Article

Autonomic Function Recovery in Post-Covid-19 Young Adults After Immunization: An Observational Follow up Case-control Study

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Abstract: Coronavirus disease 2019 (COVID-19) has detrimental multi-system consequences. Symptoms may appear during the acute phase of infection, but literature on long-term recovery of young adults after mild-to-moderate infection is lacking. Heart rate variability (HRV) allows observation of autonomic nervous system (ANS) modulation post SARS-CoV-2 infection. Additionally, physical activity (PA) helps improve ANS modulation, where investigation of PA influence on ANS recovery is vital to reduce risk and severity of symptoms. Clinicians may use this research to aid development of non-medication interventions. At baseline, 18 control (CT) and 20 post-COVID-19 (PCOV) participants were observed where general amnamnesis was performed, followed by HRV and PA assessment. 10 CT and 7 PCOV subjects returned for follow-up (FU) evaluation 6 weeks after complete immunization (2 doses) and assessments were repeated. Over the follow-up period, decrease in sympathetic (SNS) activity (mean heart rate: p=0.0024, CI=-24.67- -3.26; SNS index: p=0.0068, CI=-2.50- -0.32) and increase in parasympathetic (PNS) activity (mean RR:p=0.0097, CI=33.72-225.51; PNS index: p=0.0091, CI=-0.20-1.47) were observed. At follow-up, HRV was not different between groups (p>0.05). Additionally, no differences were observed in PA between moments and groups. This study provides evidence of ANS recovery after SARS-CoV-2 insult in young adults over a follow-up period, independent of changes in PA.

Keywords: COVID-19; SARS-CoV-2; exercise; autonomic nervous system; sympathetic nervous system; parasympathetic nervous system; COVID-19 vaccination; post-acute sequelae of COVID-19; communicable diseases

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1. Introduction

The coronavirus disease (COVID-19) is a rapidly spreading condition caused by the SARS-CoV-2 virus that infiltrated global populations at alarming rates (1). Despite the severe complications that may arise through contracting COVID-19, a significant number of individuals did not require intensive care (mild-to-moderate cases) and continued to present with physical (2), neurological(3, 4), and other autonomic nervous system (ANS) related dysfunctions (2, 4-7) four to six months after diagnosis(2, 6).

Autonomic modulation was shown to be impaired even in young adults short after mild and moderate COVID-19 (8-10). The presence of SARS-CoV-2 virus within the carotid body, can be a possible mechanism explaining the observed silent hypoxemia and thus providing a pathway for nervous system infiltration and the subsequent ANS dysfunction (2, 8, 11, 12). Also, disruption in autonomic regulation by a viral pathogen is associated with a cytokine storm immune response, which results in oxidative stress leading to cell damage (13).

It is well established that ANS plays a major role in modulating homeostasis through influencing systemic bodily functions. Dysfunction of the ANS could results in detrimental effects of downstream physiological processes associated with respiratory, vascular, immune, hematological, and renal processes (14, 15). Increases in SNS activity have also been indentified to be an independent predictor of mortality in association with several diseases (16, 17). Considering previous studies that reported persistent symptoms and impairments as a results of post-acute sequelae of COVID-19 (PASC), it is vital to investigate ANS behavior for longer periods of time following infection by the SARS-CoV-2 (8, 18, 19).

Additionally, investigating factors that can influence better ANS outcomes after COVID-19 is essential to advancements in care and intervention strategies. Previous research has found that physical activity (PA) levels can directly influence autonomic modulation (20, 21) and can be associated with reduced risk and severity of COVID-19 symptoms (9, 18, 19). These findings highlight the magnitude of PA in regulating multiple autonomic system routes to maintain homeostasis and by which may provide a protective effect from symptoms experienced from SARS-CoV-2 infection. An improved understanding of the influences of the long term effects of COVID-19 on ANS and consequently, multiple body systems is vital for developing public health strategies that can reduce the impact of the disease. Clinicians may consider evidence from this literature as means for guidance to improve clinical assessment and develop non-medication intervention strategies in the prevention and recovery from infection in a young adult population.

Recently, our group demonstrated the short term effects of COVID-19 on ANS in young adults (9) but long-term effects of COVID-19 still needs research. Young adults are an overlooked population, possibly due to the fact that they are the least at risk for severe negative outcomes. However, the high incidence rate in young adults (22) emphasizes the need for research into the effects of COVID-19 on this population. Therefore, the primary aim of this study was to observe the effects of mild-to-moderate COVID-19 on ANS function over a follow-up period, including before and after a 6-week complete immunization period, in young adults. Secondarily, we aim to identify PA behavior over that period and analyze possible correlations with ANS modulation.

2. Materials and Methods

2.1. Ethical approval

The Declaration of Helsinki's ethical guidelines were followed in conducting the present study. All participants gave written informed permission to all protocols after being apprised of the study's goals and procedures. The study was approved by the Sao Paulo State University Ethical Institutional Review Board (approval number: 38701820.0.0000.5402).

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2.2. Study design

This study followed an observational prospective case-control design. The results presented in this research are part of FIT-COVID Study (23). Prior to the beginning of the study the protocol was previously registered at the Brazilian Clinical Trials Registry (registration number: RBR-5dqvkv3). The reports presented along the study followed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (24).

We recruited individuals who were infected with COVID-19 to take part in the study using local media, including television, radio and social media. Data from the database of the Municipal Health Secretariat of Presidente Prudente São Paulo, Brazil was also accessed. The database showed in May 2021 the following data: 231,953 inhabitiants; human development index, 0.806; 23,657 confirmed COVID-19 cases, moving average of 135 cases.

Male and female subjects between the ages of 20 and 40 who had been diagnosed with mild or moderate clinical COVID-19, had a prior positive polymerase chain reaction (PCR) test, and had mild clinical symptoms like fever or respiratory symptoms but hadn't been admitted to the intensive care unit met the inclusion criteria.

