( $r=-0.68$ ,  $p=0.03$ ) in men.

As shown in Table 3, there were unadjusted differences between men and women in TNF- $\alpha$  and IL-6, whereas

**Table 3.** Comparisons of biochemical, inflammatory/oxidative stress markers, heart rate variability (HRV) and heart rate recovery (HRR) by sex.

	Women	Men	p-Value	Diff. 95% CI		ES (d)	1-β
				Lower	Upper		
Blood Glucose (mg·dL <sup>-1</sup> )	96.9 ± 10.5	100.7 ± 9.24	0.22	-0.11	0.02	0.38	0.21
TC (mg·dL <sup>-1</sup> )	222.8 ± 42.7	195.7 ± 40.7	0.56	-0.72	55.0	0.64	0.51
TG (mg·dL <sup>-1</sup> )	105.5 ± 60.6	135.6 ± 61.1	0.84	-0.68	0.04	0.49	0.33
LDL-C (mg·dL <sup>-1</sup> )	135.0 ± 35.1	126.1 ± 33.6	0.44	-13.9	31.8	0.26	0.12
<b>HDL-C (mg·dL<sup>-1</sup>)</b>	<b>64.8 ± 10.4</b>	<b>48.4 ± 10.4</b>	<b>&lt;0.001</b>	<b>9.28</b>	<b>23.49</b>	<b>1.57</b>	<b>0.99</b>
HbA <sub>1c</sub> (%)§	5.5 (0.6)	5.6 (0.5)	0.75	-	-	-	-
Fibrinogen	436.4 ± 82.17	412.6 ± 114.4	0.47	-40.3	87.89	0.24	0.18
Ln CRP (mg·L <sup>-1</sup> )	-1.82 ± 0.84	-1.93 ± 1.14	0.76	-0.64	0.86	0.61	0.11
Ln IL-1β (pg·mL <sup>-1</sup> )	1.55 ± 0.46	1.81 ± 0.75	0.23	-0.71	0.18	0.41	0.18
INF-γ (pg·mL <sup>-1</sup> )	4.17 ± 2.22	3.33 ± 1.57	0.29	-0.77	2.44	0.43	0.19
<b>TNF-α (pg·mL<sup>-1</sup>)</b>	<b>6.27 ± 3.21</b>	<b>3.44 ± 2.54</b>	<b>0.01</b>	<b>0.72</b>	<b>4.94</b>	<b>0.97</b>	<b>0.79</b>
<b>Ln IL-6 (pg·mL<sup>-1</sup>)</b>	<b>2.16 ± 0.95</b>	<b>1.13 ± 1.43</b>	<b>0.02</b>	<b>0.14</b>	<b>1.92</b>	<b>0.84</b>	<b>0.58</b>
Ln MCP-1 (pg·mL <sup>-1</sup> )	6.09 ± 0.73	6.10 ± 0.62	0.95	-0.49	0.46	0.01	0.05
Ln IL-8 (pg·mL <sup>-1</sup> )	3.23 ± 1.43	3.21 ± 0.76	0.95	-0.81	0.86	0.01	0.05
IL-10 (pg·mL <sup>-1</sup> )	3.10 ± 1.97	2.16 ± 2.03	0.25	-0.70	2.57	0.46	0.20
IL-18 (pg·mL <sup>-1</sup> )	205.9 ± 104.9	207.3 ± 121.1	0.97	-74.9	72.1	0.01	0.05
IL-23 (pg·mL <sup>-1</sup> )	27.01 ± 20.6	21.7 ± 17.9	0.45	-8.9	19.5	0.27	0.11
CAT (nmol·min <sup>-1</sup> ·mL <sup>-1</sup> )	21.80 ± 2.80	20.93 ± 1.61	0.41	-1.26	3.00	0.27	0.09
GPX (nmol·min <sup>-1</sup> ·mL <sup>-1</sup> )	2.67 ± 0.85	2.61 ± 0.88	0.85	-0.61	0.73	0.07	0.05
<b>SDNN (ms)</b>	<b>26.5 ± 12.3</b>	<b>34.5 ± 7.09</b>	<b>0.013</b>	-15.0	-0.21	<b>0.80</b>	<b>0.69</b>
RMSSD (ms)	24.9 ± 13.4	28.1 ± 9.3	0.43	-11.23	0.49	0.27	0.13
PNS <sub>INDEX</sub>	-0.75 ± 0.79	-0.47 ± 0.65	0.26	-0.77	0.21	0.38	0.22
SNS <sub>INDEX</sub> §	0.96 (1.86)	0.48 (1.14)	0.11	-	-	-	-
<b>Ln LF (ms<sup>2</sup>)</b>	<b>5.39 ± 1.02</b>	<b>6.37 ± 0.58</b>	<b>0.002</b>	-1.57	-0.38	<b>1.18</b>	<b>0.95</b>
Ln HF (ms <sup>2</sup> )	5.01 ± 1.25	5.31 ± 0.86	0.58	-0.96	0.55	0.28	0.14
<b>Ln LF/HF</b>	<b>0.34 ± 0.82</b>	<b>1.12 ± 1.06</b>	<b>0.012</b>	-1.39	-0.18	<b>0.82</b>	<b>0.71</b>
SampEn	1.58 ± 0.28	1.44 ± 0.26	0.13	-0.04	0.32	0.51	0.35
SD1 (ms)	17.9 ± 9.7	19.9 ± 6.6	0.49	-7.80	3.82	0.24	0.11
<b>SD2 (ms)</b>	<b>33.3 ± 15.8</b>	<b>44.1 ± 8.8</b>	<b>0.007</b>	-18.4	-3.07	<b>0.84</b>	<b>0.74</b>
α1	1.06 ± 0.26	1.23 ± 0.28	0.07	-0.35	0.01	0.62	0.49
<b>α2</b>	<b>0.43 ± 0.13</b>	<b>0.33 ± 0.12</b>	<b>0.017</b>	<b>0.02</b>	<b>0.18</b>	<b>0.80</b>	<b>0.69</b>
Ln HRR τ (s)	4.20 ± 0.34	4.34 ± 0.29	0.21	-0.35	0.08	0.44	0.27
HR <sub>0</sub> (bpm)	100 ± 12	94 ± 13	0.16	-2.39	14.19	0.48	0.31
HRRamp (bpm)	42 ± 13	39 ± 18	0.64	-7.81	12.42	0.19	0.09
HRR30 (beats·min <sup>-1</sup> )	126 ± 12	120 ± 12	0.80	-0.85	14.51	0.5	0.33
HRR1 (beats·min <sup>-1</sup> )	117 ± 12	112 ± 11	0.23	-3.02	12.04	0.43	0.26
HRR2 (beats·min <sup>-1</sup> )	109 ± 12	106 ± 8	0.47	-4.47	9.54	0.29	0.14
HRR3 (beats·min <sup>-1</sup> )	104 ± 12	100 ± 9	0.34	-3.89	10.89	0.37	0.21
HRR4 (beats·min <sup>-1</sup> )	102 ± 12	98 ± 9	0.28	-3.35	11.28	0.37	0.21
HRR5 (beats·min <sup>-1</sup> )	101 ± 13	95 ± 9	0.13	-1.84	14.34	0.53	0.38
Δ30 (beats·min <sup>-1</sup> )	15 ± 6	15 ± 6	0.87	-4.29	3.64	0.00	0.05
Δ1 (beats·min <sup>-1</sup> )	24 ± 8	22 ± 6	0.39	-2.69	6.67	0.28	0.14
Δ2 (beats·min <sup>-1</sup> )	32 ± 10	27 ± 8	0.10	-1.03	10.89	0.55	0.39
Δ3 (beats·min <sup>-1</sup> )	37 ± 10	32 ± 9	0.23	-2.58	10.51	0.52	0.36
Δ4 (beats·min <sup>-1</sup> )	38 ± 12	35 ± 8	0.33	-3.64	10.64	0.29	0.14
Δ5 (beats·min <sup>-1</sup> )	40 ± 13	45 ± 31	0.42	-19.32	8.18	0.21	0.10

