

Methods Supplement

Institutional Review Board Approval & Consent

Two separate protocols (2018P00753, 2021P00064) approved by the Mass General Brigham (MGB) Institutional Review Board (IRB) cover all aspects of this study. One protocol covers the prospective clinical CPET database from which the matched reference athlete cohort was obtained. The protocol outlines for accessing patient's medical records to ascertain health conditions and view other cardiac testing. The need for informed consent from patients whose clinical CPETs are included in the database was waived by the IRB, as there is no contact or interaction with patients beyond that which is required for their clinical care. A second protocol covers all aspects of the prospective longitudinal study of post-COVID athletes, including the symptoms surveys and second research CPETs presented here. All post-COVID athletes participating in the longitudinal study underwent informed consent as outlined by the IRB-approved protocol.

Athlete & COVID-19 Illness Definitions

Competitive athletes were defined as those completing dedicated exercise training for competitive individual or team-based goals; recreational athletes were defined as those completing dedicated exercise training without competitive goals. Predominate sport type was categorized into team (e.g. football, soccer, volleyball, basketball, or lacrosse), endurance (e.g. long-distance running, cycling, triathlon, rowing and/or swimming), or mixed / other (participating in multiple sports, or participating in a sport not meeting endurance or team definitions).

Confirmed SARS-CoV-2 infection was defined as a positive polymerase chain reaction (PCR) or antigen test. Acute presentation with COVID-19 was defined as mild if only fatigue, gastrointestinal symptoms (nausea, vomiting, diarrhea), headache, anosmia, ageusia, rhinorrhea, sore throat, or nasopharyngeal congestion were present; moderate if chills, fever or myalgias were present; or cardiopulmonary if exertional intolerance, chest pain, dyspnea, palpitations, lightheadedness, syncope, or cough were present.¹ If an athlete had symptoms in multiple categories, they were assigned the most severe category with cardiopulmonary symptoms considered of greater severity than moderate.

Study Population: Matched Healthy Athletes

To generate a reference group to compare CPET findings in the post-COVID athletes, we matched each post-COVID-19 athlete (n=21) with two healthy athletes from our research database. From all athlete tests in the database (n=1837), a healthy cohort of 566 athletes was available for potential matching. Unless clinically contraindicated, patients referred to the program undergo a CPET in conjunction with their clinical intake visit. All healthy athletes had been referred for clinically indicated CPETs either prior to the pandemic (n=39) or during the pandemic but without a clinical history and/or testing consistent with COVID-19 (n=3). As previously published in detail, rigorous exclusion criteria derived from the CPET itself, the medical history including diagnoses made as the result of the CPET, and transthoracic echocardiography were used to generate the cohort of athletes free of cardiac disease available for matching (n=566/1837, **Supplemental Figure**).² The one difference from the published workflow is that we allowed patients with abnormal spirometry into the cohort for matching.

A group of potential reference athletes was generated for each post-COVID-19 athlete that was exactly matched for sex, test type (treadmill vs. cycle ergometer), sport type as defined above, and diagnosis of current asthma. Within these groups, reference athletes were sought for each post-COVID-19 athlete that matched age ± 1 year and weight ± 5 kilograms. If there were more than two matched reference athletes, those two reference athletes with the closest weight were chosen as the final matches. Successfully matched reference athletes were removed from the pool of potential matches. If two matches were not available for a given post-COVID-19 athlete, additional reference athletes were sought that matched age ± 2 years and weight ± 15 kilograms. Again, if there were more than two total matched reference athletes, those two reference athletes with the closest weight were chosen as the final matches. Using this algorithm, two healthy reference athletes were matched for each post-COVID athlete.

The most common reasons for CPETs in reference athletes (n=42) were palpitations (33%), chest pain (21%), syncope (14%), and presyncope/lightheadedness (14%). As per exclusion criteria (**Supplemental Figure**), the presenting symptom(s) were found to be due to a clinically benign and/or non-cardiac entity (e.g., non-cardiac chest pain, neurocardiogenic syncope, ectopic beats).