After a positive PCR test diagnosis for at least 15 days and up to 120 days, participants were included (25). Additionally, a healthy, age-matched control group that tested negative for COVID-19 was enlisted. The control group underwent a lateral flow test for SARS-CoV-2 Immunoglobin G (IgG) and Immunoglobin M (IgM) antibodies using amplified chemiluminescence and chemiluminescence serological techniques to screen for proven or suspected prior SARS-CoV-2 infection.

The following criteria was used for exclusion for this study: (1) the existence of any chronic noncommunicable diseases; (2) smoking; (3) a history of continuous drug use; (4) the use of medications such as anti-inflammatory drugs, antibiotics, and other drugs known to have an effect on the ANS; (5) participants who had received intensive care during COVID-19 treatment.

2.3. Evaluations

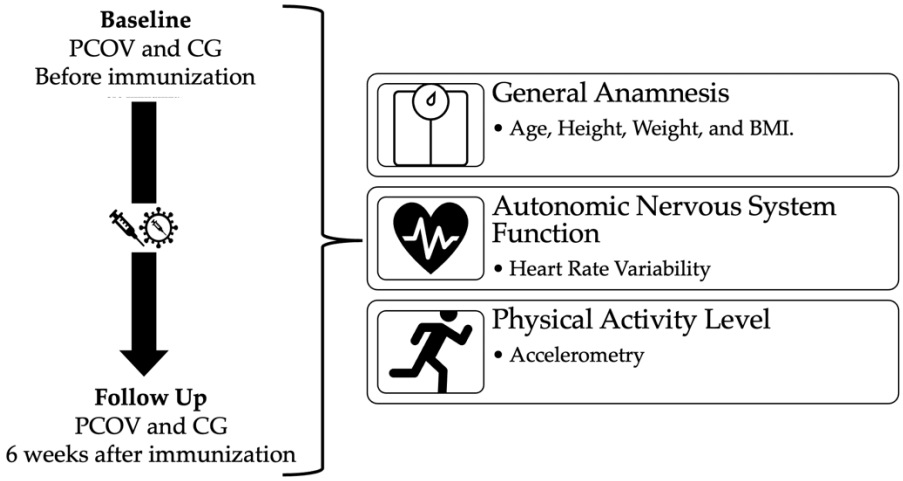

Figure 1. Study design. Evaluations performed on Post-COVID19 (PCOV) and Control group (CG) at baseline and after a minimum six weeks following complete immunization (follow-up).

Initially, a general anamnesis was obtained to determine sociodemographic information, self-rated health, and medical history. After that, participants', Physical Activity level (26), and body mass index (BMI) were evaluated (27). Autonomic nervous system was assessed via Heart rate Variability analysis. Additionally, we also investigated

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symptoms that were present during acute phase of SARS-CoV-2 infection and recurrent symptoms (28). Finally, six weeks after receiving the full dose of the SARS-CoV-2 vaccine, the subjects repeated all assessments on a follow-up visit (23). Figure 1 shows the study design. For the control group (CG) and Post COVID-19 group (PCOV), the difference between follow-up and baseline assessments was 161.6045.94 days and 168.4224.26 days, respectively (approximately 5 months).

2.4. Body mass index

BMI was determined based on prior research. We defined BMI as the product of height (m) squared and weight (kg). Subjects wore light clothing and were barefoot while being weighed on an electronic, calibrated scale (Kratos-Cas, So Paulo, SP, Brazil). A portable anthropometer (Kratos-Cas, So Paulo, SP, Brazil) was used to measure height (29).

2.5. Heart rate variability

Heart rate variability (HRV), which is regarded as an easy, reliable, and noninvasive technique, was used to test ANS function (30). Participants were requested to come to an outpatient clinic for this examination and were told to come fasting at least 4 hours following light meal, having avoided exercise, caffeine, chocolate, and alcohol for at least 24 hours before to the evaluation. Evaluations were carried out in a quiet environment with a controled temperature of 23°C. To prevent unwanted effects of circadian variations, HRV analysis was always assessed in the morning (30).

In order to assess the cardiac autonomic modulation, heart rate was measured beat-to-beat. A cardio-frequency meter (Polar RS800CX, Polar Electro, Kempele, Finland) was used to this assessment using a sampling rate of 1kHz. Participants were positioned in a sitting position wearing a chest strap and a monitor, and were instructed breathe naturally for 25 minutes. The most stable section of the tachogram was used to conduct HRV analysis on 256 consecutive RR intervals, or the time between successive heartbeats. Only series with less than 5% error were deemed appropriate for analysis.

The HRV analysis was carried out using Kubios HRV program (Biosignal Analysis and Medical Image Group, Department of Physics, University of Kuopio, Finland) (31, 32) HRV was evaluated in both the time and frequency domains. The mean RR intervals, which reflect worldwide variability, were employed for the temporal domain. The indices listed in Table 1 were calculated.

Table 1. Description of heart rate variability (HRV) indexes evaluated. Adapted from(33).

