## Title

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MAJOR ARTICLE

# Reduced Exercise Capacity, Chronotropic Incompetence, and Early Systemic Inflammation in Cardiopulmonary Phenotype Long Coronavirus Disease 2019

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***Background.*** Mechanisms underlying persistent cardiopulmonary symptoms after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (postacute sequelae of coronavirus disease 2019 [COVID-19; PASC] or "long COVID") remain unclear. This study sought to elucidate mechanisms of cardiopulmonary symptoms and reduced exercise capacity.

***Methods.*** We conducted cardiopulmonary exercise testing (CPET), cardiac magnetic resonance imaging (CMR) and ambulatory rhythm monitoring among adults >1 year after SARS-CoV-2 infection, compared those with and those without symptoms, and correlated findings with previously measured biomarkers.

***Results.*** Sixty participants (median age, 53 years; 42% female; 87% nonhospitalized; median 17.6 months after infection) were studied. At CPET, 18/37 (49%) with symptoms had reduced exercise capacity (<85% predicted), compared with 3/19 (16%) without symptoms ($P = .02$). The adjusted peak oxygen consumption ($VO_2$) was 5.2 mL/kg/min lower (95% confidence interval, 2.1–8.3; $P = .001$) or 16.9% lower percent predicted (4.3%-29.6%; $P = .02$) among those with symptoms. Chronotropic incompetence was common. Inflammatory markers and antibody levels early in PASC were negatively correlated with peak $VO_2$. Late-gadolinium enhancement on CMR and arrhythmias were absent.

***Conclusions.*** Cardiopulmonary symptoms >1 year after COVID-19 were associated with reduced exercise capacity, which was associated with earlier inflammatory markers. Chronotropic incompetence may explain exercise intolerance among some with "long COVID."

**Keywords.** SARS-CoV-2; cardiac magnetic resonance imaging; cardiopulmonary exercise testing; chronotropic incompetence; postacute sequelae of COVID-19.

Following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, some individuals experience persistent symptoms of "long COVID" (LC), a type of postacute sequelae of COVID-19 (PASC) [1]. Within months after infection, cardiac function is generally normal on echocardiogram [2]. Cardiac magnetic resonance (CMR) imaging has revealed early changes suggestive of cardiac inflammation without consistent associations with symptoms or differences from controls [3–7]. Therefore, other techniques are needed to identify physiologic correlates of symptoms.

Cardiopulmonary exercise testing (CPET), the reference standard for measuring exercise capacity (peak oxygen consumption [$VO_2$]), has demonstrated reduced exercise capacity among those with PASC [8]. With CPET, exercise limitations may be classified as likely ventilatory, cardiac, peripheral (oxygen extraction/utilization), or deconditioning. Some CPET protocols allow assessment of exertional heart rate (HR), or chronotropy, a major determinant of exercise capacity [9]. During clinical stress testing, failure to reach 85% of age-

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predicted maximum HR without ischemic electrocardiographic changes identifies chronotropic incompetence. With CPET, an adjusted HR reserve (AHRR) <80% (which accounts for resting HR), lower-than-expected exercise capacity, maximal effort, and no other pattern of limitations is more specific for chronotropic incompetence [9].

We designed the Long-Term Impact of Infection with Novel Coronavirus (LIINC) study (NCT 04362150) to evaluate physical and mental health after SARS-COV-2 infection by including individuals representing the full spectrum of acute illness and postacute recovery [10]. The purpose of this substudy was to elucidate mechanisms underlying cardiopulmonary symptoms >1 year after SARS-CoV-2 infection by comparing symptomatic and recovered individuals ,using advanced cardiopulmonary testing and correlating findings with blood-based markers.

## METHODS

As previously reported, LIINC is a San Francisco-based post-COVID cohort [10]. After our echocardiogram-based study did not reveal cardiac mechanisms of symptoms [2], we amended our protocol to conduct a second visit 1 year later for cross-sectional cardiopulmonary testing, including CPET, CMR imaging, and ambulatory rhythm monitoring.

### Participants

LIINC participants with PCR-confirmed SARS-CoV-2 infection who completed an echocardiogram visit were eligible irrespective of symptoms. Those with pregnancy, significant cardiopulmonary disease (see Supplementary Methods), and conditions precluding cycle ergometry were excluded. Individuals with noncompatible implants or claustrophobia were excluded from CMR imaging, and those with an estimated glomerular filtration rate <30 mL/min/1.73m$^2$ were excluded from gadolinium.

### Symptoms

Participants completed structured interviews about medical history, acute infection, cardiopulmonary diagnoses, and symptoms. Our primary case definition of "symptoms" included chest pain, dyspnea, palpitations, or fatigue in the 2 weeks preceding the study visit. Self-reported reduced exercise capacity was not included a priori but was added in sensitivity analyses. Consistent with the World Health Organization definition, all classified as symptomatic were >3 months after SARS-CoV-2 infection with new symptoms and without cardiopulmonary diagnoses [11].

### Blood-Based Markers

Participants had venous blood samples collected and processed for serum and plasma at the echocardiogram visit. Samples were batch processed for measurement of high-sensitivity troponin I, N-terminal prohormone b-type natriuretic protein, and high-sensitivity C-reactive protein (hsCRP). A subset had antibodies and additional markers from 2 earlier time points (<90 and 90–150 days after infection) assayed by Monogram Biosciences, using the Quanterix Simoa platform and blinded to patient and clinical information [12]. Epstein-Barr virus (EBV) early antigen diffuse immunoglobulin G (IgG) and nuclear antigen IgG were measured by ARUP Laboratories from specimens collected 90–150 days after infection [13].

### CPET Protocol

CPET was performed by an exercise physiologist and cardiology nurse practitioner blinded to participant data, according to standard protocol and using a metabolic cart (Medical Graphics Corporation Ultima CardiO$_2$) and cycle ergometer (Lode Corival CPET) with continuous 12-lead electrocardiographic, blood pressure, and pulse oximetry monitoring. After rest measurements, participants exercised to maximal exertion with work increased in 1-minute steps. We determined the work increase from the maximum voluntary ventilation targeting a 10-minute test, rounded to increments of 5 W/min (range, 10–30). The full protocol is described in the Supplementary Methods.

We report exercise capacity using relative peak $VO_2$ , absolute peak $VO_2$, and percent predicted peak $VO_2$. We classified peak $VO_2$ <85% predicted as reduced. We defined chronotropic incompetence as peak $VO_2$ <85% predicted, AHRR <80% ($[HR_{peak} - HR_{rest}]/[220 - age - HR_{rest}]$), and no alternative limitation [9]. We used AHRR as a continuous measure of chronotropy. CPET results were interpreted independently by 2 cardiologists, with discrepancies resolved by consensus.