Cardiopulmonary Exercise Testing Methods

All participants underwent an intensity graded, maximal effort-limited exercise test with continuous gas exchange on either the treadmill (Woodway Pro 27, Woodway USA, Waukesha, Wisconsin) or the upright cycle ergometer (Sport Excalibur Bicycle Ergometer, Lode, Holland) as previously described.² Briefly, the exercise modality was chosen by the participant and exercise physiologist with the goal of matching testing to a participant's primary form of exercise. The cycle ergometry test protocol consisted of 3 minutes of free-wheel cycling followed by continual increase in resistance (varying from 10 to 40 watts per minute) until test completion. Treadmill tests began with a 5-minute warm-up at 3.0 to 7.5 miles per hour and 1% grade followed by a progressive increase in incline (0.5% grade increase every 15 seconds) at a fixed speed until exhaustion. The intensity of the cycle ergometry ramp and the speed of treadmill testing were determined by the overseeing exercise physiologist in conjunction with the participant with a goal of reaching a 10 minute total ramp time. The exercise modality was chosen by the participant and exercise physiologist with the goal of matching testing to a participant's primary form of exercise.

Gas exchange was measured on a breath-by-breath basis using a Hans Rudolph V2 Mask (Hans Rudolph, Inc, Shawnee, Kansas), a commercially available metabolic cart and gas exchange analyzer (Ultima CardiaO₂; Medgraphics Diagnostics, St. Paul, Minnesota) and analyzed using Breeze Suite software (Medgraphics Diagnostics, Version 8.2, 2015). Continuous 12-lead ECG monitoring (Mortara Instrument X12+ wireless ECG transmitter, Milwaukee, Wisconsin) was performed and blood pressures were measured using a manual sphygmomanometer before exercise, at three- minute intervals during exercise, at peak exercise, and during recovery. Participants were instructed to report the development of any cardiopulmonary symptoms during testing, and at test termination were asked to rate the maximal severity of these symptoms during testing on a 1-10 Likert scale, with 1 being minimal and 10 being worst possible severity.

Test termination was determined by volitional exhaustion and maximal effort was confirmed by a peak respiratory exchange ratio >1.05 and a maximal heart rate of > 85% age/gender predicted peak values. Peak oxygen consumption ($\dot{V}O_2$) was defined as the highest oxygen uptake over a period of 30 seconds over the last

minute of effort-limited exercise and was assessed for normality using the Jones equations derived in the general population.^{3,4} An abnormally low $\dot{V}O_2$ was defined as <80% predicted, and a “low-normal” $\dot{V}O_2$ was defined as $\geq 80\%$ but <90% predicted. The peak respiratory exchange ratio was defined as exhaled carbon dioxide divided by oxygen consumption using the same 30 second average. The ventilatory threshold was determined using gas exchange data, specifically the V-slope method with complementary assessment of ventilatory equivalents and end-tidal gases as previously described.⁵ Resting sinus tachycardia was defined as resting heart rate (HR) of > 100 beats per minute. An abnormal blood pressure (BP) response was defined as blunted BP augmentation (systolic BP fall or rise < 20 mmHg)⁶⁻⁸ or a rapid fall in the first minute of post-exercise BP, with accompanying reproduction of presenting symptoms. Normal heart rate recovery was defined as a reduction of >24bpm at 2 minutes into recovery. Abnormal breathing reserve was defined as <10% at peak exercise and calculated as $(\text{Maximal } \dot{V}E / (40 * FEV_1))$. The peak oxygen pulse was calculated as $\dot{V}O_2 / \text{peak HR}$. The chronotropic index was calculated as: $((\text{Peak HR} - \text{Baseline HR}) / (\text{Predicted Peak HR} - \text{Baseline HR})) / ((\text{PVO}_2 - \text{Baseline } \dot{V}O_2) / (\text{Predicted PVO}_2 - \text{Baseline } \dot{V}O_2))$.⁹

Spirometry was performed both immediately prior to exercise according to American Thoracic Society (ATS)/European Respiratory Society (ERS) acceptability and repeatability criteria¹⁰, using a standard mouthpiece with a preVent flow sensor (Medgraphics Diagnostics, St. Paul, Minnesota) attached to the metabolic cart. Normality of the best forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) pre-exercise was assessed using predicted equations from the Global Lung Function Initiative (GLI).¹¹ FEV_1 , FVC, and FEV_1/FVC were considered abnormal if <5th percentile (-1.645 Z-score). If the FEV_1/FVC was abnormal, the degree of obstruction was graded as mild ($FEV_1 \geq 70\%$ predicted), moderate (FEV_1 60-69% predicted), moderately severe (FEV_1 50-59% predicted), severe (FEV_1 35-49% predicted), and very severe ($FEV_1 < 35\%$ predicted).

