

Acute exercise increases immune responses to SARS CoV-2 in a previously infected man

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Abstract:

Evidence is emerging that exercise and physical activity provides protection against severe COVID-19 disease in patients infected with SARS-CoV-2, but it is not known how exercise affects immune responses to the virus. A healthy man completed a graded cycling ergometer test prior to and after SARS-CoV-2 infection, then again after receiving an adenovirus vector-based COVID-19 vaccine. Using whole blood SARS-CoV-2 peptide stimulation assays, IFN- γ ELISPOT assays, flow cytometry, *ex vivo* viral-specific T-cell expansion assays and deep T-cell receptor (TCR) β sequencing, we found that exercise robustly mobilized highly functional SARS-CoV-2 specific T-cells to the blood compartment that recognized spike protein, membrane protein, nucleocapsid antigen and the B.1.1.7 α -variant, and consisted mostly of CD3⁺/CD8⁺ T-cells and double-negative (CD4⁻/CD8⁻) CD3⁺ T-cells. The magnitude of SARS-CoV-2 T-cell mobilization with exercise was intensity dependent and robust when compared to T-cells recognizing other viruses (e.g. CMV, EBV, influenza). Vaccination enhanced the number of exercise-mobilized SARS-CoV-2 T-cells recognizing spike protein and the α -variant only. Exercise-mobilized SARS-CoV-2 specific T-cells proliferated more vigorously to *ex vivo* peptide stimulation and maintained broad TCR- β diversity against SARS-CoV-2 antigens both before and after *ex vivo* expansion. Neutralizing antibodies to SARS-CoV-2 were transiently elevated during exercise after both infection and vaccination. Finally, infection was associated with an increased metabolic demand to defined exercise workloads, which was restored to pre-infection levels after vaccination. This case study provides impetus for larger studies to determine if these immune responses to exercise can facilitate viral clearance, ameliorate symptoms of long COVID syndrome, and/or restore functional exercise capacity following SARS-CoV-2 infection.

Keywords: Exercise immunology; long COVID syndrome; α -variant; TCR sequencing; virus specific T-cells; metabolic response, respiratory gas exchange; lactate; catecholamines; cortisol; physical activity

Highlights:

- 1) Exercise mobilizes SARS-CoV-2 T-cells and augments their *ex vivo* expansion
- 2) SARS-CoV-2 T-cells are highly exercise-responsive compared to other viral T-cells
- 3) Exercise mobilized and expanded SARS-CoV-2 T-cells maintain broad TCR- β diversity
- 4) Exercise transiently elevates neutralizing antibodies to SARS CoV-2 in serum
- 5) Vaccination restored the greater metabolic demand of exercise seen after infection

1. Introduction

As of August 5th 2021, the coronavirus disease of 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than ~200 million people worldwide resulting in over 4.2 million deaths (Dong et al., 2020). The rapid development and distribution of vaccine candidates has seen more than 4.3 billion vaccine doses administered to date, lowering rates of infection and mortality. It has been reported, however, that 10-30% of COVID-19 survivors will develop so-called long COVID syndrome, experiencing continuing symptoms of disease such as fatigue, shortness of breath, “brain fog”, fever, anxiety, depression, sleep disorders and gastrointestinal problems (Logue et al., 2021; Sudre et al., 2021; Yong, 2021). The causes of long COVID syndrome are not yet known, but viral persistence and/or a prolonged inflammatory response are likely to be involved (Yong, 2021).

It has emerged that lifestyle factors are playing a role in disease severity following SARS-CoV-2 infection, with those who are obese and/or physically inactive more likely to die, become hospitalized and/or require ventilatory support following infection (Cummins et al., 2021; Sallis et al., 2021). Nevertheless, the mechanisms by which physical activity can protect against severe infections and potentially ameliorate the symptoms of long COVID remain unknown. Each bout of exercise evokes an instantaneous mobilization and redistribution of effector lymphocytes, including CD4⁺ and CD8⁺ T-cells that recognize viral antigens. For instance, memory subsets of CD8⁺ T-cells specific to cytomegalovirus (CMV), Epstein-Barr virus (EBV) and adenovirus (AdV) are immediately mobilized to the blood compartment in response to acute leg cycling exercise (Kunz et al., 2020; Kunz et al., 2018; Spielmann et al., 2014; Spielmann et al., 2016). This response is driven by the release of catecholamines acting on β_2 adrenergic receptors expressed by the antigen-specific T-cells and is purported to facilitate anti-viral immune

surveillance in individuals who exercise regularly (Kunz et al., 2020). Moreover, exercise enhances lymphatic flow to transiently increase circulating levels of immunoglobulins (Nehlsen-Cannarella et al., 1991), but it is not known if acute exercise can increase circulating levels of neutralizing antibodies against certain viruses. It is possible that any positive changes in anti-SARS-CoV-2 immunity with exercise could play an important role facilitating viral clearance in those with long COVID syndrome. Indeed, observational studies are emerging to indicate that symptoms of long COVID syndrome improve after vaccination (Arnold et al., 2021), possibly due to vaccine-induced enhancements in neutralizing antibodies and SARS-CoV-2 specific T-cells to reduce the viral reservoir.