### CMR Imaging

Multiparametric, sequence-standardized, blinded (technician and reader) CMR imaging was performed with a 3-T system (Premier; General Electric), including assessment of left and right ventricular size and function, parametric mapping, and late gadolinium enhancement. Measurements were performed in accordance with guidelines by a single reader under supervision by a senior cardiac imager, both blinded to clinical variables (full protocol in the Supplementary Methods).

### Ambulatory Rhythm Monitoring

Ambulatory rhythm monitors (Carnation Ambulatory Monitor; BardyDx) were placed on participants' chests. They were instructed wear the monitor for 2 weeks, press the button for symptoms, and record a symptom diary. Monitors were processed according to standard procedures. Reports were overread by a cardiologist.

### Statistical Analysis

To compare participants with and without symptoms, we used logistic regression to estimate adjusted odds ratios (ORs) of parameters with symptoms and linear regression to estimate

adjusted mean differences. Adjusted models included age, sex, time since SARS-CoV-2 infection, hospitalization, and body mass index. Nonnormally distributed variables were log-transformed, and findings are reported as mean ratios (unadjusted) and per doubling (adjusted). For biomarker data, we report unadjusted Pearson's ρ correlation coefficients and adjusted linear regression models. For longitudinal data we used mixed-effects models with a random intercept per participant. Sensitivity analyses considered other symptom definitions and also adjusted for medical history (hypertension, diabetes, asthma or chronic obstructive pulmonary disease, and human immunodeficiency virus [HIV]) and echocardiographic and spirometric parameters. REDCap software was used for data entry. Analyses were performed using Stata 17.1 software. The first author (M. S. D.) had full access and takes responsibility for the integrity of data and analyses. The study was approved by the institutional review board of the University of California, San Francisco (IRB 20-33000). All participants provided written informed consent before participation.

## RESULTS

### Participant Characteristics

Sixty participants were included. Their median age (interquartile range [IQR]) was 53 ( 41–59) years, 25 (42%) were female, and 8 (13%) were hospitalized during acute infection (Table 1). The median month of infection was in June 2020 (IQR, March–November 2020), with the ancestral strain. Four participants were vaccinated before infection ("breakthrough" infections), and 57 (95%) had received ≥1 SARS-CoV-2 vaccine before advanced testing.

### Persistent Symptoms at 18 Months

At visit 1 (median interval after infection, 6 months; echocardiogram visit), 40 of 60 participants (67%) reported symptoms.

**Table 1. Baseline Characteristics (n = 60)**

| Characteristic | | Participants, No. (%)[a] | |
|---|---|---|---|
| | | With Symptoms (n = 38) | Without Symptoms (n = 22) |
| Time since infection, median (IQR), mo | | 17.7 (15.9–19.4) | 17.5 (15.8–18.6) |
| Age, median (IQR), y | | 50.5 (40–57) | 54.5 (42–61) |
| Sex | Male | 19 (50) | 16 (73) |
| | Female | 19 (50) | 6 (27) |
| Race/ethnicity | Hispanic/Latino | 10 (24) | 5 (22) |
| | White | 26 (63) | 15 (65) |
| | Black/African American | 2 (5) | 1 (4) |
| | Asian | 2 (9) | 3 (7) |
| BMI, mean (SD)[b] | | 30.2 (7.5) | 28.1 (5.0) |
| Change in BMI from visit 1 to visit 2, mean (SD)[b] | | 1.18 (1.4) | 1.32 (2.1) |
| BMI category[b] | ≤24.9 | 10 (26) | 7 (32) |
| | 25–29.9 | 11 (29) | 10 (45) |
| | 30–34.5 | 9 (24) | 4 (18) |
| | ≥35 | 8 (21) | 1 (5) |
| Medical history | Hypertension | 9 (24) | 5 (24) |
| | Diabetes | 6 (16) | 1 (5) |
| | Asthma/COPD | 10 (27) | 2 (10) |
| | HIV | 10 (26) | 6 (27) |
| | Autoimmune disease | 3 (8) | 1 (5) |
| | Cancer | 1 (3) | 1 (5) |
| | Kidney disease | 1 (3) | 0 (0) |
| | Former or current tobacco use | 13 (34) | 3 (14) |
| Hospitalized (including ICU) | | 6 (16) | 2 (9) |
| ICU | | 2/6 (33)[c] | 0 |
| Any evidence of recent EBV reactivation[d] | | 28/35 (80) | 7/17 (41) |
| EBV early antigen diffuse IgG (≥9 U/mL)[d] | | 19/35 (54) | 2/17 (12) |
| EBV high nuclear antigen (>600 U/mL)[d] | | 18/35 (51) | 6/17 (35) |

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; ICU, intensive care unit; IgG, immunoglobulin G; IQR, interquartile range; SD, standard deviation.

[a]Data represent no. (%) of participants unless otherwise specified.

[b]BMI calculated as weight in kilograms divided by height in meters squared.

[c]Percentage of those hospitalized.

[d]EBV data were missing for 3 individuals with symptoms and 5 without symptoms.

At visit 2 (median interval after infection, 17.6 months; advanced testing visit), 38 of 60 (63%) reported symptoms. Trajectories of individual symptoms were similar (Supplementary Table 1). Self-reported exercise capacity was highly associated with symptoms: 29 of 33 participants (88%) reporting reduced exercise capacity had symptoms versus 9 of 27 (33%) reporting preserved or improved exercise capacity (OR, 14.5 [95% CI, 3.9–54.1]; $P < .001$). Evidence of recent EBV reactivation after SARS-CoV-2 infection was associated with symptoms (OR, 9.3 [95% CI, 2.02–43.6]; $P = .004$).

#### Maximal CPET

Of 60 participants who attended a CPET visit, 59 completed CPET, at a median 17.6 months after SARS-CoV-2 infection (IQR, 15.8–19.4 months). One participant was too hypertensive to undergo CPET, 1 was excluded owing to β-blocker use, and 2 had submaximal tests, leaving 56 CPET procedures for analysis. Three tests were stopped for hypertensive response (at >100% predicted); all others were symptom-limited maximal tests. No included participants were taking chronotropic medications or antianginal medications.

#### Reduced Exercise Capacity With CPET

Exercise capacity was reduced (peak $VO_2$ <85% predicted) among 18 of 37 participants (49%) with symptoms versus 3 of 19 (16%) without symptoms ($P = .02$). A 5 mL/kg/min decrease in peak $VO_2$ was associated with 2.75 times higher odds of symptoms (95% CI, 1.39–5.44; $P = .004$). Including EBV reactivation yielded a similar OR (3.04 [95% CI, 1.31–6.93]; $P = .01$). Those with symptoms completed less work despite higher perceived effort and similar respiratory exchange ratio (Table 2). Most other CPET parameters were not associated with symptoms (Table 2 and Supplementary Table 2).

