

Original article

COVID-19 vaccination produces exercise-responsive SARS-CoV-2 specific T-cells regardless of infection history

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Abstract

Background: The mobilization and redistribution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific T-cells and neutralizing antibodies (nAbs) during exercise is purported to increase immune surveillance and protect against severe coronavirus disease 2019 (COVID-19). We sought to determine if COVID-19 vaccination would elicit exercise-responsive SARS-CoV-2 T-cells and transiently alter nAb titers.

Methods: Eighteen healthy participants completed a 20-min bout of graded cycling exercise before and/or after receiving a COVID-19 vaccine. All major leukocyte subtypes were enumerated before, during, and after exercise by flow cytometry, and immune responses to SARS-CoV-2 were determined using whole blood peptide stimulation assays, T-cell receptor (TCR)- β sequencing, and SARS-CoV-2 nAb serology.

Results: COVID-19 vaccination had no effect on the mobilization or egress of major leukocyte subsets in response to intensity-controlled graded exercise. However, non-infected participants had a significantly reduced mobilization of CD4⁺ and CD8⁺ naive T-cells, as well as CD4⁺ central memory T-cells, after vaccination (synthetic immunity group); this was not seen after vaccination in those with prior SARS-CoV-2 infection (hybrid immunity group). Acute exercise after vaccination robustly mobilized SARS-CoV-2 specific T-cells to blood in an intensity-dependent manner. Both groups mobilized T-cells that reacted to spike protein; however, only the hybrid immunity group mobilized T-cells that reacted to membrane and nucleocapsid antigens. nAbs increased significantly during exercise only in the hybrid immunity group.

Conclusion: These data indicate that acute exercise mobilizes SARS-CoV-2 specific T-cells that recognize spike protein and increases the redistribution of nAbs in individuals with hybrid immunity.

Keywords: Anti-viral; COVID-19; Exercise immunology; SARS-CoV-2; T-Cells; Vaccine

1. Introduction

Enhanced immunity against viral infection has been attributed to healthy lifestyle factors such as engagement in

regular physical activity.^{1,2} Participation in moderate-to-vigorous intensity exercise has been shown to elicit several responses that improve anti-viral immune surveillance, including exchange of natural killer (NK) cells and virus-specific T-cells (VSTs) between the tissues and peripheral circulation as well as the augmented redistribution of immunoglobulins.^{1,3–5} Indeed, the protective benefits of exercise against viral disease have been demonstrated in animal models^{6–8} and have become evident in

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humans during the coronavirus disease 2019 (COVID-19) pandemic, as individuals who regularly exercise have shown improved clinical outcomes and reduced symptoms following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.^{9–14}

Single exercise bouts elicit transient immune responses that may protect against infection. Among these is the extensive, rapid mobilization and redistribution of effector lymphocytes (e.g., NK cells, CD8⁺ T-cells, and $\gamma\delta$ T-cells) with high lytic function and migratory capacity.^{1,4} More specifically, exercise preferentially mobilizes VSTs with enhanced proliferative properties that may increase lymphocyte trafficking and inhibit the reactivation of latent viruses (e.g., cytomegalovirus and Epstein–Barr virus).^{15–17} These preferentially mobilized VSTs exhibit increased responsiveness to viral peptide stimulation *ex vivo* and, therefore, may exert potent anti-viral responses upon reintroduction to viral antigens.^{16,17} Consequently, the summation of acute exercise bouts (i.e., regular exercise) may routinely increase immunosurveillance against viral pathogens, providing beneficial anti-viral health effects over the long term.^{1,5} Existing evidence indicates that regular exercise may not only reduce the risk of acquiring respiratory infections but also decreases the severity of symptoms.^{18,19}

While exercise has been shown to promote anti-viral immunity, the mechanisms by which exercise can ameliorate symptoms and enhance immunity against SARS-CoV-2 are still unknown. Our group was of the first to report on the immune responses to acute exercise following natural SARS-CoV-2 infection and reported that exercise mobilizes functional SARS-CoV-2 specific T-cells and increases transportation of neutralizing antibodies (nAbs).^{20,21} However, it is unknown whether vaccination in the absence of infection elicits similar responses.