TC: total cholesterol; TG: triglycerides; HbA<sub>1c</sub>: glycosylated hemoglobin; CRP: C-Reactive protein; INF-γ: interferon gamma; TNF-α: tumor necrosis factor alpha; MCP-1: monocyte chemoattractant protein 1; CAT: catalase; GPX: glutathione peroxidase; SDNN: standard deviation of the R-R series; RMSSD: square root of the mean of the sum of the squares of differences between adjacent R-R intervals; LF: low frequency power; HF: high frequency power; SD1: the long-term modulation from Poincare Plots; SD2: short-term modulation from Poincare Plots; SampEn: sample entropy; α1: short-term detrended fluctuations; α2: long-term detrended fluctuations; HRR: heart rate recovery; HRRτ: time constant of the exponential fit for the HRR; HRRamp: amplitude of the exponential fit; ES: effect size; 1-β: observed statistical power.

Data are presented as means ± SD or medians (IQR). Significant results are highlighted in bold.

§Man-Whitney test.

adjusting for covariates such as WC, Age and CRF, yielded differences in ln-CRP [ $B=1.18$ , 95% CI=0.27 to 2.10,  $p=0.013$ ] in women versus men. The adjusted analysis for PA cluster showed high levels of IL-6 for the group with low LPA and low MVPA [ $B=0.56$ , 95% CI=0.12 to 2.46,  $p=0.032$ ] with no effect of sex as covariate. Post hoc analysis showed that High MVPA Low LPA cluster had the lowest levels ( $p=0.029$ ) of ln-IL-6 (Figure 1). Lower values of ln-TNF- $\alpha$  and ln-IL-10 were found in the high CRF group and a tendency for lower values of IL-6 (Table 4, Figure 1).