Exercise capacity was lower among participants with symptoms (Figure 1). The adjusted difference in peak $VO_2$ was 5.2 mL/kg/min (95% CI, 2.1–8.3; $P = .001$), 0.4 L/min (.09–.73; $P = .02$), and 16.9% lower percent predicted (4.3%–29.6%; $P = .02$). Results were robust to including diabetes and hypertension (4.5 mL/kg/min [95% CI, 1.40–7.50]; $P = .005$) and also to including asthma or chronic obstructive pulmonary disease, HIV, spirometry, and echocardiographic parameters (3.9 mL/kg/min [.6–7.3]; $P = .02$) and EBV reactivation (5.0 mL/kg/min [1.50–8.48]; $P = .006$) but not to symptom classification (Supplementary Table 3). In an adjusted model excluding symptoms as a mediator, EBV reactivation was associated with lower exercise capacity, but this association was not statistically significant (2.6 mL/kg/min [95% CI], −.65 to 5.93; $P = .11$).

#### Chronotropic Incompetence Among Symptomatic Participants

Among 56 participants with maximal CPET, 21 (37%) had peak $VO_2$ <85% of predicted; no participants had ventilatory limitation, 3 had cardiac limitation, and 1 had a hypertensive response. Four had findings most consistent with deconditioning or obesity, and 1 reached 84% predicted with no other abnormalities (possible deconditioning). Twelve (21% overall and 57% with reduced exercise capacity) had chronotropic incompetence. Among participants with symptoms, 11 of 37 (30%) had chronotropic incompetence, compared with 1 of 19 (5%) without symptoms ($P = .04$).

Chronotropic incompetence was highly associated with symptoms (OR, 17.6 [95% CI, 1.43–216]; $P = .03$). Compared with those with normal exercise capacity and chronotropy, those with chronotropic incompetence had a peak HR lower by 49 beats/min (119 vs 170 beats/min; [95% CI, 40–60]; $P < .001$; Figure 2). They completed 100 W less work (95% CI, 49–152; $P < .001$), achieved 12.2 mL/kg/min lower peak $VO_2$ (6.5–17.9; $P < .001$), and had reduced HR recovery (7.9 beats/min less at 1 minute [1.3–14.6]; $P = .02$).

In absolute terms, those with chronotropic incompetence generated a mean peak $VO_2$ of 1.59 L/min compared with 2.35 L/min in those with normal exercise capacity (difference, 0.76 L/min [95% CI, .23 to 1.28]; $P = .007$); a linear regression model with only rest and peak HR explains 54% of the difference in relative $VO_2$ (in milliliters per kilogram per minute) and 34% of the difference in absolute $VO_2$ (in liters per minute).

Factors associated with chronotropic incompetence in exploratory univariate analyses include diabetes ($P < .001$), hypertension ($P = .04$), body mass index ($P = .002$), HIV ($P = .001$), EBV reactivation ($P = .01$), and possibly smoking ($P = .05$) but not age or sex. Correlations of early inflammatory markers with AHRR were of borderline statistical significance (hsCRP, $P = .04$; tumor necrosis factor [TNF], $P = .06$; interleukin 6 [IL-6], $P = .05$). In 52 of 56 participants with EBV antibodies assessed, all 11 individuals with chronotropic incompetence had evidence of EBV reactivation. This is driven by differences in early antigen IgG, a more specific marker of reactivation, with 81% with chronotropic incompetence positive compared with 55% with high nuclear antigen IgG. AHRR is 17% lower among those with early antigen IgG (95% CI, 5.6%–29.1%; $P = .005$), but not significantly different by high nuclear antigen IgG (−1.6% [−14 to 11]; $P = .80$). HIV is also associated with chronotropic incompetence (OR, 9.0 [95% CI, 2.17–37.4; $P = .002$).

#### Normal Cardiac Structure and Function at CMR Imaging

Forty-three participants completed CMR imaging, including 2 without gadolinium (1 owing to estimated glomerular filtration rate <30 mL/min/1.73m$^2$ and 1 owing to inability to place an intravenous line). CMR imaging demonstrated normal chamber sizes and ejection fraction. Smaller right ventricular volumes were associated with higher odds of symptoms (Table 3). No participants had late gadolinium enhancement suggestive of replacement fibrosis, and markers of inflammation or interstitial fibrosis were not associated with

**Table 2. Cardiopulmonary Exercise Testing Parameters by Symptom Status (n = 56)**

| Variable | Measure | Value, Mean (SD)[a] | | Adjusted OR (95% CI; *P* Value)[b] | Adjusted Difference (95% CI; *P* Value)[b] |
|---|---|---|---|---|---|
| | | Participants With Symptoms (n = 37) | Participants Without Symptoms (n = 19) | | |
| Exercise capacity | Peak $VO_2$, mL/kg/min | 22.7 (8.1) | 29.6 (7.0) | 2.75 per −5 mL/kg/min (1.39–5.44; *P* = .004)[c] | −5.2 (−8.3 to −2.1; *P* = .001)[c] |
| | Peak $VO_2$, % predicted | 92.0 (22.0) | 107.3 (22.0) | 1.22 per −5% (1.04–1.43; *P* = .01)[c] | −17 (−30 to −4.3; *P* = .01)[c] |
| | Peak $VO_2$, mean L/min | 1.9 (0.6) | 2.4 (0.7) | 1.18 per −0.1/min (1.03–1.37; *P* = .02)[c] | −0.41 (−.73 to −.09; *P* = .01)[c] |
| | Reduced capacity (<85% predicted), no. (%) | 18 (49) | 3 (16) | 7.97 (1.56 to 40.8; *P* = .01)[c] | … |
| Ventilatory | Peak respiratory rate, breaths/min | 37.5 (8.7) | 40.8 (9.7) | 0.95 (.88–1.02; *P* = .17) | −3.8 (−10.0 to 2.4; *P* = .23) |
| | Breathing reserve (MVV-$V_{Emax}$), median (IQR) | 44.5 (38.3–59.0) | 32.8 (24.0–44.8) | 1.04 (1.00–1.07; *P* = .05) | 14.8 (.7 to 29; *P* = .04)[c] |
| | Ventilatory efficiency ($V_E/VCO_2$ slope)[d] | 27.5 (3.7) | 25.8 (3.7) | 1.18 (.98–1.44; *P* = .09) | 2.0 (−.4 to 4.4; *P* = .10) |
| Peripheral | $VO_2$/work slope, mL/Watt | 9.9 (3.0) | 10.9 (3.5) | 0.94 (.76–1.17; *P* = .60) | −0.5 (−2.6 to 1.5; *P* = .60) |
| Cardiac | $VO_2$ pulse, mL/beat | 13.1 (3.3) | 15.4 (4.5) | 0.85 (.69–1.03; *P* = .11) | −1.6 (−3.8 to .5; *P* = .13) |
| | SBP peak, mm Hg | 173.9 (31.7) | 189.2 (23.3) | 0.86 per 5 mm Hg (.73–1.03; *P* = .10) | −13.2 (−31.3 to 4.8; *P* = .15) |
| HR | Rest, beats/min | 78.3 (14.5) | 75.2 (10.4) | 1.01 (.96–1.06; *P* = .75) | .9 (−6.9 to 8.7; *P* = .83) |
| | Peak, beats/min | 147.2 (25.7) | 154.1 (21.7) | 0.97 (.94–1.01; *P* = .10) | −9.3 (−21 to 2; *P* = .11) |
| | Peak, % age predicted | 86.2 (11.9) | 94.1 (9.3) | 0.92 (.85–.99; *P* = .02)[c] | −8.5 (−15.2 to −1.8; *P* = .02)[c] |
| | AHRR achieved, % | 73.6 (22.0) | 84.9 (21.2) | 0.97 (.94–1.00; *P* = .05) | −12.1 (−24.6 to .5; *P* = .06) |
| | HR recovery at 1 min, beats/min | 14.4 (7.4) | 14.0 (10.0) | 1.04 (.95–1.14; *P* = .39) | 2.0 (−2.9 to 6.9; *P* = .42) |
| Exertion | Work, Watts | 140.8 (60.6) | 196.2 (68.2) | 1.20 per −10 W (1.04 to 1.40; *P* = .01)[c] | −49.6 (−86.2 to −13.0; *P* = .009)[c] |
| | Perceived exertion, Borg scale 6–20[e] | 16.2 (1.8) | 14.9 (2.2) | 1.64 (1.05–2.57; *P* = .03)[c] | 1.3 (.1 to 2.6; *P* = .03)[c] |
| | Peak respiratory exchange ratio ($VCO_2/VO_2$), median (IQR) | 1.18 (1.12–1.23) | 1.20 (1.12–1.30) | 1.01 per −0.1 (.48–2.12; *P* = .98) | 0.03 (−.09 to .02; *P* = .23) |