In the present study, we compared the exercise-induced immune response to SARS-CoV-2 in fully vaccinated individuals that were either naturally infected (hybrid immunity) or uninfected (synthetic immunity) prior to vaccination. We demonstrate that: (a) recent vaccination in uninfected individuals elicits shifts in T-cell maturation and blunts the mobilization of naive CD4⁺ and CD8⁺ T-cells with exercise; (b) exercise mobilizes T-cells that react to spike (S) protein after vaccination regardless of infection history; and (c) nAb titers in blood are elevated during exercise after vaccination in individuals with hybrid immunity.

2. Methods

2.1. Participants

We recruited 18 participants (8 males, 10 females) to participate in this investigation. Participants were young, healthy individuals (21–44 years) free of any cardiovascular, pulmonary, or autoimmune diseases. Eleven virus-naïve participants received the Pfizer 2-dose COVID-19 vaccine regimen during this study period and were considered to have “synthetic” immunity. An additional 7 participants who were previously infected and subsequently recovered also received a COVID-19 vaccine (Johnson and Johnson, $n=5$; Pfizer, $n=2$) and were considered to have

“hybrid” immunity. All post-vaccination trials took place 3–4 weeks after final inoculation. All participants completed a brief medical history questionnaire as well as the American Heart Association/American College of Sports Medicine Pre-participation and Screening Questionnaire²² to ensure they were identified as “low risk” for cardiovascular disease and able to perform vigorous-intensity exercise. In addition, participants were eligible if they reported an active-physical activity rating >4 .²³ Research participants were non-users of tobacco products, consumed <10 standard alcoholic beverages per week, and were not taking prescription medications that could affect cardiovascular, metabolic, or immune responses to exercise. After screening, all participants provided written and oral informed consent, and all experimental procedures were conducted in accordance with the ethical guidelines provided by the Belmont Report. The University of Arizona Institutional Review Board granted ethical approval, and the trial was registered at www.clinicaltrials.gov (NCT05019456).

2.2. Experimental design

The exercise protocol and metabolic responses to exercise used in these research participants have been previously described.²⁴ In brief, participants first completed a submaximal graded exercise test on a cycle ergometer (Velotron, Quarq Technology, San Diego, CA, USA) to determine predicted maximal oxygen consumption ($\dot{V}O_{2max}$) (Quark CPET, COSMED, Pabona di Albona Laziale, Italy). Upon successful completion of submaximal testing, individualized power outputs for the experimental trials were determined via simple linear regression between $\dot{V}O_2$ and power (W). Participants then returned to the laboratory twice more for experimental trials, once before and once after COVID-19 vaccination. Trials consisted of 4 incremental 5-min stages with power outputs corresponding to 50%, 60%, 70%, and 80% of the individual predicted $\dot{V}O_{2max}$. Blood was collected at rest, during the 60% $\dot{V}O_{2max}$ stage, during the 80% $\dot{V}O_{2max}$ stage, and 1 h after exercise cessation. To maintain consistency, the absolute cycling power outputs for each individual were identical during the experimental trials. Heart rate and rating of perceived exertion (Modified Borg Scale) were recorded during the final 15 s of each stage. A schematic of the experimental trial design is displayed in Fig. 1 (Created with BioRender.com).

2.3. Blood processing and immunophenotyping

Heparinized blood was utilized for a 24 h whole blood peptide stimulation assay and for isolation of peripheral blood mononuclear cells using standard density gradient centrifugation protocols. Ethylenediaminetetra-acetic acid whole blood samples were stained and analyzed via flow cytometry (MACSQuant 10, Miltenyi Biotec, Bergisch Gladbach, Germany) to determine lymphocyte counts and immune cell subsets using the following conjugated antibodies: CD8-VioBlue, CD14-VioGreen, CD3-VioGreen, CD3-FITC, CD4-FITC, CD4-PE, CD62L-PE, CD20-PerCP, PD-1-PerCP, CD45RA-PerCPVio770, CD45-APC, and CD56-APCVio770 (Miltenyi Biotec, Bergisch Gladbach, Germany). The percentage of all CD45⁺ lymphocytes expressing the surface marker of interest were multiplied by the total lymphocyte