We demonstrate in an otherwise healthy man that acute exercise transiently increases circulating neutralizing antibodies and instantaneously mobilizes highly functional SARS-CoV-2 specific T-cells after natural infection and again following COVID-19 vaccination. We also report that natural infection increased the metabolic response to defined exercise workloads, which was reversed after COVID-19 vaccination. This case study provides impetus for future research to determine if the frequent mobilization and redistribution of SARS-CoV-2 specific T-cells and increased transportation of neutralizing antibodies with each bout of exercise can increase viral clearance, ameliorate symptoms of long COVID syndrome, and/or restore functional exercise capacity after infection.

2. Methods

2.1 Study Participant and Procedures

A 42-year-old healthy male (height: 180.5cm; body mass: ~73.5kg) with a background in competitive endurance sports (e.g. running, cycling, duathlon) contracted SARS-CoV-2 on

December 28th 2020 and subsequently received the Johnson and Johnson (J&J) human adenovirus-vector COVID-19 vaccine on March 31st 2021. He reported testing positive for SARS CoV-2 the day after his live-in wife received a positive test and described experiencing symptoms consistent with acute SARS CoV-2 infection (e.g. fever, chills, muscle aches, runny nose and a temporary loss of taste and smell) that lasted for 48-72h. For the remainder of the study period (up to April 21st 2021) he described experiencing increased levels of fatigue/malaise, particularly in the late afternoon when he would oftentimes have to nap for 30-60-minutes on most days. He reported participating in vigorous exercise 4-6 times/week for 45-90 minutes/session with no major changes in his exercise habits throughout the study period (May 22nd 2020 – April 21st 2021). Body mass measured on 4 separate occasions across the study timepoints was recorded as 71.4, 73.9, 74.3 and 74.5kg, respectively). As part of an ongoing IRB approved research study in our laboratory at the University of Arizona, he completed a graded exercise test to volitional exhaustion on a cycle ergometer (Velotron, RacerMate, USA) on May 22 2020, to determine his peak oxygen consumption ($\dot{V}O_{2peak}$). Subsequently, he performed an incremental continuous cycling bout consisting of 4x5-minute stages at power outputs corresponding to 50% (50W), 60% (95W), 70% (150W), and 80% (200W) of his pre-determined $\dot{V}O_{2peak}$ (20-minutes total) on November 18th 2020, 5.7 weeks prior to contracting SARS-CoV-2 (pre-infection; Pre-I). This exercise test was repeated using identical absolute power outputs on February 12th 2021 (6.6 weeks after infection; Post-I) and again on April 21th 2021 (3.0-weeks after vaccination/16.3-weeks after infection; Post-V). During each exercise trial, he arrived at the laboratory at 08:00 following an overnight fast and an intravenous catheter was inserted to allow blood to be drawn at rest, during exercise at the 60% and 80% $\dot{V}O_{2peak}$ stages, and at 1h post (+1h) exercise. Respiratory gas exchange (Quark CPET,

COSMED, Italy) and heart rate (Garmin, USA) were monitored continuously at rest and during exercise by indirect calorimetry (breath-by-breath) and short-range telemetry, respectively.

2.2 Blood Processing & Flow Cytometry

Frozen serum samples from each exercise timepoint were analyzed for SARS-CoV-2 neutralizing antibodies (Cayman Chemical, Michigan, USA), cortisol (Invitrogen, MA, USA), lactate (Sigma-Aldrich, Missouri, USA), and catecholamines (2-CAT ELISA, LDN, Germany) using commercially available ELISA kits. Heparinized blood was utilized for 24 –hour whole blood peptide stimulation assays and to isolate peripheral blood mononuclear cells (PBMCs) using standard density gradient centrifugation procedures. PBMCs were used for IFN- γ ELISPOTs, IFN- γ capture assays, and the *ex vivo* expansion of SARS-CoV-2 virus-specific T-cells (VSTs). EDTA whole blood samples collected from each timepoint were stained and analyzed by 7-color flow cytometer (MACSQuant 10; Miltenyi Biotec Inc., Germany) using the following directly conjugated antibodies: CD8-VioBlue, CD14-VioGreen, CD3-FITC, CD4-PE, CD20-PerCP, CD45-APC, CD56-APC-Vio770 (Miltenyi). The percentage of all CD45⁺ lymphocytes expressing the surface markers of interest were multiplied by the total lymphocyte count (determined using a volumetric flow cytometry-based whole blood assay) to enumerate each lymphocyte subtype per unit of whole blood.

2.3 Whole Blood Peptide Stimulation

Whole blood (1mL) treated with lithium heparin and 2mg glucose was stimulated with saline (1st negative control), PHA (10 μ g/mL; Sigma Aldrich; positive control), an actin pepmix (1 μ g/mL;

JPT Peptide Technologies, Germany; 2nd negative control), or the SARS-CoV-2 spike (S), membrane (M), nucleocapsid (N), and α -variant pepmixes, each overlapping by 11 amino acids (10 μ g/mL; Miltenyi). Whole blood was also stimulated with pooled S, M and N pepmixes, or with pooled pepmixes derived from other common viruses (10 μ g/mL; Miltenyi) including, Epstein-Barr virus (EBV; consensus), cytomegalovirus (CMV; IE-1, pp65), adenovirus (ADV; hexon, penton), respiratory syncytial virus (RSV; nucleoprotein), BK virus (BKV; LT, VP-1), JC virus (JCV; LT, VP-1), herpes simplex virus-1 (HSV-1; Envelope Glycoprotein D), influenza (HA, NA, MP-1, NP), and varicella-zoster virus (VZV; IE-63) (5 μ g/mL; JPT). After 24-hour incubation at 37°C, plasma was removed and stored at -80°C until analysis. Interferon gamma (IFN- γ) concentration was then determined using a commercially available ELISA kit (R&D Systems, MN, USA).