Abbreviations: AHRR, adjusted heart rate reserve; CI, confidence interval; HR, heart rate; MVV, maximal voluntary ventilation; OR, odds ratio; SBP, systolic blood pressure; SD, standard deviation; $VCO_2$, carbon dioxide production; $V_D/V_T$, dead space ratio; $V_E$, minute ventilation; $V_{Emax}$, maximum $V_E$; $VO_2$, oxygen consumption.

[a]Data represent mean (SD) values unless otherwise specified.

[b]We present both the ORs for the association between cardiopulmonary exercise testing parameters and symptoms—estimated using logistic regression with adjustment for age, sex, time since coronavirus disease 2019 (COVID-19), hospitalization for acute COVID-19, and body mass index category—and the estimated adjusted mean differences between participants with or without symptoms, using linear regression adjusting for the same covariates. Sensitivity analysis incorporating history of hypertension, diabetes, and lung disease showed no substantive changes in effect sizes or CIs.

[c]Significant at *P* < .05.

[d]The $VE/VCO_2$ slope could not be determined for 1 participant without symptoms.

[e]The Borg rating of perceived exertion ranges from 6 to 20, with 15–20 considered vigorous intensity, and it was assessed every 2 minutes; these values represent the last measurement before the test was stopped.

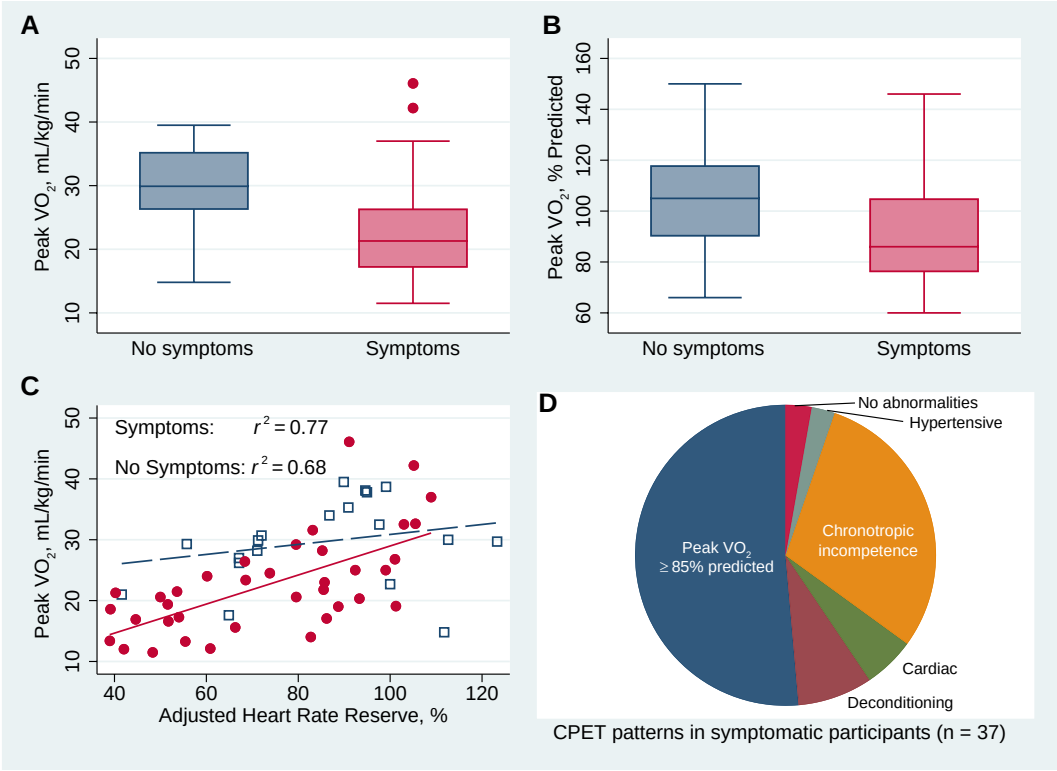


**Figure 1.** Exercise capacity by symptoms and chronotropic response to exercise. Box and whisker plots represent peak oxygen consumption ($VO_2$) among those without (*left*) or with (*right*) symptoms 17.6 months after severe acute respiratory syndrome coronavirus 2 infection (top). *A, B,* The mean peak $VO_2$ (standard deviation) was 22.7 ( 8.1) and 29.6 (7.0) mL/kg/min among those with and those without symptoms (*A*), respectively, a difference of 6.9 mL/kg/min (95% confidence interval, 2.5–11.3; $P = .003$) and 92% versus 107% percent predicted, respectively (*B*), a 15% difference ($P = .02$). *C,* Peak $VO_2$ plotted by adjusted heart rate reserve (AHRR) to demonstrate the cluster of symptomatic individuals with low peak $VO_2$ and chronotropic incompetence (*bottom left, circles = symptoms, squares = no symptoms*). *D,* Cardiopulmonary exercise testing (CPET) patterns among participants with long coronavirus disease 2019 symptoms: half achieved ≥85% predicted peak $VO_2$, and chronotropic incompetence was the most common pattern among those with reduced exercise capacity.

symptoms. Some participants (10 of 43 [23%]) had trace or small pericardial effusions, with no difference by symptoms ($P = .59$).