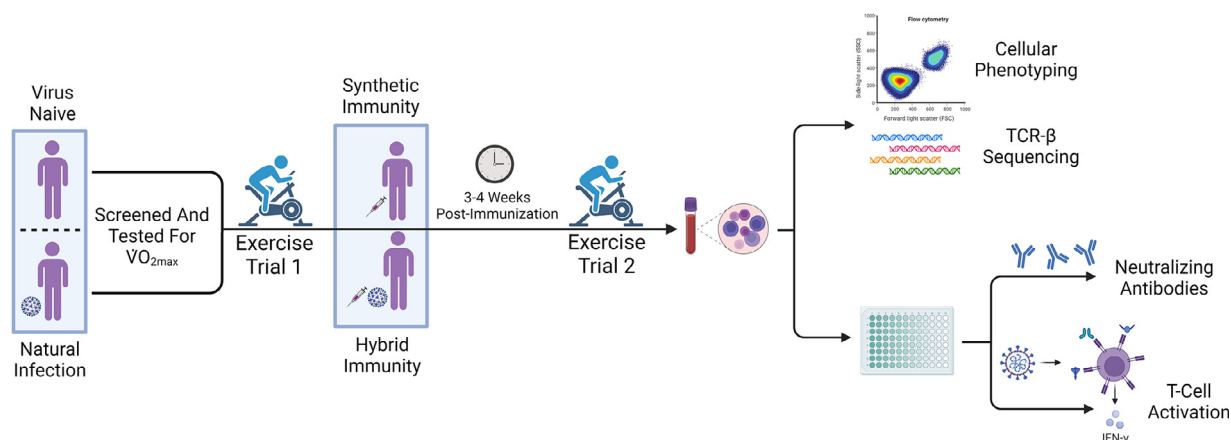


Fig. 1. Schematic of experimental design. Virus-naïve ($n = 9$) and naturally infected ($n = 5$) participants were screened and performed submaximal graded exercise testing prior to their first main exercise trial (pre-vaccination). Participants performed a second exercise trial 3–4 weeks post-vaccination. Those who were virus-naïve and subsequently vaccinated were included in the synthetic immunity group, with the addition of 2 participants who only performed the exercise trial post-vaccination ($n = 11$). Those who were naturally infected and subsequently vaccinated were included in the hybrid immunity group, with the addition of 2 participants who only performed the exercise trials post-vaccination ($n = 7$). Blood samples were taken at rest, during the exercise trial, and 1 h post-exercise for further analysis utilizing flow cytometry, TCR-sequencing, whole-blood peptide stimulation, and ELISA. Diagram was created with BioRender.com. ELISA = enzyme-linked immunosorbent assay; TCR = T-cell receptor; $\dot{V}O_{2max}$ = maximal oxygen consumption.

count to enumerate each lymphocyte subset per unit of whole blood. Serum was isolated from each timepoint and frozen for future analysis to determine SARS-CoV-2 nAb titers using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical, Ann Arbor, MI, USA).

2.4. Whole blood peptide stimulation

Whole blood (1 mL) samples were utilized for whole blood peptide stimulation as previously described.²⁰ In brief, whole blood was stimulated with the following: saline (negative control), phytohemagglutinin (10 $\mu\text{g/mL}$; positive control; Sigma Aldrich, St. Louis, MO, USA), actin pepmix (1 $\mu\text{g/mL}$; peptide control; JPT Peptide Technologies, Berlin, Germany), or SARS-CoV-2 S, membrane (M), and nucleocapsid (N) pepmixes (10 $\mu\text{g/mL}$; Miltenyi). After 24 h of incubation at 37°C and 5% CO_2 , the samples were centrifuged and plasma was stored at -80°C for future analysis. After thawing, interferon gamma (IFN- γ) concentrations within the plasma were determined via a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA).