2.4 Generation of SARS-CoV-2 virus-specific T-cells

SARS-CoV-2 VSTs were expanded from PBMCs in the Post-I trial only using a previously described VST expansion protocol (Keller et al., 2020). Briefly, 1x10⁷ PBMCs were pulsed with 700ng each of S, M, and N SARS-CoV-2 pepmix pools (JPT) in 100 μ L RPMI culture medium consisting of 10% fetal bovine serum and 1% penicillin-streptomycin for 1h at 37°C with 5% CO₂. After incubation, culture media supplemented with IL-4 (1000IU/mL) and IL-7 (10ng/mL) was added to a final concentration of 1x10⁶ cells/mL culture medium and cells were placed within gas permeable G-REX devices for 7-days. On day 7, cells were enumerated and resuspended at 1x10⁶ cells/mL in media supplemented with IL-4 (1000IU/mL), IL-7 (10ng/mL), and IL-15 (5ng/mL) until day 10 whereby cells were harvested, enumerated, phenotyped, and used for functional assays.

2.5 Enumeration of SARS-CoV-2 specific T-cells by IFN- γ ELISPOT

The number of functional SARS-CoV-2 specific T-cells producing IFN- γ in response to virus-specific peptide stimulation were determined by enzyme-linked immunospot (ELISPOT) assays (Mabtech AB, Sweden) as previously described (Keller et al., 2020). Frozen PBMCs (100,000 cells/well) and expanded SARS-CoV-2 VSTs (100,000 cells/well) were stimulated with media only (1st negative control), (1 μ g/mL), actin (2nd negative control), 1 μ g/mL anti-CD3 mAb (1st positive control), 2 μ g/mL PHA (2nd positive control), or 2 μ g/mL each of the individual SARS-CoV-2 (JPT) pepmixes in separate wells. The number of IFN- γ spot-forming-cells (SFCs) were enumerated by Zellnet Consulting (NJ, USA) and, for the PBMCs collected on Day 0, adjusted for the total blood T-cell count (SFC/ml).

2.6 SARS-COV2 IFN- γ Assay Methods

SARS-CoV-2 specific T-cells and expanded VSTs were assessed for CD3, CD4 and CD8 surface marker expression using an IFN- γ Secretion Assay Kit (Miltenyi) and flow cytometry. Briefly, PBMCs or VSTs were washed and resuspended in RPMI+5% human serum at a final concentration of 1×10^6 cells/mL; 200IU/mL IL-2 was then added to the culture medium to rest overnight. On day 2, the cells were resuspended in culture medium at a concentration of 1×10^7 cells/mL in a 96-well plate (150 μ L/well). Each sample received 3 μ L of cytoestim (positive control), 1 μ L of MOG (negative control), and 10 μ LS, M or N SARS-CoV-2 pepmixes followed by 3h incubation at 37°C with 5% CO₂. Cells were then harvested, washed, and IFN- γ capture reagent was added. After 45 minutes at 37°C with 5% CO₂, the cells were washed and monoclonal antibodies were added. After 30 minutes in the dark, cells were washed, and phenotyped by flow cytometry.

2.7 TCR- β sequencing

The DNeasy blood Extraction Kit (Qiagen, USA) was used to extract genomic DNA from PBMCs collected at rest, during-exercise (80% intensity only), and 1h post-exercise in the Post-I and Post-V trials only. Genomic DNA was also extracted from the SARS-CoV-2 VSTs that were expanded after these trials. TCR- β deep sequencing was performed by Adaptive Biotechnologies (Seattle, WA, USA) and TCR- β rearrangements specific to SARS-CoV-2 were determined using the immunoSEQ T-MAP COVID ImmuneCODE database (Adaptive Biotechnologies, USA).

3. Results

3.1 Exercise mobilizes diverse SARS-CoV-2 specific T-cells to the blood compartment and transiently elevates neutralizing antibodies.

Changes in the numbers of circulating monocytes, lymphocytes and the major lymphocyte subsets in response to each exercise trial are shown in Table 1. The effects of exercise on the mobilization of SARS-CoV-2 specific T-cells after infection and vaccination are depicted in Figure 1. Specifically, exercise increased the whole blood IFN- γ response to pooled SARS-CoV-2 pepmix stimulation in an intensity dependent manner (Figure 1a). These results were corroborated via IFN- γ SFCs measured by ELISPOT (Figure 1a) (the alpha-variant pepmix was not used to stimulate whole blood in the Post-I trial as they were not available at this time). There was an enhanced IFN- γ response after exercise Post-V, but not in an intensity dependent manner (Figure 1a). A similar exercise response was observed with SARS-CoV-2 specific neutralizing antibodies (Figure 2b). The IFN- γ response to peptide stimulation (whole blood and ELISPOT) was increased after