Sympathetic Nervous System Activity	**Unit**	
Mean HR	bpm	Average heart rate
Stress Index	~	Baevsky's stress index, a geometric measure of HRV
LF	nu	Relative power between 0.04 Hz to 0.15 Hz
SNS Index	~	Calculated based on the mean HR, Baevsky's stress index, and SD2 in normalized units
Peripheral Nervous System Activity		
Mean RR	ms	Average time of R-R intervals
RMSSD	ms	Square root of mean squared difference between adjacent RR intervals
HF	nu	Relative power between 0.15 Hz to 0.4 Hz
pNN50	%	Percentage of successive RR intervals that differ by more than 50 ms
SD1	ms	Poincaré plot standard deviation perpendicular the line of identity

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PNS index	~	Calculated based on the mean RR, RMSSD, and SD1 in normalized units
Global Variability		
SDNN	ms	Standard deviation of all normal RR intervals
RR Triangular Index	~	Integral of the density of the RR interval histogram divided by its height
TINN	ms	Width of RR interval histogram
LF/HF	%	Ratio of low and high frequency power
SD2	ms	Poincaré plot standard deviation along the line of identity

HR: heart rate; LF: low-frequency component; SNS index: sympathetic nervous system index; RMSSD: root mean square of differences between adjacent normal RR intervals in a time interval; HF: high-frequency component; pNN50: percentage of adjacent RR intervals with a difference in duration >50 ms; SD1: standard deviation of instantaneous beat-to-beat variability; SD2: standard deviation of long-term intervals between consecutive heartbeats; PNS index: parasympathetic nervous system index; SDNN: standard deviation of all normal RR intervals recorded in a time interval; TINN: triangular interpolation of NN interval; ms: millisecond; nu: normalized unit; HRV: heart rate variability; Hz: Hertz.

2.6. Physical activity level

PA level was measured using a triaxial accelerometer (GT3X+; ActiGraph, LLC, Pensacola, FL, USA). Participants were instructed to wear the accelerometer above the waist for seven consecutive days during waking hours. A minimum of four days with at least 10 hours per day was considered valid accelerometer data. Participants were instructed not to use the accelerometer while bathing, sleeping, or performing water activities. Moreover, every morning, a researcher sent a WhatsApp message reminding the participant to use the accelerometer.

Non-wear periods were defined as time intervals of at least 60 consecutive minutes of zero counts, with an activity interruption allowance of 0-100 counts per minute lasting a maximum of 2 consecutive minutes (34). Counts per minute were calculated using the sum of the total activity counts in the vertical axis divided by the valid number of days. Sedentary time was defined as values <100 counts per minute, light PA as values between 100 and 2019 counts per minute, and moderate-vigourous PA (MVPA) as values >2020 counts/minute. Data were processed using ActLife software (version 6.9.2, Pensacola, FL, USA)(35).

2.7. Statistical Analysis

GraphPad Prism 9 was used for the statistical analysis (version 9.3.1; GraphPad Software, San Diego, CA, USA). The Shapiro-Wilk test was used to examine the distribution of the data. Depending on the distribution of the data, the unpaired t-test or Mann-Whitney U-test was employed for the primary analysis of intergroup comparisons between the Post-COVID-19 (PCOV) and control groups (CG). The Welch's correction was further utilized to compare variables with different standard deviations.

For intragroup paired analysis, paired T test or Wilcoxon test were performed. The Chi-square test was used to assess for differences within the categorical variables of PA. The Fisher exact test was performed to assess for differences between sex distribution.

Effect size (ES) was calculated using the difference between moments to determine the magnitude of differences over time. ES was represented by Cohen's d values and was classified as follows: negligible (<0.01), small (0.01-0.29), medium (0.3-0.49), and large (≥0.5) (36).

Secondarily, the data were analyzed using analysis of covariance (ANCOVA) to better understand the influences of PA on the change in HRV indexes (37). The dependent variable was represented by each HRV index and MVPA was used as the covariate in this multivariable regression. Statistical significance was considered $p < 0.05$.

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3. Results

239 control and 154 post-COVID subjects were assessed for eligibility for this study. 92 control and 33 post-COVID subjects were deemed eligible to participate in this study and 268 individuals did not meet the inclusion criteria. 57 of these subjects were evaluated at baseline. After 19 exclusions due to errors in heart rate variability recordings, 38 subjects with complete data were included in the baseline analysis where 18 subjects were in CT and 20 in the PCOV group. Subjects then returned six weeks after receiving the second shot of COVID-19 immunization. At this stage, 20 participants were excluded due declining a follow-up assessment and one individual was excluded due to SARS-CoV-2 reinfection. The number of individuals at each stage of study are presented in Figure 2.