#### Palpitations Are Not Explained by Arrhythmias at Ambulatory Rhythm Monitoring

Thirty-eight participants contributed ambulatory rhythm monitor data. Consistent with CPET findings, lower maximum HR, age-predicted maximum HR, and AHRR were associated with symptoms (Supplementary Table 4). One symptomatic individual had a single episode of nonsustained ventricular tachycardia without symptoms recorded or button push; no other clinically significant arrhythmias, including atrial fibrillation, were present in either group. The burdens of sinus tachycardia and supraventricular tachycardias did not differ significantly by symptoms. Premature ventricular contractions were associated with symptoms (especially palpitations), and we could not exclude an association between premature atrial contractions and symptoms. Symptomatic individuals pressed the button 3.2 times more often (95% CI, 2.1–4.7; $P < .001$). Button pushes occurred mostly during sinus rhythm, sinus tachycardia, or supraventricular ectopy (Supplementary Figure 2).

#### Ambulatory Rhythm Monitoring Correlates of Chronotropic Incompetence at CPET

Peak HR at CPET was correlated with ambulatory maximum sinus HR (Pearson's $\rho = 0.71$; $P < .001$). Ambulatory peak HR was lower among those with chronotropic incompetence (29 beats/min [95% CI, 13–45]; $P < .001$). Chronotropic incompetence was associated with higher minimum HR (12.6 beats/min [95% CI, 3–22]; $P = .01$) and lower HR variability (59 ms standard deviation n-to-n [24–95]; $P = .002$) (Supplementary Table 5). PR intervals were not significantly longer among those with chronotropic incompetence (171 vs 168 ms; $P = .72$), and no participants had second-degree Mobitz type 2 or third-degree heart block.

#### Association Between Markers of Inflammation Early in PASC and Exercise Capacity >1 Year Later

Markers of inflammation (hsCRP, IL-6, and TNF) and SARS-CoV-2 receptor-binding domain IgG level, but not

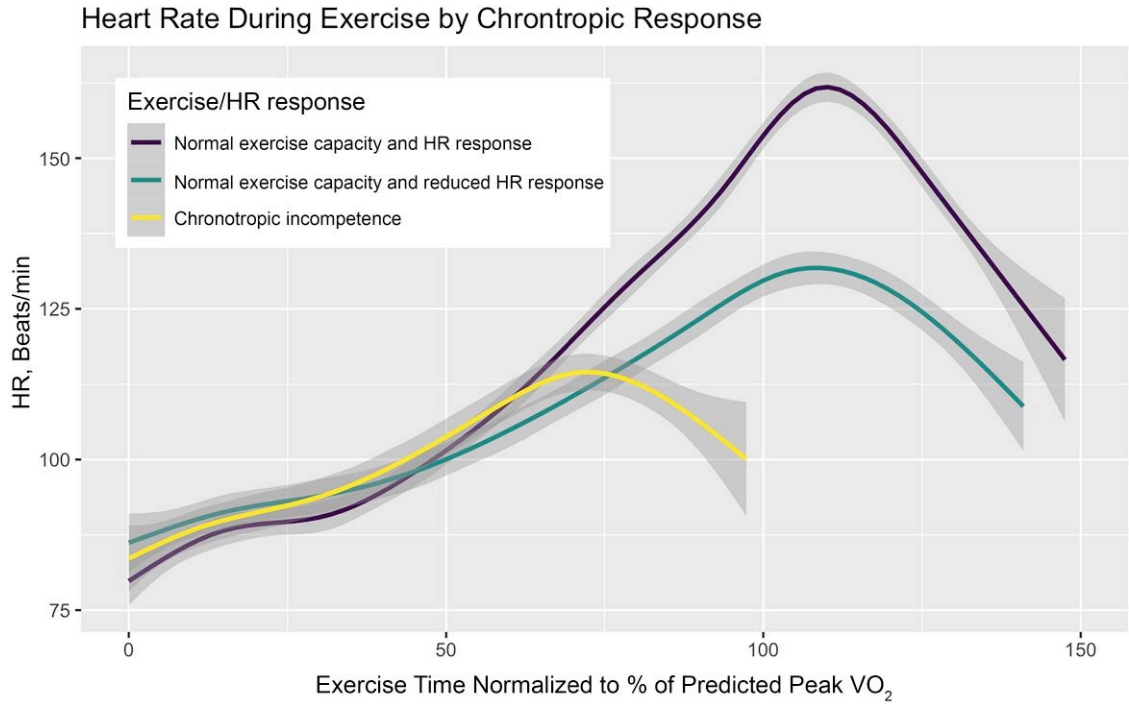


**Figure 2.** Heart rate (HR) during exercise by chronotropic response to exercise. The mean HR is plotted as a function of exercise time normalized to the percent of predicted peak oxygen consumption ($VO_2$). The top line represents participants with normal exercise capacity (peak $VO_2$ ≥85% predicted) and normal HR response (n = 16); middle line, those with normal exercise capacity (peak $VO_2$ ≥85%) and blunted HR response (adjusted HR response <80%) (n = 8); bottom line, those with chronotropic incompetence (n = 9), as described in Supplementary Table 4.

high-sensitivity troponin or N-terminal prohormone b-type natriuretic protein, measured approximately 6 months after infection, are negatively correlated with peak $VO_2$ >1 year later (Figure 3 and Supplementary Figure 3). Peak $VO_2$ was lower per doubling of TNF (6.2 mL/kg/min [95% CI, .6–11.8]; $P = .03$) and hsCRP (1.8 mL/kg/min [.8–2.9]; $P = .001$).

Longitudinal markers of inflammation, neurologic injury, and SARS-CoV-2 receptor-binding domain IgG were measured before vaccination at <90 days (median 52) and between 90 and 150 days (median, 124) after infection in 35 participants (Supplementary Figure 4). Reduced exercise capacity was associated with higher markers at <90 days, including SARS-CoV-2 IgG (2.99-fold higher mean ratio [95% CI, 1.41–6.33]; $P = .004$) and TNF (1.34-fold higher mean ratio [1.11–1.61]; $P = .002$). At 90–150 days, only SARS-CoV-2 IgG (2.12-fold higher [95% CI, 1.02–4.43]; $P = .04$) remained statistically significant, although not with adjustment (1.1 mL/kg/min per doubling, 95% CI, −.3 to 2.4; $P = .11$). We could not exclude an effect of IL-6 (1.34-fold higher [95% CI, .92–1.96; $P = .11$]; 2.1 mL/kg/min per doubling [−.5 to 4.7; $P = .11$]). All biomarkers except IL-6 decreased over time regardless of eventual exercise capacity.