2.5. T-cell receptor- β sequencing

Genomic DNA was extracted from peripheral blood mononuclear cells collected at rest, 80% $\dot{V}O_{2max}$, and 1 h post-exercise in post-natural infection and post-vaccination trials (DNeasy Extraction Kit, Qiagen, Germantown, MD, USA). Genomic DNA was sent to Adaptive Biotechnologies (Seattle, WA, USA), where subsequent T-cell receptor β (TCR- β) sequencing was performed and analysis was carried out utilizing the ImmunoSEQ (Adaptive Biotechnologies) data platform. TCR- β rearrangements specific to SARS-CoV-2 were determined using the ImmunoSEQ T-MAP COVID ImmuneCODE database. The public database VDJdb (<https://vdjdb.cdr3.net>) was utilized to determine the major histocompatibility complex (MHC) class of each identified SARS-CoV-2

productive rearrangement. This included only those SARS-CoV-2 specific T-cell clones with a confidence threshold score of 3 (very high confidence) or 2 (high confidence).

2.6. Statistical analysis

Differences in participant characteristics were assessed using unpaired t tests. For general immune cell phenotype changes across time, 2-way repeated measures analysis of variance were used with Bonferonni multiple comparisons. To determine immune cell phenotype changes across time, a mixed model was used with Bonferonni multiple comparisons. Lastly, 2-way repeated measures analysis of variance with Bonferonni multiple comparisons was used to assess differences in nAb titers and cytokine secretion across time. All statistical analyses were performed using GraphPad Prism (Version 9.4; GraphPad Software, San Diego, CA, USA). Significance was accepted at $p < 0.05$, and all data are presented as means \pm standard error of mean.

3. Results

3.1. Participant characteristics

The demographics of the 18 participants in this investigation can be viewed in Table 1. Nine individuals who were SARS-CoV-2-naïve (confirmed negative via SARS-CoV-2 spike IgG titers) were assessed before and after mRNA-based vaccination (Pfizer, New York, NY, USA), with the addition of 2 individuals who were only assessed post-vaccination (Pfizer). Five individuals who were previously infected with SARS-CoV-2 were assessed before and after mRNA-based vaccination (Pfizer), with the addition of 2 individuals who were only assessed post-vaccination (Johnson and Johnson, New Brunswick, NJ, USA). There were no significant differences in demographics between groups; SARS-CoV-2 spike IgG was significantly elevated from baseline after vaccination in the entire cohort ($p < 0.0001$).

Table 1
Participant demographics and serostatus (mean \pm SE).

	Pre-vaccination ^a	Post-vaccination ^b	<i>p</i>
Female	6/14	10/18	>0.999
Age (year)	32.0 \pm 5.9	31.2 \pm 6.3	0.723
Height (cm)	171.5 \pm 10.2	172.8 \pm 10.1	0.730
Weight (kg)	68.9 \pm 12.7	69.5 \pm 12.3	0.896
VO _{2max} (mL/kg/min)	41.5 \pm 9.4	40.2 \pm 9.4	0.701
PAR score (1–7)	5.6 \pm 1.4	5.4 \pm 1.5	0.633
CMV serostatus	6/14	8/18	0.293
SARS-CoV-2 serostatus	5/14	18/18	0.469
SARS-CoV-2 spike IgG (μ g/mL)	2.4 \pm 3.1	14.9 \pm 0.0	<0.0001

^a Includes 5 participants who have prior natural infection.

^b Includes 4 participants who did not test prior to vaccination.

Abbreviations: CMV = cytomegalovirus; PAR = physical activity rating; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; VO_{2max} = maximal oxygen consumption.

3.2. Naive CD4+ and CD8+ T-cell mobilization during exercise is blunted after vaccination in virus-naïve but not previously infected individuals

All participants successfully completed the exercise trials as prescribed. The effect of acute cycling exercise on the number of circulating lymphocyte subsets and monocytes is displayed in Fig. 2. Considering all participants regardless of infection status, there were no differences in the circulating number of total lymphocytes, monocytes, T-cells, B-cells, or NK cells at any timepoint after vaccination. All immune cell subsets assessed displayed a strong mobilization across time, with cell numbers returning to baseline 1 h post-exercise ($p < 0.0001$). Overall, vaccination status did not significantly affect general immune cell subset numbers at rest, during exercise, or during recovery.