exercise in the Post-I trial with the strongest effect seen for T-cells recognizing S protein (and α -variant), followed by M, and then N structural proteins (Figure 1c). The IFN- γ response to peptide stimulation (whole blood and ELISPOT) increased at the 60% intensity Post-V, with the strongest effect seen for T-cells recognizing S protein (and α -variant), followed by N, and then M structural proteins (Figure 1d). In the Post-I trial, the absolute change in IFN- γ secretion to combined SARS-CoV-2 whole blood peptide stimulation was greater than that observed after stimulating with pooled peptides derived from nine other viruses (Figure 1e). Phenotypic characterization of T-cells responding to SARS-CoV2 peptides among PBMCs Post-I revealed a relative dominance of CD4⁺ T-cells, regardless of exercise time point or SARS-CoV-2 antigen specificity (S, M, N) (Figure 1 f/g/h). The percentages of CD4⁺ and CD8⁺ T-cells remained relatively stable across time. Interestingly, the percentage of ‘double negative’ (CD4⁻CD8⁻) T-cells responsive to each individual pepmix, increased at 80% $\dot{V}O_{2peak}$; CD4⁻CD8⁻ T-cells increased from 3.61% to 19.46% (S), 3.06% to 10.3% (M), and 5.22% to 11.84% from (N) rest to 80% $\dot{V}O_{2peak}$ (Figure 1 f/g/h). Deep TCR- β sequencing in the Post-I trial revealed that exercise mobilized cells maintain diversity (Figure 1i), although there was a discernible increase in the number of unique clones recognizing M glycoprotein, ORF6 and N phosphoprotein in PBMCs collected during exercise compared to PBMCs collected at rest and 1h post-exercise (Figure 1i/j/k/l).

Table 1: The total number (cells/ μ L) of lymphocytes, CD3⁺ T-cells, CD4⁺ T-cells, CD8⁺ T-cells, ‘double-negative’ T-cells, NK-cells, B-Cells, and monocytes found in peripheral blood before (rest), during (60% and 80%), and 1h after each exercise trial.

Leukocyte Subsets (cells/ μ L)	Rest	60%	80%	+1h
Lymphocytes				
<i>Pre-I</i>	1744.54	2218.37	2735.60	2167.97
<i>Post-I</i>	1587.88	1832.95	2806.57	1512.49
<i>Post-V</i>	1710.38	1893.01	2387.63	1291.64
CD3⁺ T-cells				
<i>Pre-I</i>	1295.67	1576.15	1856.38	1677.79
<i>Post-I</i>	1190.91	1288.01	1729.13	1098.82
<i>Post-V</i>	1271.33	1274.75	1443.08	964.08
CD4⁺ T-cells				
<i>Pre-I</i>	875.09	1021.66	1203.49	1144.59
<i>Post-I</i>	761.47	812.87	1033.85	727.64
<i>Post-V</i>	817.08	754.91	821.98	623.86
CD8⁺ T-cells				
<i>Pre-I</i>	387.41	509.10	605.55	491.43
<i>Post-I</i>	391.33	436.76	607.10	344.26
<i>Post-V</i>	421.06	486.57	581.27	315.45
CD4⁺CD8⁻ T-cells				
<i>Pre-I</i>	27.47	33.41	46.97	34.90
<i>Post-I</i>	29.42	31.30	44.09	20.88
<i>Post-V</i>	28.35	29.19	34.63	21.79
NK-cells				
<i>Pre-I</i>	195.74	338.30	454.66	168.23
<i>Post-I</i>	249.46	336.71	662.91	243.81
<i>Post-V</i>	304.79	429.71	709.84	201.50
B-cells				
<i>Pre-I</i>	94.73	176.58	205.17	189.91
<i>Post-I</i>	116.55	161.12	227.05	159.87
<i>Post-V</i>	161.29	162.80	175.25	137.17
Monocytes				
<i>Pre-I</i>	204.26	300.09	352.17	318.71
<i>Post-I</i>	213.78	272.79	354.13	256.97
<i>Post-V</i>	219.8	257.39	310.80	174.79