## DISCUSSION

We demonstrate that clinically meaningful reductions in exercise capacity are associated with LC symptoms >1 year after SARS-CoV-2 infection. Our findings suggest that chronotropic incompetence contributes to exercise limitations in LC. We found that elevated inflammatory and antibody levels early in PASC are associated with reduced exercise capacity more than a year later. Evidence of EBV reactivation was found in all individuals with chronotropic incompetence. We did not find evidence of myocarditis, cardiac dysfunction, or clinically significant arrhythmias. Finally, our study validates that CPET allows objective measurement of patient-reported exercise intolerance and therefore may be useful for interventional trials of therapeutics for LC.

Our findings are consistent with those of other studies that have reported reduced exercise capacity in PASC, which we summarized in a systematic review and meta-analysis [8]. Our study builds on these by (1) measuring exercise capacity later after infection, (2) including multimodality evaluation, (3) adjusting for confounders, (4) including recovered persons as comparators, (5) demonstrating associations with longitudinal biomarkers, and (6) noting hypothesis-generating findings regarding chronotropic incompetence and EBV reactivation.

Differences in classification of exercise limitations across CPET studies of PASC may arise from selection bias, confounding, CPET protocols, and interpretation algorithms. Although reductions in physical activity after COVID-19 contribute to deconditioning, a common CPET finding in PASC,

**Table 3. Cardiac Magnetic Resonance Imaging Parameters (n = 43) by Symptom Status**

| Parameter | | Value, Mean (SD)[a] | | |
|---|---|---|---|---|
| | | Participants With Symptoms (n = 25) | Participants Without Symptoms (n = 18) | Adjusted OR (95% CI; *P* Value)[b] |
| Time since SARS-CoV-2 infection, mo | | 15.9 (3.8) | 15.9 (3.9) | … |
| Hematocrit, % | | 43.0 (3.5) | 44.5 (4.1) | 0.86 (.67 to 1.09; *P* = .21) |
| LV size and function | LVEDi, mL/m$^2$ | 63.6 (13.9) | 69.3 (12.3) | 0.97 (.91–1.03; *P* = .27) |
| | LVESi, mL/m$^2$ | 24.4 (6.9) | 25.3 (7.0) | 1.00 (.89–1.12; *P* = .97) |
| | LVEF, % | 61.8 (5.9) | 63.3 (5.8) | 0.93 (.82–1.05; *P* = .26) |
| | LV mass index, gm/m$^2$ | 47.6 (7.9) | 51.6 (7.6) | 0.98 (.90 to 1.06; *P* = .60) |
| | Stroke volume, mL[c] | 77.2 (18.1) | 84.2 (18.1) | 0.97 (.93–1.01; *P* = .20) |
| RV size and function | RVEDi, mL/m$^2$ | 65.3 (13.5) | 75.6 (12.4) | 0.92 (.86 to .99; *P* = .02)[d] |
| | RVESi, mL/m$^2$ | 27.1 (6.4) | 31.3 (7.6) | 0.86 (.75 = .99; *P* = .04)[d] |
| | RVEF, % | 58.9 (5.0) | 58.4 (5.0) | 0.75 (.86 to 1.14; *P* = .93) |
| Markers of cardiac inflammation | Mapping time, median (IQR), ms | | | |
| | T1 native mapping time | 1202 (1141–1253) | 1219 (1153–1248) | 1.00 (.99–1.00; *P* = .51) |
| | Postcontrast T1 mapping time | 603 (507–634) | 624 (577–655) | 1.00 (.99 to 1.01; *P* = .64) |
| | T2 native mapping time | 46.5 (44.4–51.0) | 48.0 (44.0–51.4) | 0.94 (.81–1.10; *P* = .45) |
| | Extracellular volume, % | 26.7 (6.3) | 24.2 (5.3) | 1.08 (.92–1.26; *P* = .35) |
| Cardiac fibrosis (LGE), no. | | 0 | 0 | … |
| Possible pericardial inflammation (pericardial effusion), no. (%) | | 6 (24) | 4 (22) | 0.33 (.04–9.70; *P* = .28) |

Abbreviations: CI, confidence interval; IQR, interquartile range; LGE, late gadolinium enhancement; LV, left ventricular; LVEDi, LV end-diastolic volume indexed to body surface area; LVEF, LV ejection fraction; LVESi, LV end-diastolic volume indexed to body surface area; OR, odds ratio; RV, right ventricular; RVEDi, RV end-diastolic volume indexed to body surface area; RVEF, RV ejection fraction; RVESi, RV end-diastolic volume indexed to body surface area; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation.

[a]Data represent mean (SD) values unless otherwise specified. (Nonnormally distributed variables are given as median (IQR).

[b]Logistic regression was used to estimate the odds of having symptoms for a given change in each parameter, adjusted for age, sex, body mass index category, hospitalization, and time since infection. Linear regression was used to estimate the mean differences between those with and those without symptoms, adjusted for the same likely confounders.

[c]LV stroke volumes are reported, but there is a high correlation between LV and RV stroke volumes (Pearson's $\rho = 0.96$).

[d]Significant at $P < .05$.

deconditioning commonly demonstrates an accelerated HR response [14].

Our finding of chronotropic incompetence is consistent with findings of 5 prior studies in PASC [15–19]. Cardiometabolic risk factors and smoking are associated with chronotropic incompetence apart from PASC and with PASC [20, 21]. Apart from PASC, chronotropic incompetence has prognostic implications: it is associated with incident cardiovascular disease and mortality [22–25], but the long-term implications and potential for reversibility in PASC are unknown.

Impaired peripheral oxygen extraction, best assessed with invasive CPET with arterial and pulmonary artery catheterization, may also contribute to exercise limitations [26], perhaps via changes in autonomic regulation of microcirculatory function or altered metabolism. We did not find differences in $VO_2$/work slope, a noninvasive correlate of oxygen extraction. Although not observed among our participants, dysfunctional (rapid, erratic) breathing and hyperventilation may contribute to dyspnea in PASC [27].

Though not ubiquitous, chronotropic incompetence may provide clues to mechanisms of PASC. Our study extends prior findings that inflammatory markers are negatively correlated with peak $VO_2$ early after COVID-19 hospitalization to >1 year after infection and in those not hospitalized [28]. This correlation may reflect a common cause for inflammation and exercise limitations in PASC (ie, viral persistence or immune activation), or these markers could be on the causal path from SARS-CoV-2 infection to symptoms (Figure 4). Animal models suggest that inflammatory markers, including IL-6 and TNF, impair chronotropy and endothelial function [29, 30].