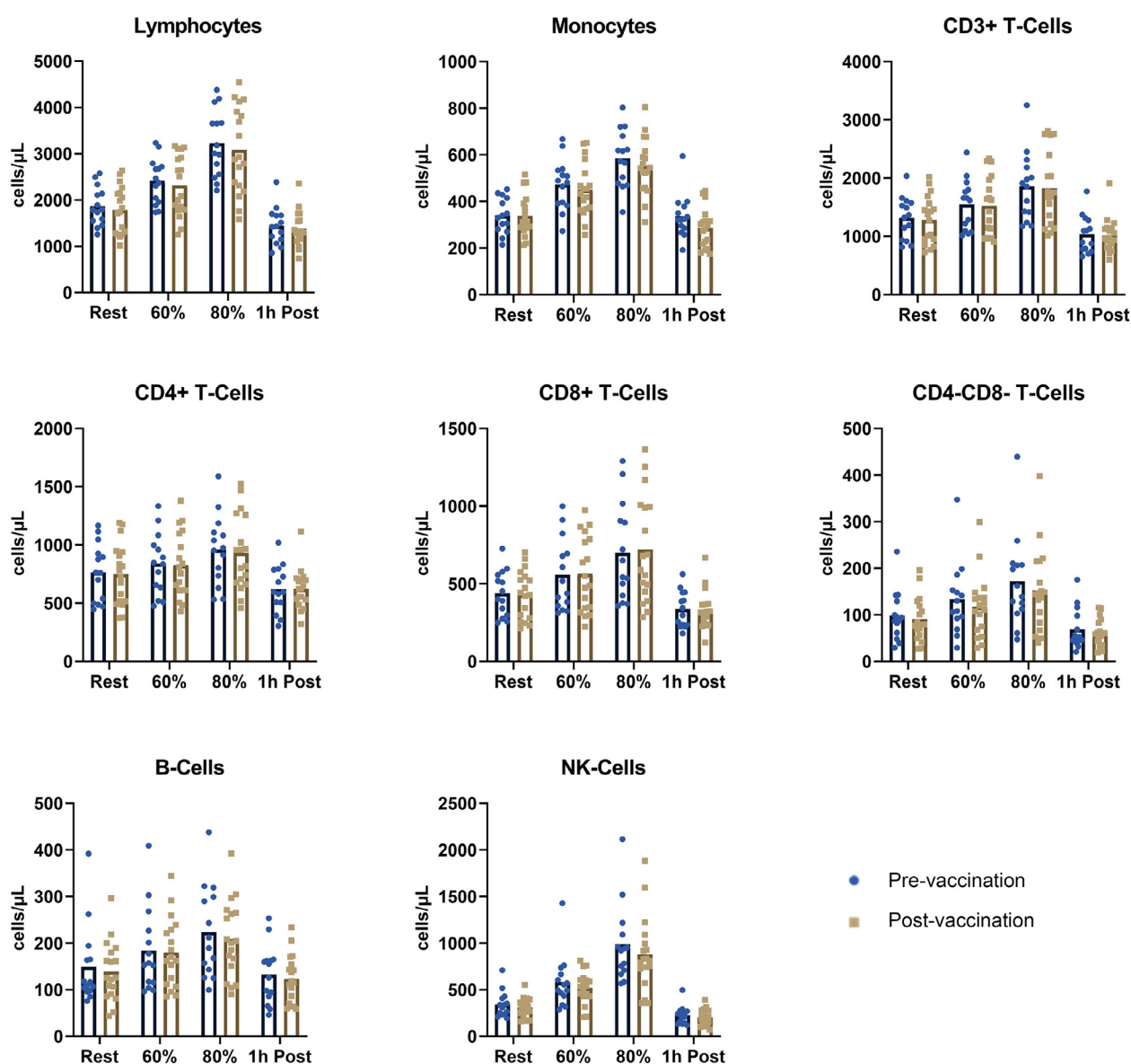


Fig. 2. Recent COVID-19 vaccination does not affect major immune subsets during exercise-induced leukocytosis. Major immune cell subsets are displayed across time for all study participants regardless of natural infection. A significant effect of time was found for all subsets analyzed ($p < 0.0001$) with no differences at any specific timepoints between pre-vaccination ($n = 14$) and post-vaccination ($n = 18$). COVID-19 = coronavirus disease 2019; NK = natural killer.