### Physical activity, sedentary time, heart rate variability, and heart rate recovery

There were no adjusted associations between any of the HRV indices and PA or SEDT. However, sex, CRF, and age emerged as covariates in several models. As shown in Table 3, women exhibited lower values for SDNN, LF, LF/HF and SD2. Also, there were differences in SDNN ( $F_{2,39}=3.54$ ,  $p=0.038$ ), ln LF ( $F_{2,39}=6.77$ ,  $p=0.003$ ) and SD2 ( $F_{2,39}=3.80$ ,  $p=0.031$ ) between age groups. Post hoc analysis revealed that for SDNN and SD2 these differences were between the younger and older groups (ES=1.13 and 1.23, respectively), while for the ln LF the three groups were different. In addition, an association between MVPA and SampEn was found in the younger group [ $B=0.01$ ,  $p=0.007$ ].

There were adjusted associations between  $\Delta 3$  and  $\Delta 4$  with LPA (Table 2). In addition, differences between PA cluster groups were found in HRR30,  $\Delta 30$ ,  $\Delta 2$ ,  $\Delta 3$ , and  $\Delta 4$  (Figure 2). Women showed moderate correlations between LPA and  $\Delta 3$  ( $r=0.41$ ,  $p=0.03$ ),  $\Delta 4$  ( $r=0.43$ ,  $p=0.02$ ) and  $\Delta 5$  ( $r=0.43$ ,  $p=0.02$ ) while in men exhibited moderate to high correlations between MVPA and  $\Delta 2$  ( $r=0.76$ ,  $p=0.002$ ),  $\Delta 3$  ( $r=0.76$ ,  $p=0.002$ ) and  $\Delta 4$  ( $r=0.77$ ,  $p=0.001$ ). Also, correlations were observed between MVPA and HRR4 ( $r=-0.71$ ,  $p=0.048$ ) and HRR5 ( $r=-0.72$ ,  $p=0.047$ ) in the High CRF group, and between LPA and  $\Delta 4$  ( $r=0.50$ ,  $p=0.047$ ) in the Low CRF group. Meanwhile controlling by age cluster yielded moderate correlations between MVPA and  $\Delta 30$  ( $r=0.53$ ,  $p=0.034$ ),  $\Delta 2$  ( $r=0.58$ ,  $p=0.022$ ),  $\Delta 3$  ( $r=0.66$ ,  $p=0.007$ ) and  $\Delta 4$  ( $r=0.62$ ,  $p=0.014$ ) in the younger group but no in the other two groups.

### Inflammatory and oxidative stress markers and HRV/HRR

After controlling for potential confounders, associations were observed among inflammatory and oxidative stress markers and HRV indices. Adjusted associations were found between ln IL-10 versus PNS<sub>INDEX</sub>; ln-HF versus ln-IL-23 and ln-CAT (Table 2). Furthermore, CRF, age, and sex were significant covariates. In addition, multivariate

linear models showed an association between HRRamp versus ln IL-23; HRR5 versus GPX (Table 2). Also, associations were found for  $\Delta 3$  and  $\Delta 4$  with ln CAT, while  $\Delta 5$  was associated with ln CRP (Table 2). There was a tendency for an association between  $\Delta 3$  and ln IL-10 [ $B=5.62$ ,  $p=0.05$ ].

### Discussion

The present study aimed to explore the associations between PA/SEDT and CRF with inflammatory and oxidative stress markers and HRV/HRR indices, while controlling for potential confounders. Although previous reports have addressed these relationships individually, to the best of our knowledge this is the first time that all these variables are evaluated together.

Among the main results of this study, after controlling for several potential confounders, we found an association between SEDT and inflammatory markers. Previous research consistently reported associations between sedentary behavior and inflammatory cytokines.<sup>18,19,29,30</sup> For instance, Cho et al.<sup>29</sup> described that the most sedentary group had higher odds for several inflammatory markers although with minimal differences between sexes. This is consistent with our findings, although we did find higher unadjusted levels of TNF- $\alpha$  and IL-6 and higher adjusted CRP levels in women compared to men, with women spending more time in sedentary behavior and less sedentary breaks than men. In this sense, it has been reported that these associations appear to be sex-dependent and independent of PA levels<sup>18</sup> with the relationship between inflammation and sedentary behavior being stronger in women compared to men.<sup>30</sup> It is worth noting that results from the study of Bergens et al.<sup>18</sup> differ from our results in terms of the inflammatory markers (e.g. IL-10 and Fibrinogen vs CRP and MCP-1, respectively). Of interest is IL-10 as it is known to have an anti-inflammatory effect, particularly related to PA and exercise.<sup>31</sup> In this regard, while Bergens et al.,<sup>18</sup> reported an inverse relationship of IL-10 with SEDT, we did not observe such association. However, we did find an effect of CRF, with lower IL-10 and TNF- $\alpha$  values, and a tendency to lower IL-6 levels in those with higher CRF (Table 4). Given that IL-10 can inhibit the synthesis of inflammatory cytokines such as TNF- $\alpha$ <sup>31</sup> and the fact that we observed a high correlation between IL-10 versus TNF- $\alpha$  ( $r=.91$ ,  $p<.001$ ) and versus IL-6 ( $r=.93$ ,  $p<0.001$ ), it can be suggested that the elevated levels of IL-10 those with low CRF are a response to the elevated levels of TNF- $\alpha$  and IL-6. However, it is also plausible that these discrepancies between our findings and those reported by Bergens et al.,<sup>18</sup> could be attributed to differences in the population studied and the modeling of the variables. Additionally, it has been suggested that physical inactivity leads to an expansion of visceral adipose tissue increasing circulating

**Table 4.** Biochemical, inflammatory, and oxidative stress markers by levels of cardiorespiratory fitness. Data are presented as means  $\pm$  SD or medians (IQR).