Chronic inflammation in other conditions is associated with adrenergic activation, chronotropic incompetence, and reduced exercise capacity. Reduced β-receptor responsiveness, a prominent feature of chronotropic incompetence, increases adrenergic activation, which activates inflammatory pathways [9, 31, 32]. Elevated sympathetic activation at rest occurs after SARS-CoV-2 infection [33] and is associated with reduced exercise capacity and vascular dysfunction in PASC [34]. Coronary microvascular dysfunction occurs in early PASC [35], and endothelial dysfunction is associated with chronotropic incompetence and inflammation [32, 36]. Thus, chronic inflammation and adrenergic activation could blunt chronotropy, vascular function, and exercise capacity without autonomic nervous system or sinus node pathology.

Altered autonomic function and sinus node remodeling are alternative hypotheses. Altered autonomic function could explain

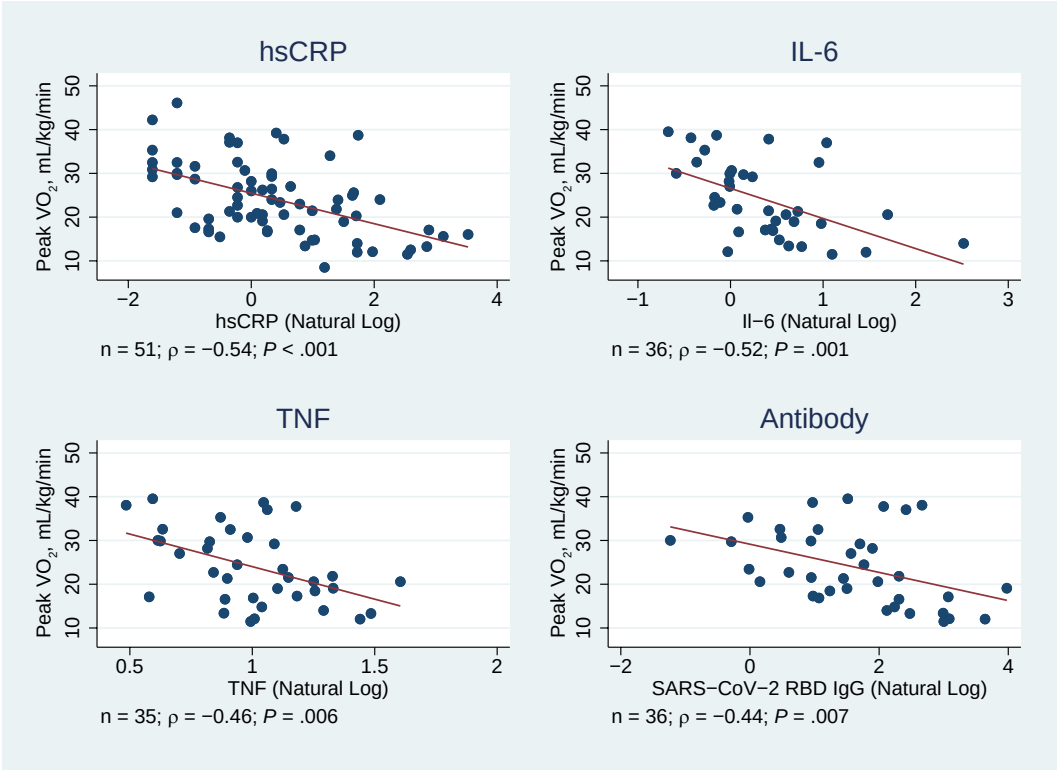


**Figure 3.** Correlation between peak oxygen consumption ($VO_2$) and previously measured inflammatory markers and antibody levels. Scatterplots and linear trend lines represent peak $VO_2$ (measured at about 18 months) by natural log of selected biomarker levels, with unadjusted Pearson's ρ correlations and *P* values listed. High-sensitivity C-reactive protein (hsCRP) was measured a median of 6 months after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection; interleukin 6 (IL-6), tumor necrosis factor (TNF), and SARS-CoV-2 receptor binding domain (RBD) immunoglobulin G (IgG) were measured a median of 4 months after infection. Prior hsCRP, IL-6, TNF, and SARS-CoV-2 antibody levels were correlated with subsequent peak $VO_2$. Additional biomarkers are plotted in Supplementary Figure 2.

others' CPET findings in PASC, including preload failure [17, 27, 37] and disordered breathing [27]. Orthostatic intolerance occurs in PASC [38], perhaps related to small fiber neuropathy [39]. Although we did not find evidence of cardiac fibrosis or sinus node dysfunction, sinus node involvement could theoretically affect chronotropy. Hamster models suggest that SARS-CoV-2 can infect sinoatrial node cells and in vitro pacemaker cells, resulting in altered calcium handling, activated inflammatory pathways, and induced ferroptosis [40]. As far as we know, human autopsy studies have not examined sinus node tissue, nor have invasive electrophysiology studies been performed in PASC.

To our knowledge, our study is the first to report evidence of EBV reactivation among those with chronotropic incompetence in PASC, a hypothesis-generating finding that needs replication. EBV reactivation may be associated with PASC [13, 41], similar to early reports of elevated early antigen IgG in chronic fatigue syndrome/myalgic encephalitis, where the role of EBV remains controversial >3 decades later.

CMR imaging findings suggestive of myocarditis without cardiac dysfunction may be present early after SARS-CoV-2 infection [3, 6]. Consistent with studies later after infection [4, 5], we did not find evidence of cardiac dysfunction, scar, or inflammation. Therefore, myocarditis or structural cardiac changes are unlikely to explain symptoms for most with PASC.

Our findings are consistent with those of 2 studies that did not find arrhythmias in early PASC [42, 43]. Inappropriate sinus tachycardia was present only in 1 individual (without symptoms), in contrast to earlier findings [44]. Premature atrial and ventricular contractions may contribute to palpitations in PASC, but clinically significant arrhythmias and inappropriate sinus tachycardia are unlikely major contributors.

Currently, LC has no proven treatments. Vaccination likely reduces risk of PASC [45], and newer variants may have lower risk [46]. Antivirals, anti-inflammatories, and anticoagulants have not been evaluated in PASC. Therefore, translational and proof-of-concept clinical research to characterize distinct phenotypes and mechanisms of PASC is urgently needed to identify potential therapies.