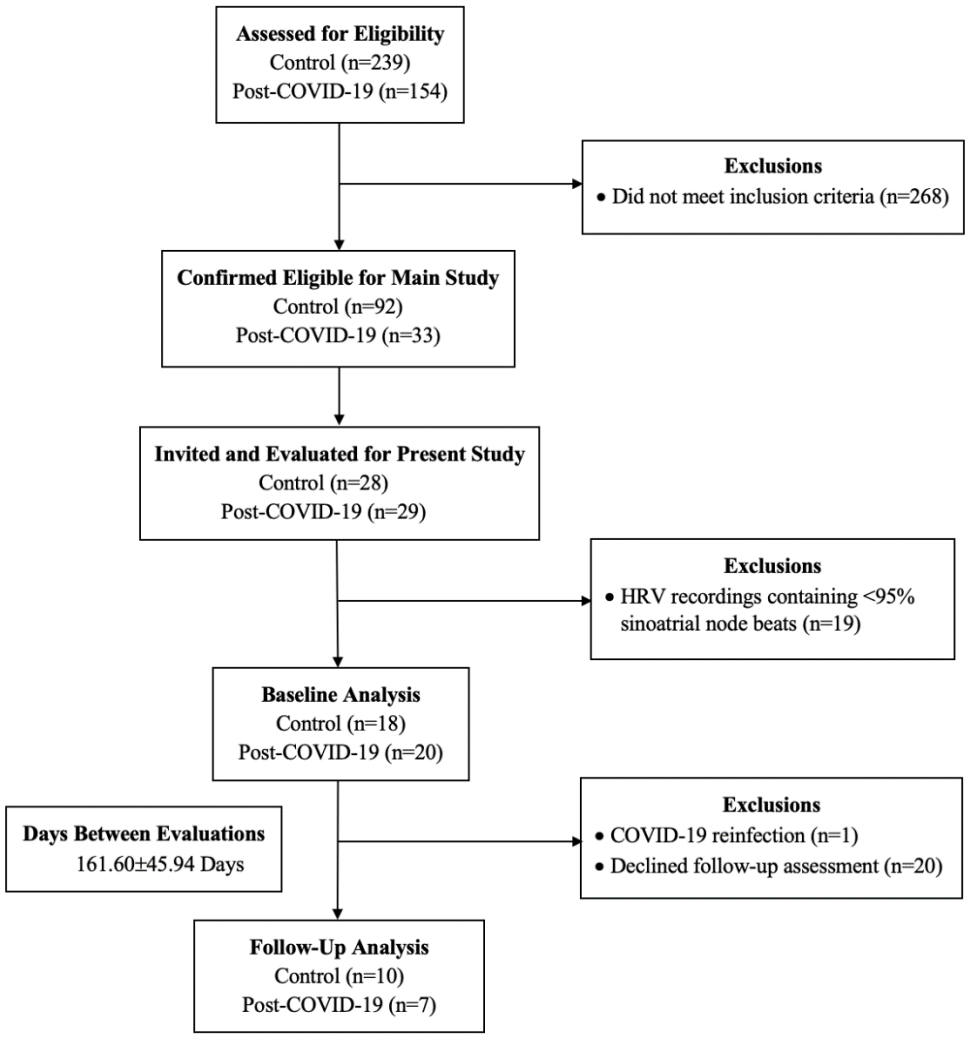

Figure 2. Flow diagram of the study.

No differences in sex distribution were observed between the groups (p=0.1119). Descriptive characteristics of age, weight, height, and body mass index were uniformly distributed and not different between groups as indicated in Table 2. Participants in CT returned for follow-up evaluation 161.60±45.94 days after baseline evaluation and PCOV returned after 168.42±24.26 days (approximately 5 months). In CT, 30% received AstraZeneca immunization, 30% received CoronaVac, and 40% received Pfizer. In PCOV, 57% received CoronaVac immunization and 43% received Pfizer. Additionally, patients in PCOV were evaluated 50±32.54 days after testing positive for SARS-CoV-2, classified as mild to moderate COVID-19 cases (25).

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There were no differences in PA between CT and PCOV groups and between baseline and follow-up moments ($p>0.05$).

Results for cross-sectional analysis with comparisons between CT and PCOV at baseline are presented in a previous study by the FIT-COVID group (9).

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Table 2. Sample characterization and physical activity data.

	Control					Post-COVID						
	Baseline (n=18)		Follow-Up (n=10)		Intragroup Analysis	Baseline (n=20)		Follow-Up (n=7)		Intragroup Analysis	Intergroup Analysis	
	Median	IQR	Median	IQR	Difference Between Means	Median	IQR	Median	IQR	Difference Between Means	95% CI	p-value
Sex (M/F)	13/5	~	5/2	~		9/11	~	3/2	~	~	~	0.1119
Age (years)	26.66	21.11-31.41	~	~	~	28.47	24.73-33.77	~	~	~	~	~
Weight (kg)	71.65	57.85-88.85	1.69	1.63-1.79	-1.67 ± 8.56	77.35	65.75-90.68	1.70	1.64-1.76	-10.31 ± 6.73	-2.20 to 6.25	0.4116
Height (m)	1.76	1.65-1.79	62.70	51.20-103.60	-0.03 ± 0.04	1.71	1.61-1.77	67.40	59.70-72.30	0.004 ± 0.04	-0.02 to 0.01	0.7348
BMI (kg/m^2)	23.92	21.11-28.20	23.60	20.00-31.98	-0.01 ± 2.29	25.45	23.13-31.34	23.53	19.76-26.41	-3.58 ± 2.47	-0.77 to 2.27	0.4318
Days between Positive Test and Follow-Up	~	~	~	~	~	37.50	24.75-70.50	~	~	~	~	~
Physical Activity												
	n=16		n=8			n=16		n=4				
	Median	IQR	Median	IQR		Median	IQR	Median	IQR			
Sedentary Activity (min/day)	578.40	534.9-676.7	499.60	467.7-664.3	-92.97 ± 98.48	499.60	467.10-593.50	601.10	493.50-647.00	-33.88 ± 146.50	-304.70 to 501.70	0.9048
Light Activity (min/day)	251.50	188.50-291.00	432.30	220.00-407.30	52.77 ± 33.62	285.10	206.80-432.30	258.50	235.20-306.70	-42.21 ± 60.08	-207.20 to 200.10	0.7302

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Moderate Activity (min/day)	17.90	5.1-29.9	33.72	6.04-15.57	-10.65 ± 8.58	13.36	9.25-33.72	27.86	23.78-32.25	4.73 ± 11.36	-41.82 to 26.99	0.5556
Vigorous Activity (min/day)	3.65	0.00-15.8	0.50	0.00-2.36	-7.73 ± 4.33	0.00	0.00-0.50	0.13	0-4.67	0.92 ± 1.00	-7.61 to 1.73	0.0794
MVPA (min/day)	23.72	12.60-46.00	34.04	6.36-17.13	-19.13 ± 11.86	14.79	9.60-34.00	31.06	24.92-32.72	5.51 ± 11.69	-44.45 to 18.91	0.4127
Steps Count (steps/day)	6571.00	4994.00-7795.00	4823.00	3715.00-8585.00	-1297.00 ± 1706.00	5232.00	3697.00-9601.00	6350.00	5996.00-7198.00	187.10 ± 1586	-7513.00 to 5084.00	0.6095
Total Time in BSB 30-60min Bouts	169.40	97.30-222.30	161.10	63.24-239.50	-18.99 ± 39.95	91.36	73.36-134.20	137.40	98.78-188.00	30.67 ± 35.92	-130.40 to 175.50	0.9143
Total Time in BSB ≥60min Bouts	34.31	19.20-99.60	46.79	4.04-69.32	-17.29 ± 22.46	17.63	8.86-29.32	30.07	2.25-57.36	-4.35 ± 25.22	-57.30 to 98.68	0.9143

IQR: interquartile range; M: male; F: female; kg: kilogram; m^2: square meter; BMI: body mass index; MVPA: moderate to vigorous physical activities;
IQR: interquartile range; min: minutes; MVPA: moderate to vigorous physical activity; BSB: breaks in sedentary behavior.