	Cardiorespiratory fitness			ANOVA <i>p</i> -Value	Brown- Forsythe	ES ( $\eta^2$ )	1- $\beta$
	Low	Moderate	High				
<b>Blood Glucose (mg·dL<sup>-1</sup>)<sup>§</sup></b>	<b>93.0 (6.0)*</b>	<b>98.0 (13.0)</b>	<b>101.0 (8.75)</b>	<b>0.044</b>	-	-	-
TC (mg·dL <sup>-1</sup> )	222.6 $\pm$ 37.0	217.3 $\pm$ 48.7	187.7 $\pm$ 39.5	0.16	0.15	0.09	0.37
TG (mg·dL <sup>-1</sup> )	114.0 $\pm$ 54.9	118.5 $\pm$ 58.8	112.7 $\pm$ 86.3	0.97	0.97	0.002	0.05
LDL-C (mg·dL <sup>-1</sup> )	137.0 $\pm$ 28.2	133.8 $\pm$ 41.2	117.5 $\pm$ 30.9	0.41	0.39	0.04	0.19
HDL-C (mg·dL <sup>-1</sup> )	60.8 $\pm$ 11.3	61.2 $\pm$ 13.7	52.3 $\pm$ 14.1	0.27	0.30	0.07	0.28
HbA <sub>1c</sub> (%) <sup>§</sup>	5.5 (0.4)	5.6 (0.6)	5.5 (0.8)	0.94	-	-	-
Fibrinogen	438.6 $\pm$ 94.2	444.6 $\pm$ 108.5	375.5 $\pm$ 59.2	0.21	0.16	0.10	0.40
<i>Inflammatory and oxidative stress</i>							
Ln PCR (mg·L <sup>-1</sup> )	-2.03 $\pm$ 0.64	-1.53 $\pm$ 1.15	-2.21 $\pm$ 0.65	0.23	0.18	0.09	0.30
Ln IL-1 $\beta$ (pg·mL <sup>-1</sup> )	1.45 $\pm$ 0.45	1.61 $\pm$ 0.43	2.17 $\pm$ 0.90	0.05	0.19	0.19	0.58
INF- $\gamma$ (pg·mL <sup>-1</sup> )	4.16 $\pm$ 1.77	4.32 $\pm$ 2.28	2.14 $\pm$ 1.32	0.10	0.07	0.14	0.45
<b>Ln TNF (pg·mL<sup>-1</sup>)</b>	<b>6.57 <math>\pm</math> 3.43*</b>	<b>5.35 <math>\pm</math> 2.79</b>	<b>2.56 <math>\pm</math> 2.56</b>	<b>0.02</b>	<b>0.02</b>	<b>0.32</b>	<b>0.93</b>
Ln IL-6 (pg·mL <sup>-1</sup> )	2.19 $\pm$ 0.66	1.91 $\pm$ 1.29	0.73 $\pm$ 1.57	0.05	0.10	0.20	0.58
Ln MCP-1 (pg·mL <sup>-1</sup> )	5.98 $\pm$ 0.54	6.06 $\pm$ 0.77	6.44 $\pm$ 0.76	0.33	0.36	0.06	0.23
Ln IL-8 (pg·mL <sup>-1</sup> )	3.14 $\pm$ 1.68	3.30 $\pm$ 0.77	3.24 $\pm$ 0.91	0.93	0.93	0.04	0.06
<b>IL-10 (pg·mL<sup>-1</sup>)</b>	<b>3.42 <math>\pm</math> 1.70*</b>	<b>3.31 <math>\pm</math> 2.05*</b>	<b>0.57 <math>\pm</math> 0.47</b>	<b>0.006</b>	<b>0.003</b>	<b>0.46</b>	<b>0.97</b>
Ln IL-18 (pg·mL <sup>-1</sup> )	5.08 $\pm$ 0.55	5.19 $\pm$ 0.65	5.33 $\pm$ 0.52	0.61	0.59	0.03	0.13
IL-23 (pg·mL <sup>-1</sup> )	2.79 $\pm$ 1.22	2.99 $\pm$ 1.10	1.34 $\pm$ 2.47	0.08	0.28	0.14	0.50
Ln CAT (nmol·min <sup>-1</sup> ·mL <sup>-1</sup> )	3.04 $\pm$ 0.10	3.04 $\pm$ 0.09	3.10 $\pm$ 0.13	0.51	0.54	0.06	0.15
GPX (nmol·min <sup>-1</sup> ·mL <sup>-1</sup> )	2.52 $\pm$ 0.69	2.92 $\pm$ 0.99	2.45 $\pm$ 0.87	0.40	0.41	0.06	0.20
<i>Heart rate variability</i>							
SDNN (ms)	29.6 $\pm$ 13.9	25.7 $\pm$ 8.4	36.8 $\pm$ 8.1	0.91	0.068	0.12	0.48
RMSSD (ms)	24.5 $\pm$ 14.8	23.7 $\pm$ 9.0	35.6 $\pm$ 7.9	0.06	0.046	0.10	0.55
<b>PNS<sub>INDEX</sub></b>	<b>-0.95 <math>\pm</math> 0.85*</b>	<b>-0.61 <math>\pm</math> 0.54*</b>	<b>-0.07 <math>\pm</math> 0.65</b>	<b>0.03</b>	<b>0.03</b>	<b>0.17</b>	<b>0.68</b>