Our study highlights the clinical challenge that many patients with symptoms have no objective findings on multimodality cardiopulmonary testing, emphasizing gaps between patient presentations and current evidence. CPET results may appear "normal" in previously athletic individuals even with life-altering reductions in exercise capacity. "Normal" results

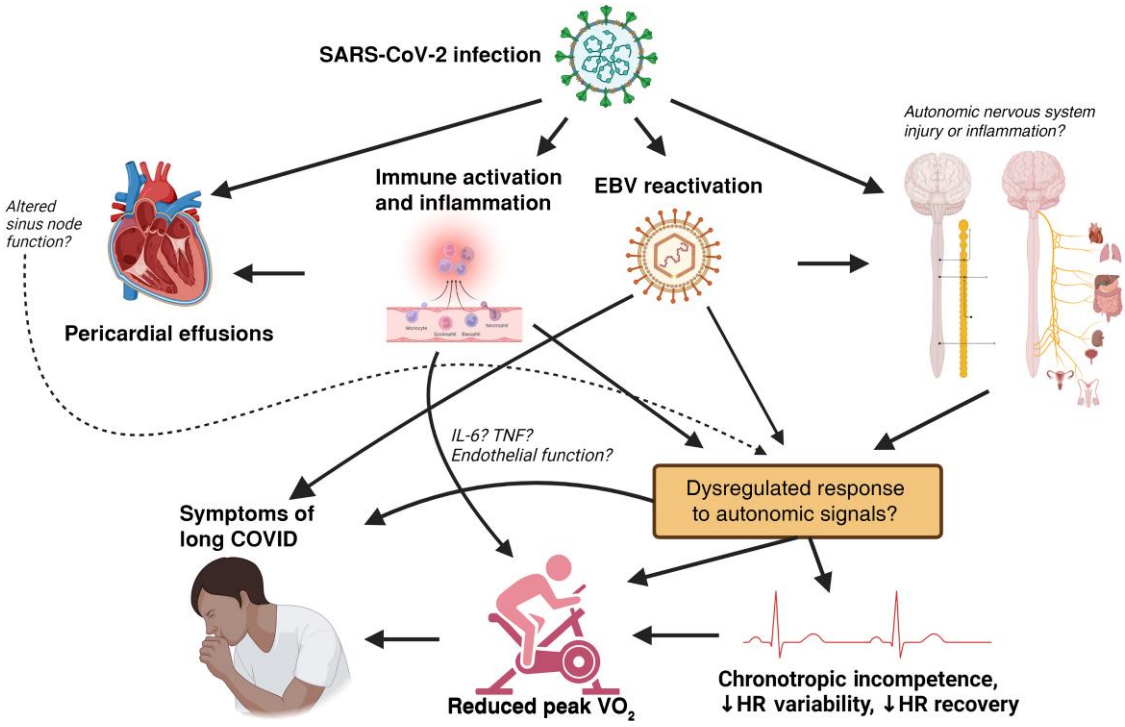


**Figure 4.** Hypothesized mechanisms of cardiopulmonary symptoms and reduced exercise capacity. We found that higher inflammatory markers (high-sensitivity C-reactive protein, interleukin 6 [IL-6], and tumor necrosis factor [TNF]) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin G antibody levels measured within the first 6 months after infection are associated with reduced exercise capacity measured with cardiopulmonary exercise testing >1 year after infection. We also found that evidence of Epstein-Barr virus (EBV) reactivation was common, including among all individuals with chronotropic incompetence. We propose that persistent immune activation and systemic inflammation, possibly related to EBV reactivation, may cause a dysregulated response to autonomic signaling that may manifest as chronotropic incompetence, reduced exercise capacity, and symptoms of long coronavirus disease 2019 ("long COVID"). Abbreviation: HR, heart rate. (Figure created with BioRender.com.)

may be due to pathologic changes not detectable with these techniques (eg, viral persistence, microvascular, mitochondrial, or autonomic dysfunction, or noncardiopulmonary causes). Cardiopulmonary PASC lacks a clear diagnostic signature, and even advanced cardiopulmonary testing largely rules out diagnoses that were not present in our cohort.

Although exercise is unlikely to cure LC, exercise training is the only intervention demonstrated to improve exercise capacity in chronotropic incompetence separate from PASC [47] and may improve symptoms and quality of life. Exercise training is also effective for postural orthostatic tachycardia syndrome, one phenotype of cardiopulmonary PASC. Reports of postexertional malaise or symptom exacerbation in PASC overlapping with myalgic encephalitis or chronic fatigue syndrome [48] mean that exercise-based interventions should be tested rigorously for safety and efficacy. In PASC, different exercise strategies have supportive preliminary data: a structured pacing intervention improved fitness and reduced postexertional symptom exacerbation [49], and supervised exercise training may be helpful and safe [50].

Limitations of this observational study arise from its small sample size, nonprobabilistic sampling, and cross-sectional measures. Effect sizes were sensitive to case definition, but our definition is consistent with current consensus [11]. Volunteer bias may result in overestimated prevalence of reduced exercise capacity but should not affect classification of limitations. We did not include uninfected comparators, and most participants were unvaccinated at infection. Although we excluded those with cardiac disease, adjusted for measured confounders, and conducted sensitivity analyses, unmeasured residual confounders remain, especially pre-COVID fitness. Performing noninvasive CPET without stress imaging may result in misclassification of exercise limitations. Finally, we lacked contemporaneous biomarkers to ascertain whether transient or ongoing inflammation is more likely.

In conclusion, >1 year after prevaccine SARS-CoV-2 infection, reduced exercise capacity on CPET is associated with cardiopulmonary symptoms, chronotropic incompetence, and earlier inflammatory markers but not evidence of myocarditis or arrhythmias. Further investigation into mechanisms of cardiopulmonary PASC should include evaluation of inflammatory pathways, chronotropy, EBV reactivation, and microvascular function.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not

copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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***Author contributions.*** M. S. D. designed the study, obtained institutional review board approval, acquired data, analyzed data, interpreted the findings, and wrote the first draft of the manuscript. M. J. P. assisted with study design, participant recruitment, data interpretation, and critical revision of the manuscript. P. K. and Y. J. L. conducted cardiac magnetic resonance (CMR) imaging measurements, drafted the CMR imaging methods, and helped interpret the CMR imaging findings. C. H. led the ambulatory rhythm monitoring measurements and helped interpret the findings. D. L., E. S., and M. A. A. helped with the cardiopulmonary exercise testing measurements, drafting of the cardiopulmonary exercise testing (CPET) methods, and CPET interpretation and provided input on the manuscript. S. S. and V. M. A. helped with measurements, data management, and revisions of the manuscript. D. V. G. helped with statistical design, analysis and visualization. S. L., S. A. G., and R. H. helped with participant recruitment, measurement, and data management. A. C., B. C. Y., J. W. W., and C. J. P. helped with biomarker measurement and provided input on drafts of the manuscript. J. D. K., T. J. H., and J. N. M. helped with the study design, interpretation of results, and revision of the manuscript. C. S. L. and D. J. G. provided funding and helped design the study, interpret results, and revise the manuscript. S. G. D. and P. Y. H. provided funding, oversight, and mentorship for the first author and participated in study design, interpretation of results, and critical manuscript revision.

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