Although vaccination did not significantly affect the mobilization and egress of immune cells in the collective cohort, significant differences in T-cell differentiation were observed when the group was split based on a history of past infection. A strong mobilization effect ($p < 0.0001$) was present for major T-cells subsets, but there were no differences between groups pre- and post-vaccination (Fig. 3A). In individuals with no history of SARS-CoV-2 infection who were subsequently vaccinated (synthetic immunity), vaccination elicited significant decreases in the exercise-induced mobilization of naive CD4+ and CD8+ T-cells at 60% and 80% $\dot{V}O_{2\max}$ ($p = 0.03$ and $p = 0.009$ for CD4+ T-cells; $p = 0.002$ and $p = 0.0009$ for CD8+ T-cells, respectively) (Fig. 3B). There were also significant reductions in central memory (CM) CD4+ T-cells at 60% and 80% $\dot{V}O_{2\max}$ post-vaccination ($p = 0.004$ and $p = 0.0002$, respectively). While there was an increased mobilization of effector

memory RA (EMRA) CD4+ T-cells across time in the synthetic immunity group after vaccination, it was not significant ($p = 0.11$). The reductions in circulating naive and CM T-cells were not observed in those with a previous history of infection who were subsequently vaccinated (hybrid immunity). Thus, vaccination reduced the mobilization of less-differentiated T-cell subsets in individuals who were virus-naïve while causing no changes in those who already possessed immunological memory to SARS-CoV-2.

3.3. Acute exercise increases circulating nAb concentrations and promotes the mobilization of T-cells responsive to SARS-CoV-2 spike protein

In addition to immunophenotyping, serum and whole blood collected from each timepoint were used for the quantification