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Figure 3 presents the intragroup analysis. There was no difference between baseline and follow-up moments within CT or PCOV groups (p>0.05) regarding SNS activity (Figure 3). Parasympathetic nervous system (PNS) activity increased between baseline and follow up moments, reflected by Mean RR (p=0.0312) and pNN50 (p=0.0312) for PCOV group (Figure 3). Mean RR increased from 734.50±131.80 ms at the baseline moment to 794.90±105.60 ms at the follow-up moment and pNN50 increased 4.53±4.68% to 11.74±8.81%. No differences between moments were observed in CT (p>0.05). The intragroup analysis between moments also revealed a difference in global variability, reflected by RR triangular index (7.91±2.34 at baseline to 9.60±2.17 at follow-up; p=0.0312; Figure 3).

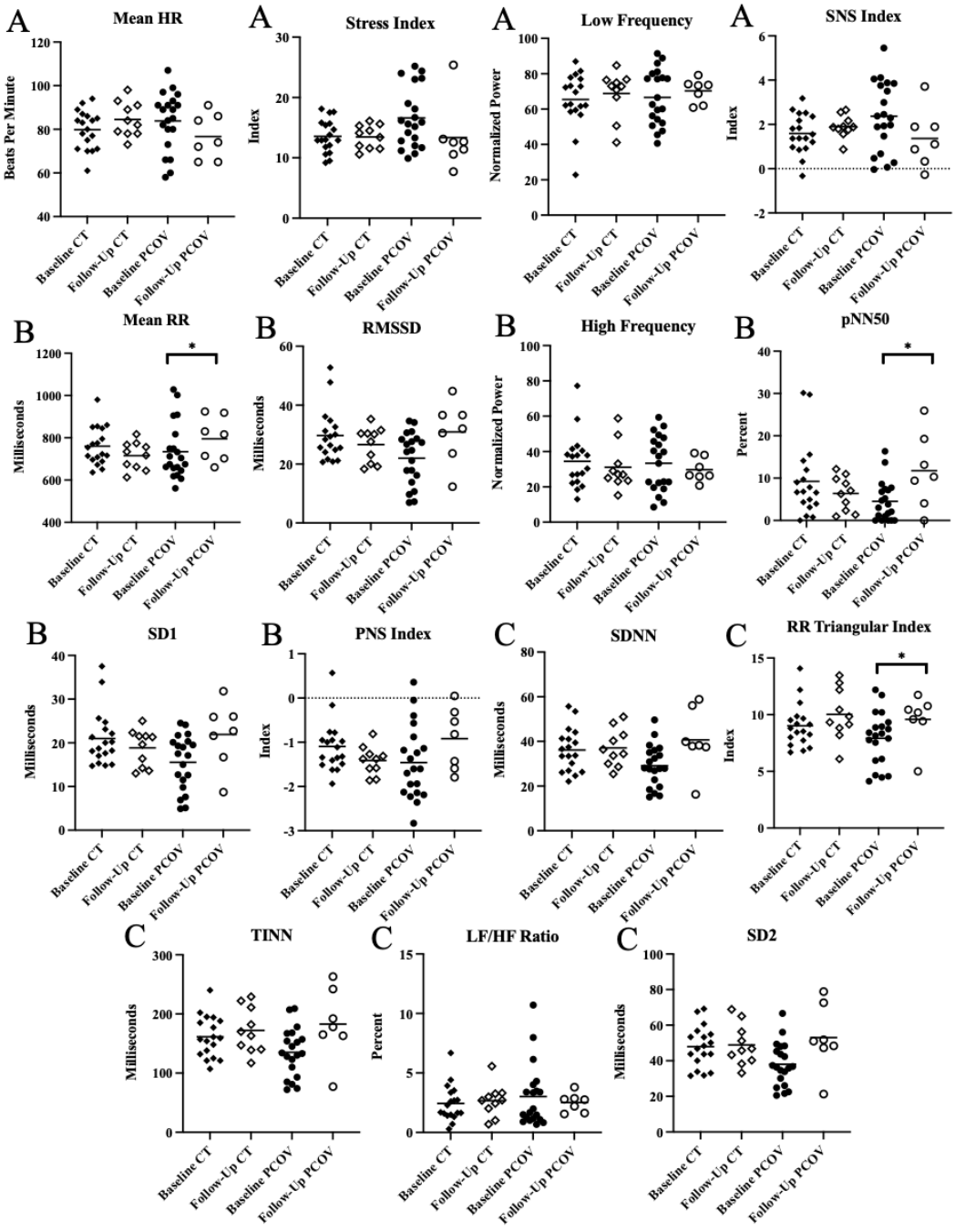

Figure 3. Scatterplot of HRV indexes for paired analysis according to group expressed as the sample mean. (**A**) sympathetic nervous system activity; (**B**) parasympathetic nervous system activity; (**C**) global variability. * Statistical difference between moments; HR: heart rate; LF: low-frequency

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component; nu: normalized unit; SNS index: sympathetic nervous system index; RMSSD: root mean square of differences between adjacent normal RR intervals in a time interval; HF: high-frequency component; pNN50: percentage of adjacent RR intervals with a difference in duration >50 ms; SD1: standard deviation of instantaneous beat-to-beat variability; ms: millisecond; PNS index: parasympathetic nervous system index; SDNN: standard deviation of all normal RR intervals recorded in a time interval; RR triangular index: Integral of the density of the RR interval histogram divided by its height; TINN: triangular interpolation of NN interval; LF/HF: ratio of low and high frequency power; SD2: standard deviation of long-term intervals between consecutive heartbeats.