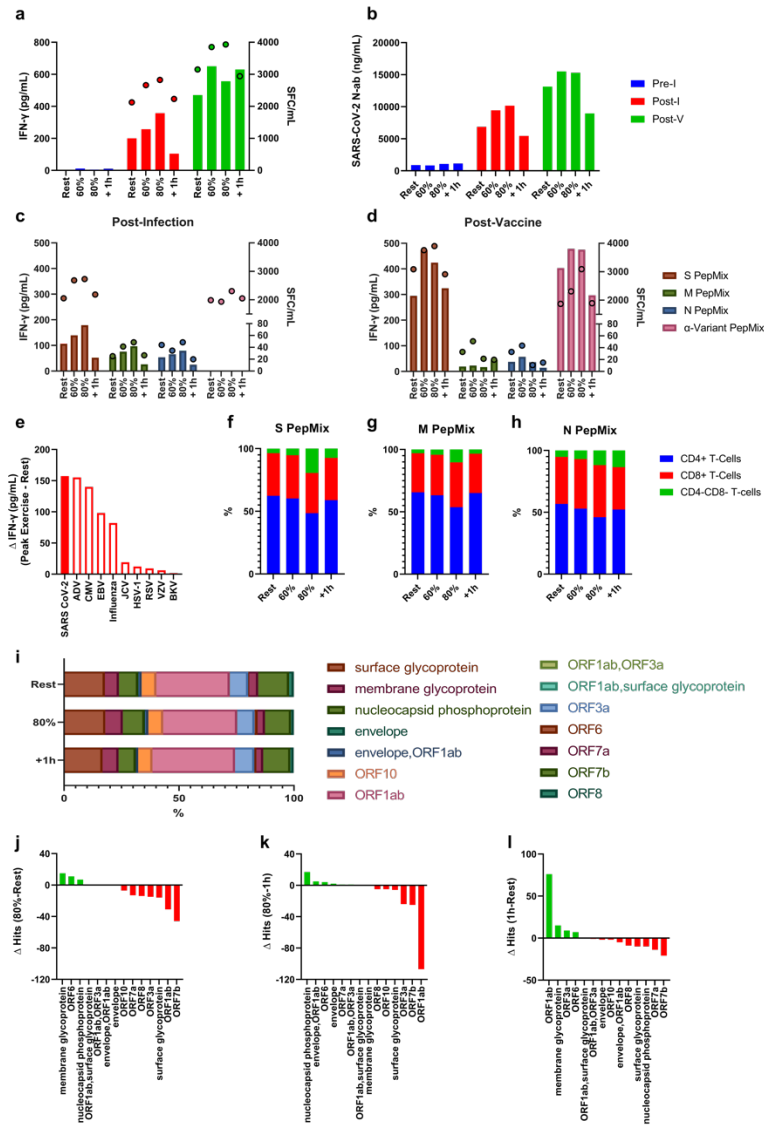


Figure 1: The effect of exercise on the mobilization of SARS-CoV-2 specific T-cells and neutralizing antibodies. (a) IFN- γ response of SARS-CoV-2 specific T-cells (S/M/N Pempmix combined) to exercise Pre-I, Post-I, and Post-V as measured by whole blood peptide stimulation (bars) and ELISPOT (dots). (b) Neutralizing antibody responses to exercise Pre-I, Post-I, and Post-V. (c) Post-I and (d) Post-V IFN- γ response, specific to S, M, N, and α -variant Pempmix individually, to exercise Pre-I, Post-I, and Post-V as measured by whole blood peptide stimulation (bars) and ELISPOT (dots). (e) The absolute change in IFN- γ (peak exercise –rest) levels in response to stimulation with pempmixes derived from SARS-CoV-2 and 9 other common viruses to which this individual demonstrated immunity to. Proportions of T-cell subsets among IFN- γ secreting PBMCs stimulated with SARS-CoV-2 (f) S, (g) M, and (h) N pempmixes, respectively. (i) SARS-CoV-2 specific TCR-diversity in response to exercise (Post-I). The absolute change of SARS-CoV-2 unique clones, (j) 80% relative to rest, (k) 80% relative to 1h, and (l) 1h relative to rest.

3.2 Exercise mobilized SARS-CoV-2 specific T-cells are more responsive to ex vivo peptide stimulation and maintain broad TCR diversity

The effect of exercise on the *ex vivo* expansion of SARS-CoV-2 VSTs in the Post-I trial is depicted in Figure 2. Exercise increased the *ex vivo* expansion of SARS-CoV-2 VSTs, with the greatest response seen at the 80% intensity (Figure 2a). After adjusting for the number of VSTs stimulated on Day 0, VSTs responding to the M structural protein, had the greatest relative expansion, followed by S protein (including α -variant) then the N structural protein. In most cases, this response occurred in an exercise intensity dependent manner (Figure 2b). *Ex vivo* expansion preferential increased the proportion of CD8⁺ T-cells that recognize the S protein (Figure 2c), M structural protein (Figure 2d), and N structural protein (Figure 2e) compared to PBMCs. The composition of the T-cell compartment after expansion was not greatly affected by exercise but there was an observable shift toward CD8 T-cell dominance against all SARS-CoV-2 antigens. Similar to PBMCs, deep TCR- β sequencing revealed that VSTs expanded after exercise maintain diversity (Figure 2f) but there was a slight skewing in T-cell clonality toward M glycoprotein and N phosphoprotein in VSTs expanded from PBMCs collected during exercise and VSTs expanded before exercise and 1h post-exercise (Figure 2g/h/i).