SNS <sub>INDEX</sub>	1.54 $\pm$ 1.76	0.99 $\pm$ 0.88	0.19 $\pm$ 0.85	0.08	0.06	0.12	0.50
Ln LF (ms <sup>2</sup> )	5.76 $\pm$ 1.21	5.46 $\pm$ 0.84	6.27 $\pm$ 0.65	0.19	0.14	0.08	0.34
Ln HF (ms <sup>2</sup> )	5.29 $\pm$ 1.08	4.99 $\pm$ 1.00	5.37 $\pm$ 1.60	0.67	0.73	0.02	0.11
Ln LF/HF	0.84 $\pm$ 0.85	0.46 $\pm$ 1.05	0.37 $\pm$ 1.05	0.41	0.43	0.04	0.19
<b>SampEn</b>	<b>1.41 <math>\pm</math> 0.24*</b>	<b>1.63 <math>\pm</math> 0.27</b>	<b>1.62 <math>\pm</math> 0.30</b>	<b>0.04</b>	<b>0.06</b>	<b>0.15</b>	<b>0.60</b>
SD1 (ms)	17.7 $\pm$ 10.7	16.7 $\pm$ 6.4	25.2 $\pm$ 5.6	0.08	0.06	0.12	0.50
SD2 (ms)	38.6 $\pm$ 17.8	32.0 $\pm$ 11.0	45.2 $\pm$ 11.1	0.10	0.08	0.11	0.45
$\alpha$ 1	1.20 $\pm$ 0.25	1.09 $\pm$ 0.30	0.98 $\pm$ 0.19	0.17	0.13	0.09	0.36
$\alpha$ 2	0.45 $\pm$ 0.17	0.36 $\pm$ 0.10	0.39 $\pm$ 0.12	0.16	0.16	0.09	0.37
<i>Post-exercise HR recovery</i>							
Ln HRR $\tau$ (min)	4.29 $\pm$ 0.39	4.19 $\pm$ 0.29	4.26 $\pm$ 0.20	0.68	0.62	0.02	0.11
<b>HR<sub>0</sub> (bpm)</b>	<b>104 <math>\pm</math> 16<sup>†</sup></b>	<b>96 <math>\pm</math> 7</b>	<b>90 <math>\pm</math> 5</b>	<b>0.017</b>	<b>0.007</b>	<b>0.19</b>	<b>0.74</b>
HRamp (bpm)	41 $\pm$ 15	41 $\pm$ 13	41 $\pm$ 9	1.00	0.99	0.00	0.05
<b>HRR30</b>	<b>131 <math>\pm</math> 12<sup>†</sup></b>	<b>121 <math>\pm</math> 9</b>	<b>114 <math>\pm</math> 9</b>	<b>0.001</b>	<b>&lt;0.001</b>	<b>0.31</b>	<b>0.97</b>
<b>HRR1</b>	<b>123 <math>\pm</math> 12<sup>†</sup></b>	<b>112 <math>\pm</math> 10</b>	<b>107 <math>\pm</math> 6</b>	<b>0.001</b>	<b>&lt;0.001</b>	<b>0.30</b>	<b>0.96</b>
<b>HRR2</b>	<b>115 <math>\pm</math> 11<sup>†</sup></b>	<b>104 <math>\pm</math> 7</b>	<b>101 <math>\pm</math> 5</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.32</b>	<b>0.97</b>
<b>HRR3</b>	<b>111 <math>\pm</math> 12<sup>†</sup></b>	<b>100 <math>\pm</math> 7</b>	<b>94 <math>\pm</math> 5</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.37</b>	<b>0.99</b>
<b>HRR4</b>	<b>109 <math>\pm</math> 13<sup>†</sup></b>	<b>98 <math>\pm</math> 7</b>	<b>92 <math>\pm</math> 5</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.34</b>	<b>0.98</b>
<b>HRR5</b>	<b>108 <math>\pm</math> 13<sup>†</sup></b>	<b>97 <math>\pm</math> 7</b>	<b>88 <math>\pm</math> 6</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.39</b>	<b>0.99</b>
$\Delta$ 30	13 $\pm$ 6	15 $\pm$ 6	17 $\pm$ 5	0.40	0.38	0.04	0.20
$\Delta$ 1	22 $\pm$ 8	23 $\pm$ 7	24 $\pm$ 6	0.75	0.72	0.01	0.09
$\Delta$ 2	30 $\pm$ 11	32 $\pm$ 9	30 $\pm$ 6	0.80	0.76	0.01	0.08
$\Delta$ 3	34 $\pm$ 12	36 $\pm$ 10	37 $\pm$ 6	0.71	0.67	0.02	0.10
$\Delta$ 4	36 $\pm$ 14	37 $\pm$ 9	39 $\pm$ 6	0.85	0.83	0.008	0.07
$\Delta$ 5	44 $\pm$ 31	39 $\pm$ 11	42 $\pm$ 10	0.83	0.80	0.01	0.08