Supplemental Table 1. Post-COVID Athletes: Clinical Presentation & Testing

Athlete	Age & Sex	Sport Type	Time to Evaluation from COVID-19 Diagnosis	Persistent Cardiopulmonary Symptoms	Laboratory Results	ECG	TTE	CMR	CPET: Symptoms During Test & Key Findings	Other Testing
1	19M	Team Sport	2.8 months	Chest pain	HS-Trop.: mildly elevated Repeat 1 wk. later: normal	Normal	Normal	Normal	+Chest Pain (4/10) • Normal test	Patch monitor: normal
2	25M	Mixed	2.2 months	Dyspnea Lightheadedness Exercise intolerance	Normal	Normal	Normal	--	+Dyspnea (7/10) +Lightheadedness (6/10) • Abrupt fall in post-exercise BP	Patch monitor: normal
3	20F	Team Sport	1.4 months	Chest pain Dyspnea	Normal	Sinus Tach.	Normal	Normal	+Chest Pain (5/10) +Dyspnea (9/10) • Resting sinus tach. • Moderately severe obstructive defect • Low breathing reserve	
4	19M*	Team Sport	2.7 months	Chest pain Palpitations Lightheadedness	Normal	Normal	Normal	Normal	+Chest Pain (7/10) +Lightheadedness (2/10) • Peak VO ₂ 89% predicted	Patch Monitor: normal
5	21F	Team Sport	4.0 months	Chest pain Palpitations Lightheadedness	Normal	Normal	Normal	--	• Moderate obstructive defect • Low breathing reserve	Patch Monitor: normal
6	18F	Endurance	1.3 months	Chest Pain Dyspnea	Elevated D-dimer	Normal	Normal	Normal	+Chest Pain +Dyspnea • Resting sinus tach.**	CT Pulmonary Angiogram: normal
7	29F	Endurance	4.9 months	Dyspnea Palpitations Lightheadedness Exercise intolerance	Normal	Normal	Normal	LGE: Subtle, mid-wall LGE in mid lateral LV segment	+Dyspnea (8/10) +Lightheadedness (5/10) • Resting sinus tach. • Low breathing reserve	Patch monitor: normal

8	31F	Endurance	8.5 months	Dyspnea Palpitations Exercise intolerance	Normal	Normal	Normal	--	+Dyspnea (5/10) • Normal test	Patch Monitor: normal
9	25M	Mixed	1.6 months	Chest pain Dyspnea Palpitations Cough Exercise intolerance	Normal	Normal	Normal	LGE: Inferior RV insertion	+Chest Pain (2/10) +Dyspnea (5/10) • Peak VO ₂ 58% predicted • Mild obstructive defect	CT Pulmonary Angiogram: normal
10	20M	Team Sport	1.3 months	Chest pain Dyspnea Palpitations Lightheadedness Exercise intolerance	Normal	Normal	Normal	Normal	+Chest Pain (8/10) +Dyspnea (6/10) +Lightheadedness (2/10) • Resting sinus tach. • Moderate obstructive defect	Patch Monitor: normal
11	30F*	Endurance	3.0 months	Dyspnea Cough Exercise intolerance		Normal	Normal	Normal	+Dyspnea (7/10) • Normal test	
12	21M	Endurance	1.2 months	Dyspnea	Normal	Normal	Normal	--	+Dyspnea (8/10) • Low breathing reserve	
13	20M	Mixed	3.4 months	Chest pain	Normal	Normal	Normal	--	• Peak VO ₂ 88% predicted • Moderately severe obstructive defect • Low breathing reserve	
14	21M	Team Sport	1.8 months	Chest pain Dyspnea Exercise intolerance	Normal	Normal	--	LGE: Patchy subepicardial and pericardial	+Chest Pain (3/10) +Dyspnea (8/10) • Mild obstructive defect • Low breathing reserve	
15	21M*	Mixed	7.3 months	Chest pain Dyspnea Palpitations	Normal	Normal	--	LGE: Inferior RV insertion	+Chest Pain (6/10) • Peak VO ₂ 87% predicted	Patch monitor: normal
16	20F*	Team Sport	5.9 months	Chest pain Dyspnea Palpitations	Normal	Sinus Tach.	Normal	--	+Chest Pain (4/10) +Dyspnea (9/10) • Resting sinus tach.	Patch monitor: normal