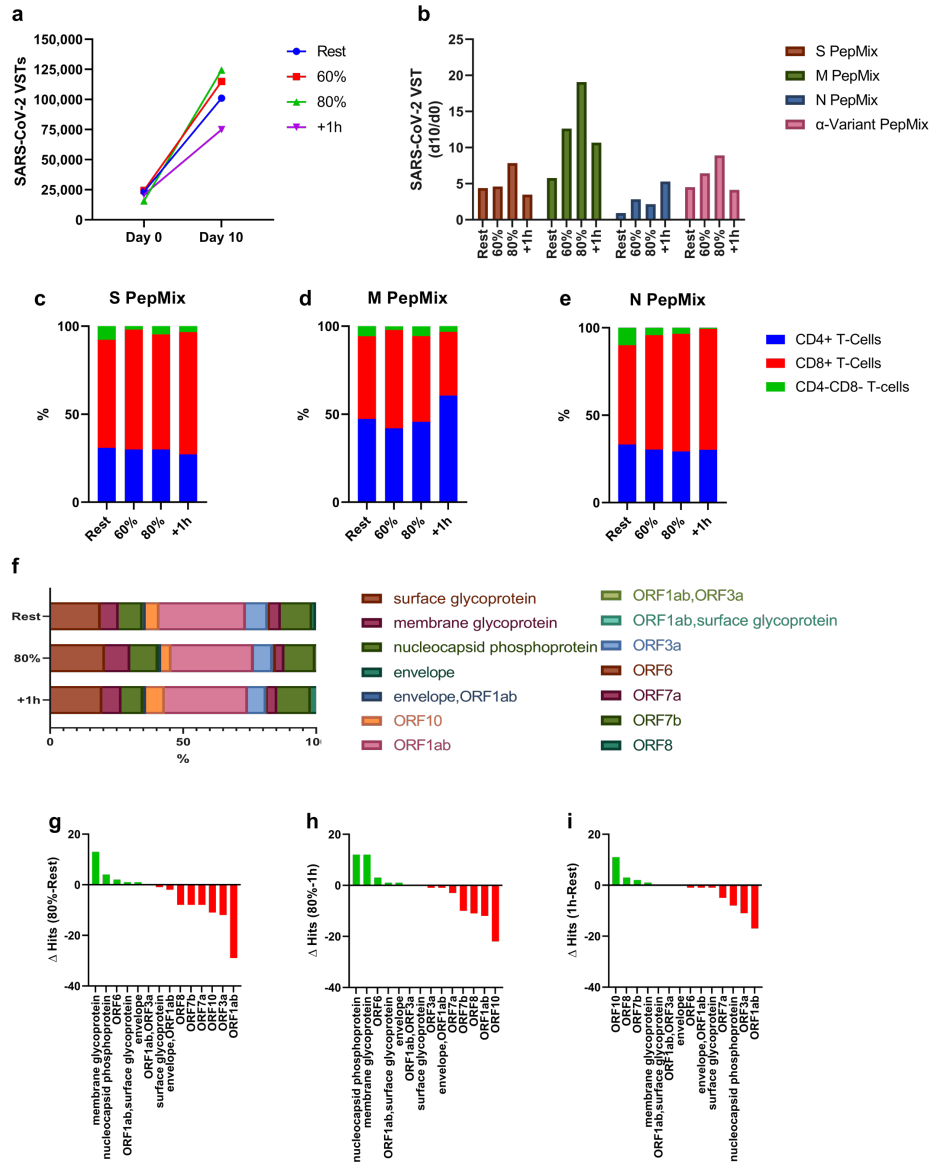


Figure 2: The effect of exercise on the *ex vivo* expansion of SARS-CoV-2 VSTs. (a) The total number of SARS-CoV-2 T-cells at the start of culture (day 0) and the total number of SARS-CoV-2 VSTs generated after 10 days of *ex vivo* expansion. (b) The number of SARS-CoV-2 VSTs generated at Day 10 divided by the number of SARS-CoV-2 T-cells in the PBMC fractions at Day 0, specific to each individual pepmix. Proportions of T-cell subsets among IFN- γ secreting expanded VSTs stimulated with SARS-CoV-2 (c) S, (d) M, and (e) N antigen pepmixes, respectively. (f) SARS-CoV-2 specific TCR-diversity in response to exercise after *ex vivo* expansion (Post-I). The absolute change in SARS-CoV-2 unique clones among the expanded VSTs, (j) 80% relative to rest. (k) 80% relative to 1h, and (l) 1h relative to rest.

3.3 SARS-CoV-2 infection was associated with an increased metabolic demand to graded exercise that was restored after COVID-19 vaccination

$\dot{V}O_2$ ratio of ventilation to carbon dioxide exhaled ($\dot{V}E/\dot{V}CO_2$) did not differ across the three exercise trials (Figure 3). However, discernable elevations in heart rate (HR), ventilation/oxygen consumption ($\dot{V}E/\dot{V}O_2$), respiratory exchange ratio (RER), V-slope, epinephrine, norepinephrine and blood lactate, with concomitant reductions in stroke volume (SV) and predicted $\dot{V}O_{2peak}$, were noted in the Post-I trial compared to the both the Pre-I and Post-V trials. The metabolic response to exercise in the Post-V trial was comparable (or slightly improved) to the Pre-I trial. Resting cortisol levels were markedly elevated in the Post-I and Post-V trials compared to the Pre-I trial.