Table 3 portrays primary analysis of the present study, exhibiting intergroup comparisons between HRV indexes in control (CT) and PCOV groups (S1). We observed that PCOV group presents significant reductions in sympathetic activity over time when compared to CT demonstrated by mean HR (p=0.0088) and SNS index (p=0.0068; Table 3; S1). PCOV also presented with a significant increase in PNS activity over time demonstrated by mean RR (-44.54±32.38 vs. 60.36±55.35 ms; p= 0.0097) and PNS index (-0.32±0.20 vs. 0.54 ± 0.35; p= 0.0091) when compared to CT (Table 3; S1). No intergroup differences were observed in global variability.

Table 3. Comparisons of change in HRV indexes in the post-COVID and control groups and between groups.

	Control (n=11)	**Post-COVID (n=7)**				
	Intragroup Analysis	**Intragroup Analysis**	**Intergroup Analysis**			
	Dif Between Means	**Dif Between Means**	**Unadjusted 95% CI**	**Unadjusted p-value**	**Adjusted 95% CI**	**Adjusted p-value**
			SNS Activity			
Mean HR	4.67 ± 3.37	-7.13 ± 5.59	-25.83 to -4.33	0.0024**	-24.67 to -3.26	0.014+
Stress Index	-0.10 ± 0.98	-3.26 ± 2.26	-0.60 to 6.51	0.0965	-6.62 to 0.57	0.093
LF (nu)	3.35 ± 5.76	3.66 ± 6.17	-31.16 to 2.17	0.0836	-2.26 to 30.39	0.086
SNS Index	0.31 ± 0.31	-1.01 ± 0.66	-2.48 to -0.29	0.0068**	-2.50 to -0.32	0.015+
			PNS Activity			
Mean RR	-44.54 ± 32.38	60.36 ± 55.35*	33.50 to 222.50	0.0097**	33.72 to 225.51	0.012+
RMSSD	-3.05 ± 3.15	8.95 ± 4.08	-17.06 to 0.80	0.1088	-0.79 to 17.35	0.071
HF (nu)	-3.35 ± 5.76	-3.62 ± 6.17	-2.33 to 31.11	0.0864	-30.33 to 2.40	0.089
pNN50	-2.84 ± 2.93	7.21 ± 2.61*	-14.62 to 0.19	0.0553	-0.21 to 14.88	0.056
SD1	-2.15 ± 2.24	6.35 ± 2.89	-12.12 to 0.56	0.1038	-0.55 to 12.32	0.07
PNS index	-0.32 ± 0.20	0.54 ± 0.35	0.20 to 1.45	0.0091**	-0.20 to 1.47	0.013+
			Global Variability			
SDNN	0.91 ± 3.63	11.54 ± 4.69	-20.92 to 0.96	0.0709	-1.31 to 21.40	0.078
RR Triangular Index	1.02 ± 0.80	1.68 ± 1.01*	-2.97 to 1.53	0.506	-1.61 to 3.06	0.517
TINN	10.67 ± 14.25	47.86 ± 20.32	-79.60 to 12.72	0.1434	-14.20 to 81.68	0.153
LF/HF	0.23 ± 0.58	-0.50 ± 1.03	-3.00 to 0.75	0.2199	-0.70 to 2.84	0.218
SD2	0.97 ± 4.55	15.03 ± 6.17	-27.16 to 1.65	0.0786	-2.16 to 27.80	0.088

Dif Between Means: difference between means (follow-up – baseline); CI: confidence interval; HR: heart rate; LF: low-frequency component; nu: normalized unit; SNS index: sympathetic nervous system index; RMSSD: root mean square of differences between adjacent normal RR intervals in a time interval; HF: high-frequency component; pNN50: percentage of adjacent RR intervals with a difference in duration >50 ms; SD1: standard deviation of instantaneous beat-to-beat variability; ms: millisecond; PNS index: parasympathetic nervous system index; SDNN: standard deviation of all normal RR intervals recorded in a time interval; RR triangular index: Integral of the density of the RR interval histogram divided by its height; TINN: triangular interpolation of NN interval; LF/HF: ratio of low and high frequency power; SD2: standard deviation of long-term intervals between

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consecutive heartbeats. *: Statistical significance ($p < 0.05$) between baseline and six-week follow-up moments. **: Statistical difference ($p < 0.05$) of difference between moments between the post-COVID-19 and control groups. +:Statistical difference ($p < 0.05$) of difference between moments between the post-COVID-19 and control groups after ANCOVA according to moderate-to-vigorous physical activity.

Intergroup comparisons between PCOV and CT at the follow-up moment revealed that there were no significant differences between the groups in any HRV index (p>0.05).

ES was determined using Cohen's d values. Variables that yielded significance in the inter-group analysis had ES reported. Within SNS activity indexes, Mean HR (ES=2.56) and SNS index (ES=2.56) both had large ESs. PNS activity indexes of Mean RR (ES=2.31) and PNS Index (ES=3.02) also yielded large ESs. No global variability indexes yielded significance during the inter-group analysis. Lastly, no PA variables presented significance in either the inter- or intra-group analysis.

The secondary analysis, including MVPA in the multivariable regression analysis model, showed that the significant group differences in mean HR, SNS index, mean RR, and PNS index were maintained (p<0.05) (Table 3).