TC: total cholesterol; TG: triglycerides; HbA<sub>1c</sub>: glycosylated hemoglobin; CRP: C-Reactive protein; INF- $\gamma$ : interferon gamma; TNF- $\alpha$ : tumor necrosis factor alpha; MCP-1: monocyte chemoattractant protein 1; CAT: catalase; GPX: glutathione peroxidase; SDNN: standard deviation of the R-R series; RMSSD: square root of the mean of the sum of the squares of differences between adjacent R-R intervals; LF: low frequency power; HF: high frequency power; SD1: the short-term modulation from Poincare Plots; SD2: short-term modulation axes from Poincare Plots; SampEn: sample entropy;  $\alpha$ 1: short-term detrended fluctuations;  $\alpha$ 2: long-term detrended fluctuations; HRR: heart rate recovery; HRR $\tau$ : time constant of the exponential fit for the HRR; amp: amplitude of the exponential fit; ES: effect size; 1- $\beta$ : observed statistical power.

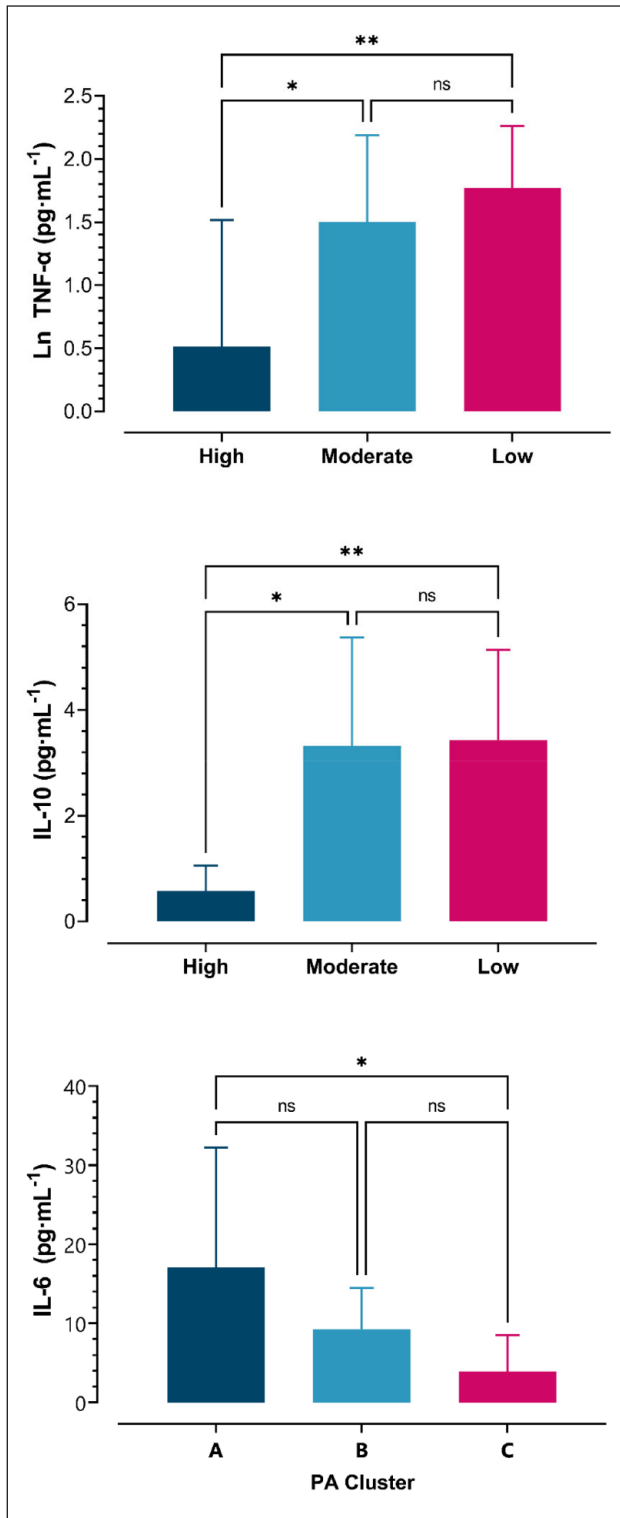
Significant results are highlighted in bold.

<sup>§</sup>Significantly different from High.