Exercise intolerance									
17	19F	Team Sport	3.5 months	Chest pain Dyspnea Palpitations Exercise intolerance	Normal	Normal	Normal	LGE: Inferior RV insertion	<ul style="list-style-type: none"> • Blunted exercise BP • Mild obstructive defect +Chest Pain (4/10) +Dyspnea (6/10) • Normal test
18	20M	Team Sport	1.5 months	Dyspnea Palpitations Exercise intolerance	Normal	Normal	Normal	--	<ul style="list-style-type: none"> • Peak VO₂ 79% predicted Patch monitor: normal
19	19M*	Team Sport	2.0 months	Dyspnea Palpitations Exercise intolerance	Normal	Normal	Normal	--	+Dyspnea (7/10) <ul style="list-style-type: none"> • Peak VO₂ 65% predicted Patch monitor: normal
20	20M	Team Sport	1.2 months	Chest pain Dyspnea Exercise intolerance Cough	Normal	Normal	Normal	Normal	+Dyspnea <ul style="list-style-type: none"> • Normal test**
21	19F	Team Sport	1.4 months	Chest pain Palpitations Exercise intolerance	Normal	Normal	Normal	Normal	+Chest Pain (2/10) <ul style="list-style-type: none"> • Mild obstructive defect • Low breathing reserve Patch monitor: normal

*Current (n=3) or childhood (n=2) asthma diagnosis

**n=2 tests on which spirometry on direct review was considered to reflect poor effort

Supplemental Table 2: Longitudinal CPET Data in Post-COVID Athletes

	First Post-COVID CPET (n=13)	Second Post-COVID CPET (n=13)	Reference Cohort (n=26)
Testing Modality			
Cycle ergometer	6 (46)	6 (46)	12 (46)
Treadmill	7 (54)	7 (54)	14 (54)
Vital Signs			
Baseline HR (beats/min)	81 ± 15	75 ± 10*	78 ± 14
Peak HR (beats/min)	188 ± 9	183 ± 9*	186 ± 10
Percent Predicted	95 ± 5	93 ± 5*	95 ± 4
Heart Rate Recovery (beats/min)	47 ± 13	46 ± 11	47 ± 10
Baseline SBP (mmHg)	122 ± 12	126 ± 16	118 ± 11
Peak SBP (mmHg)	169 ± 21	172 ± 25	168 ± 27
Baseline DBP (mmHg)	75 ± 5	75 ± 7	75 ± 8
Peak DBP (mmHg)	77 ± 5	75 ± 7	67 ± 11 ⁺
Baseline O ₂ Saturation (%)	98 ± 1	97 ± 1.5	98 ± 1
O ₂ Saturation (%) at Peak Exercise	96 ± 2	95 ± 3	96 ± 2
Spirometry			
Pre-Exercise FEV ₁ (L)	3.8 ± 1.0	4.1 ± 0.9	4.3 ± 0.9
Percent Predicted (%)	88.6 ± 16.6	94.5 ± 12.7	97.3 ± 12.2
Abnormal (Below 5 th percentile)	3 (23)	1 (8)	2 (8)
Pre-Exercise FVC (L)	5.1 ± 1.0	5.1 ± 1.1	5.0 ± 1.1
Percent Predicted (%)	100 ± 10	101.4 ± 11.8	97.3 ± 13.5
Pre-Exercise FEV ₁ /FVC	0.75 ± 0.12	0.79 ± 0.09	0.85 ± 0.06 ⁺
Abnormal (Below 5 th percentile)	4 (31)	3 (23)	1 (4) ⁺
Gas Exchange			
Respiratory Exchange Ratio	1.16 ± 0.08	1.16 ± 0.07	1.16 ± 0.08
Peak $\dot{V}O_2$ (L/min)	3.39 ± 0.69	3.62 ± 0.81*	3.54 ± 1.02
Peak $\dot{V}O_2$ (ml/kg/min)	46.7 ± 9.0	49.0 ± 9.0	47.2 ± 10.6
Percent Predicted (%)	117 ± 36	123 ± 38*	114 ± 25
Abnormal (<80% predicted)	1 (8)	0 (0)	1 (4)
$\dot{V}O_2$ at VT (ml/kg/min)	38.2 ± 10.2	40.9 ± 9.0	36.2 ± 11.0
Chronotropic Index	0.85 ± 0.23	0.77 ± 0.20*	0.83 ± 0.18
Oxygen Pulse (ml/beat)	18.0 ± 3.4	19.7 ± 4.3*	18.9 ± 5.2
Total $\dot{V}E/\dot{V}CO_2$ slope	28.5 ± 3.9	27.3 ± 4.7	28.7 ± 4.4
$\dot{V}E/\dot{V}CO_2$ slope through VT	24.5 ± 3.8	24.4 ± 4.3	24.1 ± 3.4
Peak $\dot{V}E$ (L/min)	122 ± 34	123 ± 39	126 ± 41
Breathing Reserve (%)*	17 ± 18	23 ± 20	27 ± 15
Low Breathing Reserve (<10%)	6 (46)	3 (23)	3 (12)