4. Discussion

This study observed the effects of mild to moderate COVID-19 on the ANS in young adults, before and a minimum of 6-weeks after complete SARS-CoV-2 immunization. The relationship between PA and the changes in ANS were also analyzed. The primary finding of this study is that autonomic function was recovered in young adults who were infected by SARS-CoV-2 after approximately 5 months of follow up. This change was characterized by significant decreases in SNS HRV indexes (mean HR and SNS index), while also having significant increases in PNS HRV indexes (mean RR and PNS index). Additionally, when both groups were compared at follow up moment, our results revealed that the young adults infected by SARS-CoV-2 presented similar autonomic function when compared to CT. Secondarily, no differences were observed in PA between PCOV and CT groups over the follow-up period. The changes on HRV data were maintained even after statistical adjustments using PA levels.

To our knowledge, this study is the first to observe recovery of autonomic function after mild-to-moderate Post-COVID-19 infection in a young adult population within 164.41±91.47 days. Much of the existing literature focuses on older populations who present with greater risk of negative outcomes(3-6, 38) or those infected with severe Post-COVID-19(8). Additionally, a majority of this research focuses on cross-sectional data aimed at identifying autonomic dysfunctions associated with Post-COVID-19 rather than progression and recovery, making it increasingly difficult to inference on ANS modulation over time and the relationship with PA.

Previous work by Freire et al. (9) as part of the cross sectional data from Fit-COVID study revealed the presence of autonomic dysfunction after mild to moderate post-COVID-19 in young adults (represented as baseline comparisons in the present study). The ANS dysfunction observed at baseline was characterized by increases in SNS activity and decreases in PNS when comparing groups (9). Increases of the SNS are associated with a systemic inflammatory condition characterized by perfusion of inflammatory cytokines in the bloodstream and other biomarkers associated with inflammation (3, 8, 11, 13). Additionally, increases in SNS activity are associated with the secretion of catecholamines that increase metabolism and cardiac activity, which overall work to increase cardiac stress (39). Increases in biomarkers indicating oxidative stress have also been observed in association with COVID-19 and flu-like infections (3, 12, 40). The PNS's influence on anti-inflammatory and restorative processes are also inhibited (41). Previous literature does not identify the exact mechanism behind inhibition of PNS after COVID-19, but likely a function of the reciprocal nature between the SNS and PNS. Therefore, the increase in oxidative stress and subsequent cytokine storm in tandem with reductions in

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restorative processes that are associated with SARS-CoV-2 infection could explain the alterations observed at baseline.

This study did not observe changes in PA over time in either group, nor were PA levels between PCOV and CT different from one-another. Although a lack of statistical difference to explain observations in this study which may have been a result of an underpowered sample, PA levels still may have had an influence on the improvements in ANS observed in PCOV group as median PA levels increased in both groups from baseline to follow-up. PA aids in ANS modulation as the peripheral stress induced by skeletal muscle contraction or mechanical stress on organs sends afferent signals to the central nervous system, thus inducing a catecholamine (epinephrine and norepinephrine) release to alter ANS function to meet metabolic demands of exercise (21). The release of epinephrine and norepinephrine increase SNS activity, and consequently promoting PNS activity during recovery from exercise (42).

The Center for Disease Control(CDC) recommends that adults should participate in 150 minutes of moderate PA per week or 75 minutes of vigorous PA to observe physiological benefits that protect against COVID-19 outcomes and reduce risk of many diseases- (43). At baseline, CT had a median moderate PA of 17.90 minutes per day (min/day) which equates to 125.30 minutes per week (min/wk) of moderate PA while PCOV had a median of 13.36 min/day, equating to 93.52 min/wk of moderate PA. Baseline assessments revealed that CT and PCOV did not meet CDC recommendations for moderate or vigorous PA. Not meeting the CDC, PA standard may be explained by modifications in behavior that were a result of sanitary efforts. Many countries employed lockdowns and restrictions to public areas to avoid the continued spread of COVID-19, which increased the difficulty of being physically active.

This difficulty in being physically active was reflected in observations by Huber et. al. (44), in which PA was significantly reduced in young adults during the pandemic. Studies have also found that young adults did not return to pre-pandemic PA levels, which was characterized by a combination of increased sedentary activity and a lower frequency of PA bouts (45). By the time of the follow-up assessment, both CT and PCOV increased moderate PA levels to satisfy CDC PA standards where CT presented with a median of 33.72 min/day (236.04 min/wk) of moderate PA and PCOV increased to a median of 27.86 min/day (195.02 min/wk) of moderate PA. The increase in PA in both groups can likely be explained by the relaxation of social restrictions driven by increasing SARS-CoV-2 immunization rates (46), allowing participants in this study easier access to public spaces such as gyms and community parks.

PA is important in the modulation of immune responses where increased levels of PA are associated with improved cytokine responses, and thus reducing the severity of COVID-19 disease (47). PA helps to modulate the release of anti-inflammatory factors, and thus may aid in immune response to SARS-CoV-2 infection. Specific immune cell activities are regulated by the ANS, where dysfunction in the regulatory system can lead to inadequate immune stimulation (12). In response, there are accumulation of reactive nitrogenous species (RNS) and reactive oxygen species (ROS) at the site of cell damage, where persistent inflammation can lead to cellular oxidative damage and damage to the infected host (48). PA can aid in the immune response to infection through increasing antioxidant capacity and increasing stimulation of the T-helper 2 (TH2) cells pathway (12). PA increases the level of oxidative stress in skeletal muscle as a product of metabolic processes, where a negative feedback mechanism increases levels of antioxidants as a defense to the presence of ROS and RNS (49). The tight relationship between PA level and immune response illustrates a possible mechanism as to why we observed improved ANS function in this young adult population after SARS-CoV-2 infection.