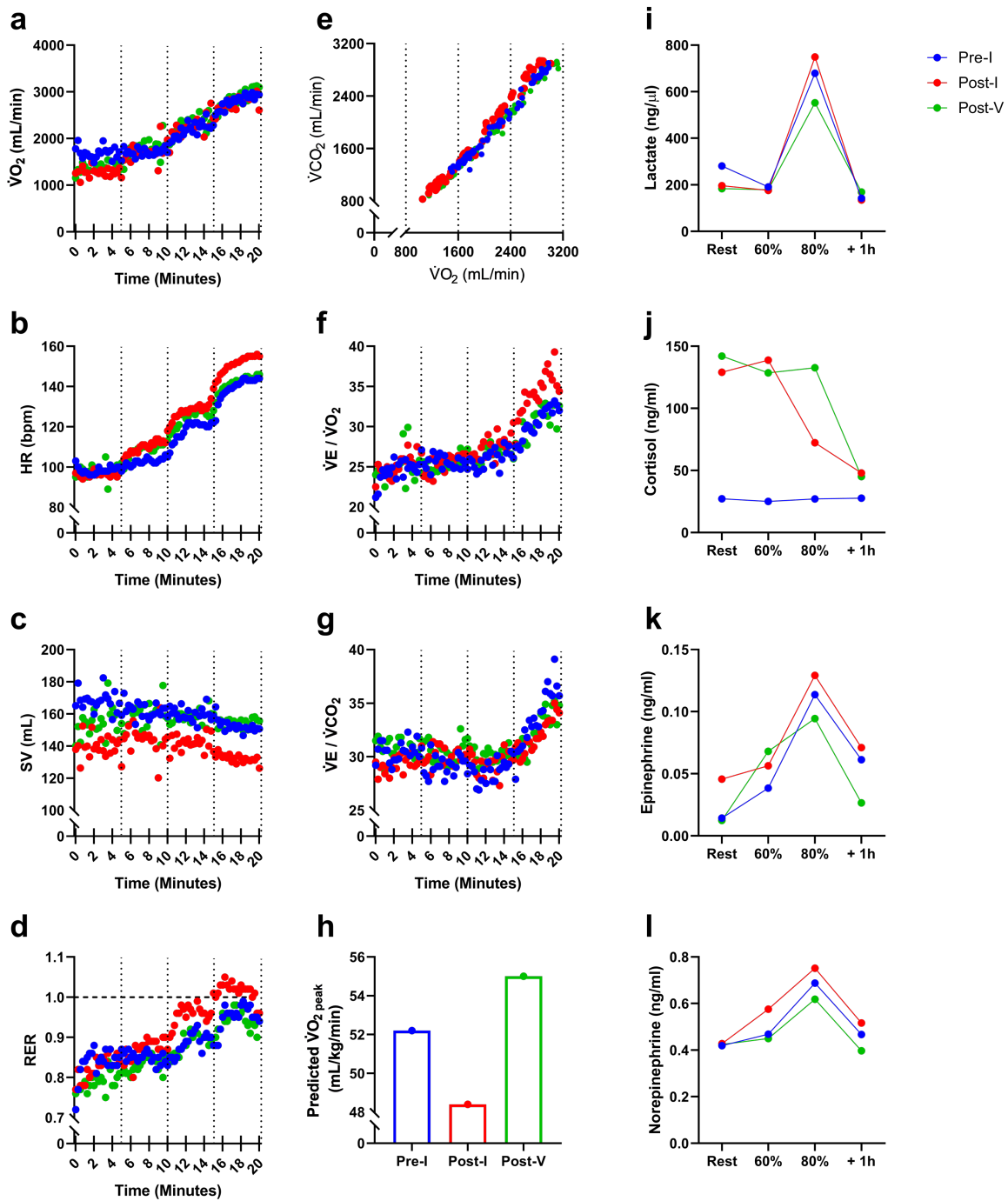


Figure 3: Metabolic responses to graded exercise before and after SARS-CoV-2 infection and COVID-19 vaccination. (a) $\dot{V}O_2$, (b) HR, (c) SV, (d) RER, (e) V-slope, (f) $\dot{V}E / \dot{V}O_2$, (g) $\dot{V}E / \dot{V}CO_2$, (h) Predicted $\dot{V}O_{2, peak}$, (i) Blood Lactate, (j) Epinephrine, and (l) Norepinephrine.

4. Discussion

Individuals that regularly exercise have improved clinical outcomes and symptomology following SARS-CoV-2 infection (Cummins et al., 2021; de Souza et al., 2021; Sallis et al., 2021). This has raised awareness on the potential for lifestyle changes, such as exercise, to safeguard against viral disease and has encouraged investigations into the role of exercise in the recovery of the so-called long COVID syndrome (McGregor et al., 2021; Udina et al., 2021). The frequent mobilization and redistribution of SARS-CoV-2 specific T-cells with each bout of exercise, combined with an increased lymphatic transportation of neutralizing antibodies, is one potential mechanism by which exercise can improve immunity against SARS-CoV-2 (Simpson and Katsanis, 2020); but how exercise affects cellular and humoral immunity against this novel coronavirus has not been determined. This case study is the first report of immune responses to exercise following SARS-CoV-2 infection and COVID-19 vaccination in an otherwise healthy individual.

We have previously shown that T-cells specific to adenovirus and common herpesviruses (CMV,EBV) are mobilized to blood in response to acute exercise, and that the effect is largely influenced by the secretion of catecholamines acting on T-cell β_2 adrenergic receptors (Kunz et al., 2020; Kunz et al., 2018; Spielmann et al., 2014; Spielmann et al., 2016). Here we show that exercise also mobilizes SARS-CoV-2 specific T-cells in an individual recently infected with the virus. The IFN- γ response to whole blood SARS-CoV-2 peptide stimulation (using overlapping peptide pools that span the S, M and N structural proteins) was elevated after exercise in an exercise-intensity dependent manner, with the greatest absolute (post – pre) response seen for T-cells recognizing S protein (including the α -variant) followed by M and N structural proteins. Strikingly, the absolute change in IFN- γ secretion to combined SARS-CoV-2 peptide stimulation was higher than the T-cell response to peptide pools derived from 9 other viruses, including known

exercise-responsive ADV, CMV and EBV-specific T-cells, as well as T-cells responding to other respiratory viruses such as influenza and RSV. This robust response of SARS-CoV-2 specific T-cells is most likely due to the exercise test being performed shortly after (~6-weeks) primary infection when increased numbers of peripheral antigen-specific T-cells are present. Exercise transiently increased the circulating levels of SARS-CoV-2 neutralizing antibodies, likely due to increased lymphatic flow and the return of protein rich fluid back to the blood compartment. We purport that increases in lymphatic flow with exercise may facilitate the transportation of neutralizing antibodies to facilitate viral clearance.