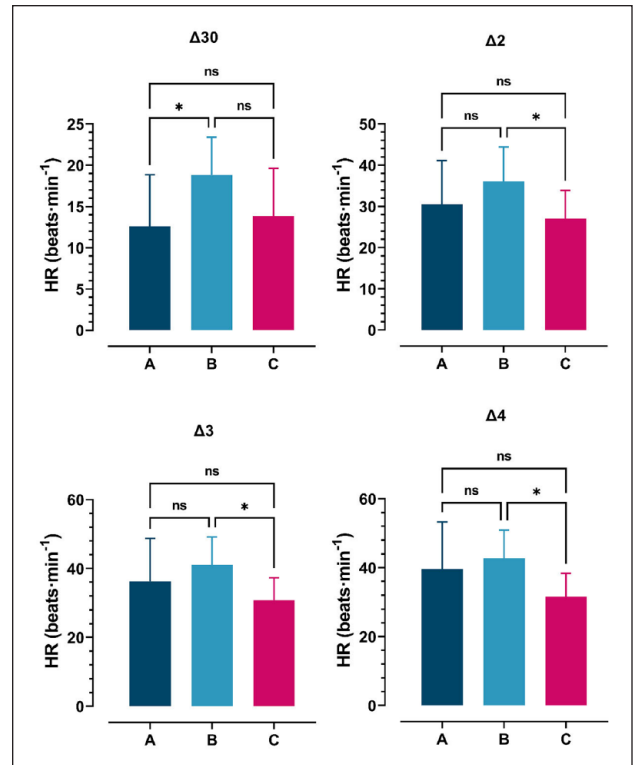
<sup>†</sup>Significantly different from moderate and high CRF.

<sup>§</sup>Kruskal-Wallis test.





**Figure 1.** Comparison of inflammatory markers, Tumor Necrosis Factor alpha (TNF- $\alpha$ ) and interleukin-10 (IL-10) between cardiorespiratory clusters (upper and middle panels) and interleukin-6 (IL-6) between physical activity clusters (lower panel). For the physical activity clusters, A=High MVPA/Low LPA, B=Low MVPA/High LPA, C=Low MVPA/Low LPA. NS: not significant. \*Significantly different at  $p < 0.05$ . \*\*Significantly different at  $p < 0.01$ .



**Figure 2.** Comparison of relative heart rate recovery ( $\Delta$ ) between physical activity clusters. A=Low MVPA/High LPA, B=High MVPA/Low LPA, C=Low MVPA/Low LPA. NS: not significant. \*Significantly different at  $p < 0.05$ . \*\*Significantly different at  $p < 0.01$ .

inflammatory cytokines.<sup>32</sup> For instance, an association between low prolonged SEDT with WC has been previously reported.<sup>19</sup> As such, it is not surprising that, in addition to SEDT, CRP was associated with WC. Moreover, the association between CRP and the cardiometabolic risk cluster (OR=7.66) reinforces the previous argument.

Regarding PA levels, we only found elevated levels of IL-6 for the PA cluster characterized for low LPA and low MVPA. Previously, Hamer et al.<sup>33</sup> in a 10-year follow-up study reported that physically active participants had lower levels of CRP and IL-6 both at baseline and at follow-up. Similarly, Vella et al.<sup>34</sup> observed lower levels of IL-6, and other inflammatory markers, in the highest quartile of MVPA after adjusting for several covariates. This later study emphasizes the importance of MVPA as we also found lower levels of IL-6 in the group with higher levels of MVPA. Moreover, it has been shown that reallocating 30 min of sedentary time or LPA with MVPA was associated with a reduction in CRP with no effect observed when SEDT was replaced with LPA.<sup>35</sup> Collectively, these findings could suggest a higher value of MVPA over LPA to ameliorate inflammation.

HRV reflects central regulation of autonomic activity and is a simple measure for assessing cardiovascular

health. In this sense, several studies have evaluated the association between PA or SEDT and HRV/HRR using different approaches.<sup>15,36,37</sup> For instance, Tonello et al.,<sup>15</sup> reported moderate correlations between vigorous and the sum of vigorous (VPA) plus very vigorous PA (VVPA) and several indices of HRV and with the HRR at 1 min. Similarly, using short-term HRV, de Sousa et al.,<sup>36</sup> stratified their participants according to weekly VVPA and reported a better HRV with increased VVPA. On the other hand, Niemelä et al.<sup>37</sup> observed an association between sedentary bouts and the RMSSD independent of covariates, such as CRF and PA. In our study, after controlling for potential confounders, we did not find associations between PA or SEDT and the different HRV indices. Besides the differences in study design and data analysis among the studies, our results showed that HRV varied between sexes and age groups. Particularly, the age cluster used in our analysis showed a reduced HRV with increasing age. Moreover, the older group in our study did not engage significant time in VPA compared to the younger group. Thus, it could be hypothesized that the combined effects of age and low VPA would explain the differences between the findings of the present and the previous studies. Conversely, an adjusted association was found between LPA and post-exercise  $\Delta$ HRR in the whole sample with a better relative HRR in the cluster with higher MVPA and correlations between HRR and LPA and MVPA in the CRF and Age clusters. These findings are similar to those reported by Tonello et al.<sup>15</sup> and Fan et al.,<sup>38</sup> who found an association between HRR versus MVPA and HRR versus VPA, respectively. Given the fact that HRR was not associated with SEDT, a finding also reported by Niemelä et al.,<sup>37</sup> and the suggestion that PA can influence HRR in the same way as a training load<sup>15</sup> it could be suggested that HRV and HRR would differentially reflect the impact of SED and PA on autonomic regulation. However, it is worth noting that research on HRR and PA/SED is limited with further studies needed to have a deeper insight on these relationships, particularly as HRR is regarded as a strong prognostic of cardiovascular health.