*p<0.05 for post-COVID athletes baseline versus follow-up CPET. ⁺p<0.05 for post-COVID athletes baseline versus reference athletes. ⁺p<0.05 for post-COVID athletes follow-up CPET versus reference athletes. **HR:** Heart Rate, **bpm:** beat per minute, **PP:** peak percentage, **HRR:** Heart Rate Recovery, **SBP:** Systolic Blood Pressure, **DBP:** Diastolic Blood Pressure, **LLN:** Lower limit of Normal, **FEV₁:** Forced expiratory volume in the first second of forced breath, **FVC:**

forced vital capacity, $\dot{V}\text{CO}_2$: carbon dioxide production, $\dot{V}\text{O}_2$: Oxygen consumption, **VT**: Ventilatory Threshold, $\dot{V}\text{E}$: Ventilation, **MVV**: Maximum Voluntary Ventilation

Figure Legends

Supplement Figure 1. Generation of Reference Athlete Cohort Free of Cardiac Disease. Our database contained 1837 CPETs in athlete patients, of which 566 were determined to healthy through the criteria above. From amongst this healthy cohort of 566, athletes who had not had COVID-19 were identified that matched with the post-COVID athletes as described in the Methods Supplement. CPET: cardiopulmonary exercise testing. RER: respiratory exchange ratio. HR: heart rate.

Supplement Figure 2. Diagnostic Approach to Athletes with Persistent Cardiopulmonary Symptoms after COVID-19.

* For example, history and physical may reveal an obvious cause for symptoms suggestive of cardiopulmonary origin such as costochondritis or pneumonia.

** ECG, Labs including HS-Troponin and TTE should be completed unless already performed as part of recent “return to play” protocol. ^CPET should be performed for evaluation of exertional symptoms unless a clinical contraindication to exercise testing, such as active inflammatory heart disease, is present. ‡ Other clinically indicated testing should be considered on a case-by-case basis. This may include ambulatory rhythm monitoring, chest CT, and full pulmonary function testing including evaluation for exercise-induced bronchoconstriction.

† High suspicion for inflammatory heart disease may be present despite normal initial testing if there is persistent unexplained exertional chest pain or tightness, significant exertional intolerance, new palpitations or syncope, and a short duration (< 3 months, particularly <1-2 months) since COVID-19 diagnosis, and no alternative diagnosis (ex. pulmonary disease) evident on initial history, physical or diagnostic testing.^{13,14}

PACS: Post-Acute COVID-19 Syndrome. ECG: electrocardiogram. TTE: transthoracic echocardiogram. CPET: cardiopulmonary exercise testing. HS-Troponin: high sensitivity troponin. CMR: cardiac magnetic resonance imaging. RTP: Return-to-play

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