This study presents significant clinical relevance as it provides further clarity on the relationship of PA behaviors on recovery after mild-to-moderate COVID-19 infection in a young adult population. We were able to observe alterations in ANS activity after a 5-month follow-up period in a young adult population, highlighted by reductions in SNS

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activity and increases in PNS activity. This observation is likely due to the PA level of this population as CT and PCOV met CDC recommendations for physical activity by the time of the follow-up assessment. This evidence better informs health professionals on non-medicinal approaches to reduce risk and severity of COVID-19 outcomes by using PA as an alternative medicine. Future research should continue to observe the effects of common population behaviors (which may be unique to age, location of residence, or occupation) on PA levels and the influence of these factors on COVID-19 outcomes. Further research may also look to observe the relationships of frequency, intensity, and duration of PA necessary to improve modulation of cytokine immune responses. Additionally, future studies may also aim to observe how behavioral changes resulting from the COVID-19 pandemic have impacted PA behavior and further explore how this may have impact ANS function.

Limitations from this study include the loss of follow-up information from baseline to follow-up. CT had 45% and PCOV had 65% loss of participants between observational moments which may be attributed to the general difficulty of subject compliance for young adults. This high loss of follow-up increases the likelihood of an underpowered sample, which would undermine the statistical findings or explain the lack of statistical differences (50). The effect of the underpowered sample on the statistical findings in this study can only be determined by referencing other literature employing similar methods.

Findings from this study serve as evidence as to the use of HRV monitoring as a reliable and accessible method to observe alterations in autonomic function. ANS dysregulation indicates deviation from homeostasis and can effectively predict cardiometabolic illness which is directly correlated with mortality in several diseases, regardless of age (15). Previous research throughout the literature has employed HRV methods that require prolonged monitoring, are subjective in nature, or require expensive equipment that is not accessible by the general population or small practice clinics. The methods employed in this study are less cumbersome on both healthcare professionals and the patient being monitored as the HRV monitoring can be completed within a single, relatively short visit. Observing changes in ANS activity may be helpful to health professionals to detect signs of infection, track viral progression, and observe dysfunctions caused by the virus which can aid in the development of individualized intervention strategies that are better informed by HRV data.

Overall, this study observed ANS improvements over a follow-up of approximately 5-month period that may be related to recovery after SARS-CoV-2 infection, in which these changes are independent to changes in PA. To our knowledge, this study is the first of its kind to investigate these outcomes within a 4-6 month period. Lastly, the methods of monitoring of ANS function could be used by healthcare professionals to observe the progression of SARS-CoV-2 infection to form individualized non-medication intervention strategies. The costs associated with equipment and the time for the HRV monitoring in this study are more accessible than other methods, which may work to improve health outcomes after COVID-19.

5. Conclusions

We observed improvements in ANS function after mild-to-moderate SARS-CoV-2 infection in young adults vaccinated over a 5 month period where ANS activity was similar to CT. Additionally, no PA changes were observed over the follow up period.

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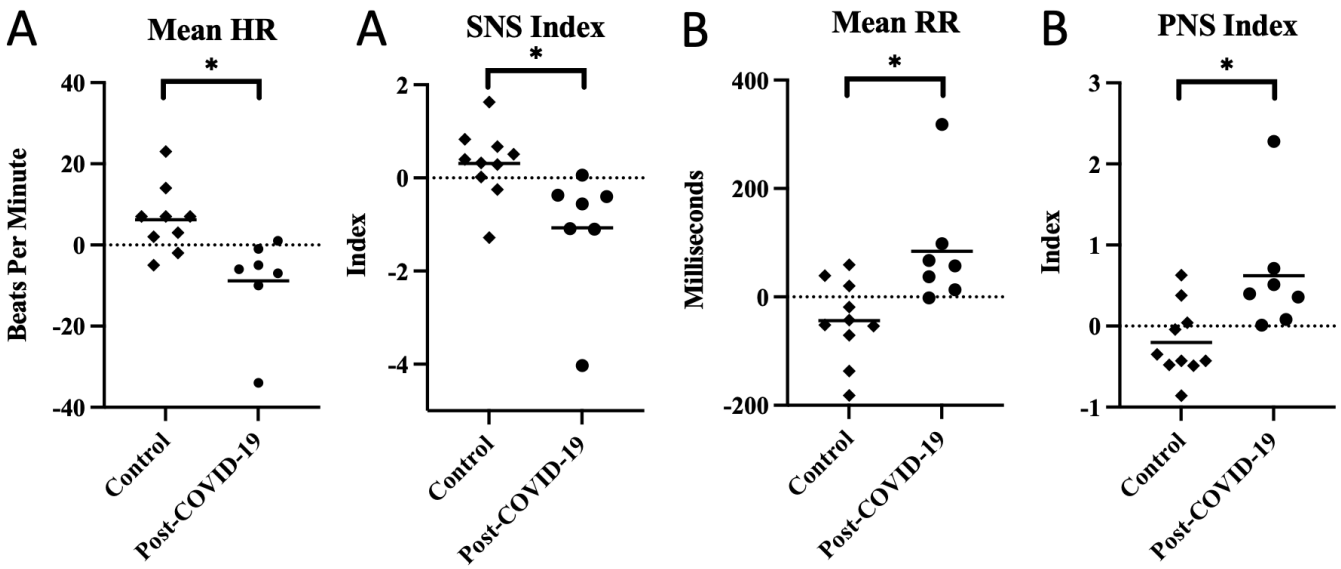
Conflicts of Interest: The authors declare no conflict of interest.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Sao Paulo State University (protocol code: 38701820.0.0000.5402; date of approval: 19 March 2021).

Informed Consent Statement: Informed consent was obtained from all participants involved in the study

Appendix A



Scheme 1. Scatterplot of HRV indexes for unpaired analysis according to group expressed as the mean difference between follow-up and baseline moments. (**A**) Sympathetic nervous system activity; (**B**) parasympathetic nervous system activity. * Statistical difference between groups; Mean HR: average heart rate measured in beats per minute; SNS Index: sympathetic nervous system index; Mean RR: time between R intervals of heartbeat; PNS index: determined by mean RR, RMSSD, and SD1.

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