The imbalance between sympathetic and parasympathetic nervous activity results in cardiac autonomic dysfunction. In this regard, an association between autonomic dysfunction and metabolic deregulation has been suggested,<sup>6</sup> with the sympathovagal imbalance being an independent risk factor for CVDs.<sup>8</sup> Furthermore, vagal activity has through discharging acetylcholine has been shown to reduce the production of proinflammatory cytokines.<sup>10</sup> In this sense, several studies have shown a reduced HRV with increased inflammatory markers.<sup>13,14,39</sup> For instance, both Cooper et al.<sup>13</sup> and Alen et al.<sup>39</sup> reported associations between HF and LF with fibrinogen, IL-6, and CRP. More recently, a meta-analysis of 51 studies conducted by Williams et al.,<sup>14</sup> indicated an overall negative relationship between HRV and markers of

inflammation. In contrast, we only found a positive association between IL-10 and  $PNS_{INDEX}$ , whereas HF was positively related to IL-23 and negatively related to CAT. Although a mechanistic explanation for these relationships is beyond the scope of the present study, it is worth noting that a role of the vagal anti-inflammatory pathway as a regulator of systemic inflammation<sup>13</sup> has been suggested and thus, the association between IL-10 and HRV should be further explored. Conversely, both HRV and HRR were related to oxidative stress markers which could be suggestive of an increased sympathetic modulation affecting the oxidative stress profile.

In conclusion, taken together our results could indicate that prolonged SEDT combined with low MVPA, would lead to an increase in an inflammatory and oxidative stress environment. This augmented inflammatory state may lead to an impaired autonomic balance increasing the risk of cardiovascular disease. All these relationships, in time, appear to be mediated by increased age and reduced cardiorespiratory fitness which highlights the value of maintaining a high CRF, especially with the advance of age and increased SED behavior.

### Study limitations

The main limitation of this study is the sample size. In this regard, we have presented the statistical power and the effect size for each analysis. While in some cases there were power values under the 0.8 threshold, in most cases the observed statistical power was of sufficient magnitude to reduce the rate of type 2 error. Also, the use of an estimation instead of a direct measure of  $VO_{2max}$  could have an impact on the reported relationships. However, it can be noted that the Åstrand-Rhyming protocol has been used previously in the literature and is considered a valid measure of the CRF. Of note, the accelerometers used in this study do not measure body position, which is considered the gold standard for sedentary behavior. Lastly, while we employed short-term HRV measures it has recently suggested that utilization of weekly HRV measures may provide a better assessment of vagal modulations for health.<sup>40</sup> As such, further studies should look at longer follow-up periods of PA/SED and HRV.

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## Author contributions

Conceptualization: SDR, NRP, and MPA; Methodology: SDR, NRP, and MPA; Data Collection: SDR and LB, Formal analysis and investigation: SDR, LB, GB, FM, YLM; Writing—original draft preparation: SDR; Writing—review and editing: SDR, LB, GB, FM, YLM, MPA, and NRP; Funding acquisition: NRP and MPA; Supervision: NRP, MPA. All authors read and approved the final manuscript and agree with the order of presentation of the authors

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Ethics approval and consent to participate

All individuals were informed about the risks and benefits of the study and signed an informed consent form. The protocol of the study was approved by the HNC ethics committee (No. 193/14), and all methods employed are in accordance with the Declaration of Helsinki for research on human subjects.

## Informed consent

The manuscript does not contain any individual person's data in any form

## Significance for public health

Cardiometabolic risks imply a complex metabolic deregulation involving inflammation, oxidative stress, and autonomic imbalance. Physical activity has a pivotal role in health promotion and disease prevention and, is a simple and low-cost intervention for the improvement of cardiorespiratory fitness, a factor strongly linked to the incidence and risk of most cardiometabolic diseases and one of the main determinants of long-term survival. Conversely, there is evidence suggesting that physical inactivity is the fourth risk factor related to worldwide mortality. In this study, we show that physical inactivity is related to a higher inflammatory environment and a lower heart rate variability independently of physical activity levels. At the same time, cardiorespiratory fitness is an important mediator of the interrelationship between sedentary time, autonomic balance, and inflammation. Moreover, our findings that some inflammatory and oxidative stress markers are related to slower post-exercise heart rate recovery, which can be an additional risk factor for cardiovascular diseases.

## Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

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