The enhanced whole blood IFN- γ response to SARS-CoV-2 peptides during exercise appears largely due to increased numbers of SARS-CoV-2 T-cells in blood, as highly sensitive IFN- γ ELISPOT assays showed that exercise increased total T-cell numbers (adjusted per volume of whole blood) recognizing all four of the SARS-CoV-2 antigen peptide pools used in this study. However, when we expanded SARS-CoV-2 VSTs, *ex vivo*, the relative expansion of VSTs after 10-days of culture was markedly elevated after exercise (particularly for T-cells recognizing S, M protein and the α -variant). The relative response in SARS-CoV-2 VST expansions were greatest for those recognizing the M protein, indicating that exercise may enhance T-cell immunity against SARS-CoV-2 antigens that are not targets of current COVID-19 vaccines. Collectively, these observations indicate that exercise mobilized SARS-CoV-2 specific T-cells are more responsive (i.e. by releasing IFN- γ and proliferating) to recall antigen exposure than equivalent cells present in resting blood. The majority of T-cells responding to SARS-CoV-2 antigens at rest were of the CD4⁺ subset (56-65%), but exercise was found to increase the proportion of CD8⁺ T-cells and ‘double negative’ T-cells recognizing all SARS-CoV-2 antigens in both PBMCs and expanded VSTs. Further, the exercise-mobilized cells maintained broad antigen specificity as confirmed by

deep TCR sequencing, although there was a notable skewing in T-cell clonality toward M glycoprotein, N phosphoprotein, and ORF6 in both PBMCs and expanded VSTs. As with the majority of T-cell subpopulations, SARS-CoV-2 specific T-cells, specifically ORF7, surface glycoprotein, and N phosphoprotein, egressed the blood compartment upon exercise cessation, falling below the pre-exercise values at 1h post-exercise (Walsh et al., 2011). It is purported that T-cells leaving the blood compartment during exercise recovery migrate to tissues that require enhanced immune surveillance following a stress response (e.g. the lungs and the intestines) (Kruger et al., 2008).

Vaccination increased total SARS-CoV-2 specific T-cells by 1.48-fold. Expectedly, vaccination induced a greater T-cell response to S and not M or N proteins as S protein is the sole target of all current mass-produced COVID-19 vaccines (Dhama et al., 2020). Vaccination did, however, increase T-cell responses to the α -variant indicating that current COVID-19 vaccines may be effective in enhancing cellular immunity against evolving SARS-CoV-2 escape variants in those previously exposed. The SARS-CoV-2 T-cell response to exercise was also increased after vaccination by 1.25-fold. For instance, T-cell IFN- γ response to S protein increased by 1.68 and 1.61-fold in response to exercise after infection and vaccination, respectively. Simultaneously, since S protein is a major inducer of neutralizing antibodies, it is not surprising vaccination increased neutralizing antibodies by 1.92-fold. Despite overall increases in neutralizing antibodies after vaccination, the exercise effect was smaller with just a 1.18-fold increase in neutralizing antibodies after exercise post vaccine compared to a 2.23-fold increase after natural infection. This could be due to a ceiling effect in neutralizing antibody titers following vaccination.

Fatigue, lethargy and low exercise tolerance are symptoms of long COVID syndrome (Jimeno-Almazán et al., 2021). The individual in this case study was highly active and presented with a

‘superior’ age-adjusted maximum aerobic capacity (52.4 mL/kg/min) as defined by the American College of Sports Medicine (American College of Sports Medicine et al., 2018). At 46-days post infection, the predicted $\dot{V}O_{2peak}$ from the graded exercise test had dropped by ~9%. This was accompanied by notable elevations in exercising HR, $\dot{V}E/\dot{V}O_2$, RER and blood lactate with a concomitant reduction in SV, all of which indicate that the metabolic demand of the exercise bout was markedly elevated after natural infection. Interestingly, vaccination was associated with a complete recovery in the metabolic response to exercise, as all respiratory gas exchange, blood lactate and catecholamine responses had returned to pre-infection values (or slightly improved) at 21-days post vaccination (114-days post infection). Whether this restoration in exercise performance was facilitated by the vaccine, the exercise habits of the individual, or to more recovery time following infection warrants further investigation. The participant did, however, report that he was still experiencing afternoon tiredness even after receiving the vaccine. It is possible that the increased fatigue/tiredness is associated with the elevated baseline cortisol levels that were observed after both infection and vaccination (Kumari et al., 2009). Cortisol did not increase during exercise, which is typical for individuals of high fitness performing relatively short bouts (<20-minutes) of exercise (Duclos et al., 1997). There did, however, appear to be a ‘cortisol clearance’ effect of exercise when baseline levels were high following SARS CoV-2 infection and COVID-19 vaccination.

In conclusion, the findings from this case study indicate that a single exercise bout mobilizes large numbers of SARS-CoV-2 specific T-cells with broad TCR diversity against COVID antigens and an enhanced ability to respond to recall antigens. Notwithstanding the obvious limitations of a case study (e.g. limited sample size, lack of statistical sampling, low external validity, limited control conditions), the findings do support the narrative that every exercise bout promotes neutralizing

antibody transportation and mobilizes and redistributes highly functional virus-specific T-cells with the potential to increase immune surveillance and facilitate viral clearance. Of particular importance is the potential that an exercise boost in anti-SARS-CoV-2 immunity could reduce the viral reservoir and improve symptomology in those with long COVID syndrome. Indeed, vaccination in people previously infected with SARS-CoV-2 has been associated with improved symptoms, possibly due to the boost in neutralizing antibodies and SARS-CoV-2 specific T-cells contributing to the clearance of residual virus (Reynolds et al., 2021). Future experimental studies with sufficient sample sizes are warranted to determine the role of exercise, and the underpinning immunological mechanisms involved, in mitigating viral propagation, reducing symptoms of viral disease and restoring functional exercise capacity following SARS-CoV-2 and other viral infections.

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