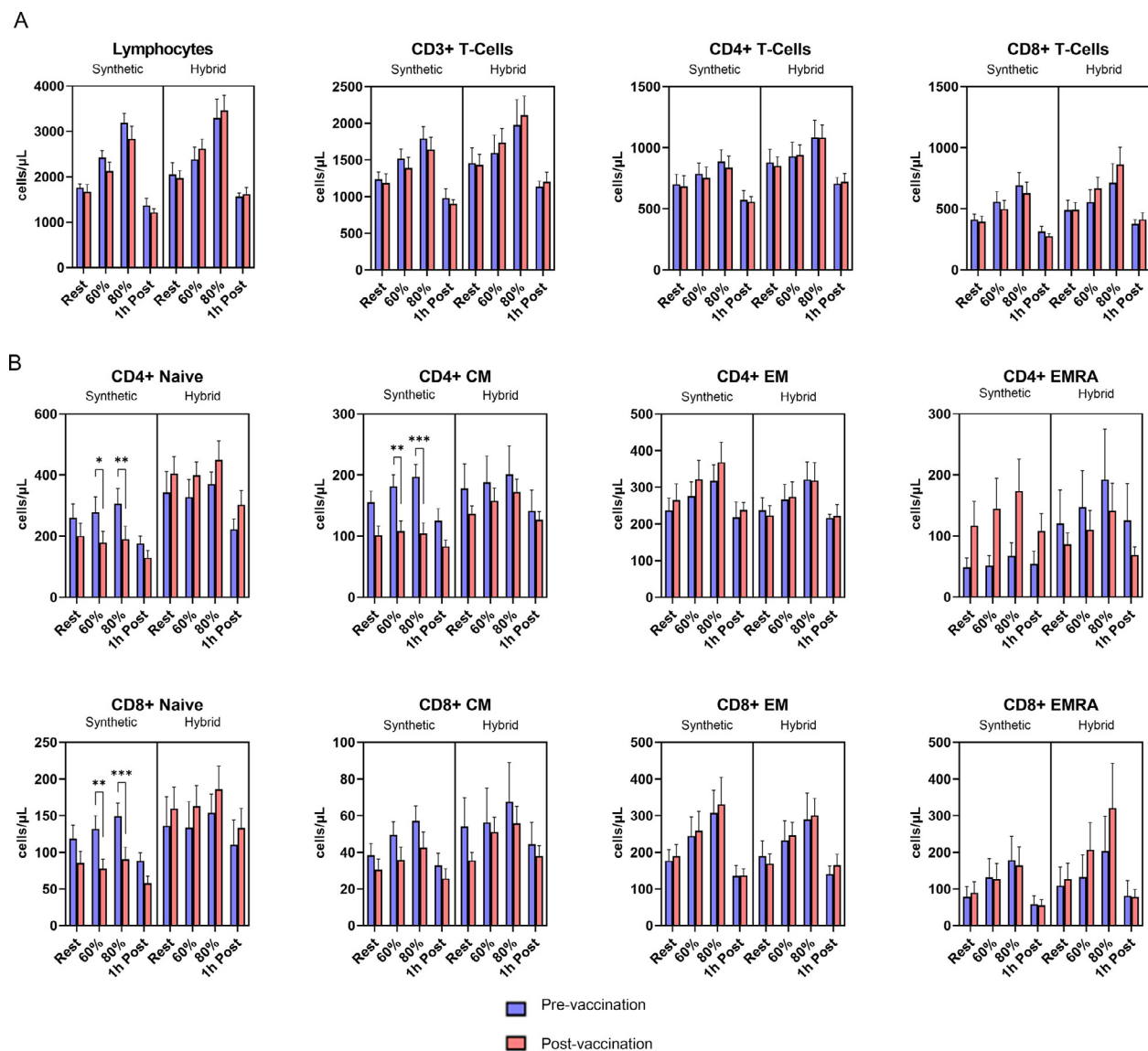


Fig. 3. Recent COVID-19 vaccination alters the T-cell compartment in those acquiring synthetic immunity. Individuals were split into synthetic ($n = 11$) and hybrid ($n = 7$) immunity groups depending on their history of previous infection to assess differences in vaccination responses. (A) Total lymphocytes and major T-cell subsets are displayed across time pre- and post-vaccination; (B) CD4+ and CD8+ T-cell differentiation status are displayed across time pre- and post-vaccination. A significant effect of time was found for all subsets analyzed ($p < 0.05$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. COVID-19 = coronavirus disease 2019; CM = central memory; EM = effector memory; EMRA = effector memory RA.

of SARS-CoV-2 nAb titers and to assess T-cell responsiveness to viral peptides *ex vivo*, respectively. For insight into B-cell responses to vaccination, nAb titers were determined at all experimental timepoints. The hybrid immunity group demonstrated significantly higher nAb titers at 80% $\dot{V}O_{2\max}$ relative to rest ($p = 0.02$). Although mean values were elevated at 80% $\dot{V}O_{2\max}$ for individuals with synthetic immunity, they were not significantly elevated from resting values (Fig. 4A).

In response to a pepmix containing S, M, and N peptides, both groups exhibited enhanced IFN- γ secretion during exercise, with values returning to normal 1 h post-exercise. For synthetic immunity responses, the total output of IFN- γ significantly increased from rest to both 60% and 80% $\dot{V}O_{2\max}$ ($p = 0.04$ and $p < 0.0001$, respectively) (Fig. 4B). Hybrid immunity responses presented enhanced IFN- γ secretion at both 60% and 80% $\dot{V}O_{2\max}$ relative to resting values ($p = 0.002$ and $p = 0.001$, respectively) (Fig. 4B). When T-cell responsiveness to peptide stimulation was assessed for individual SARS-CoV-2 components, hybrid immunity led to robust IFN- γ secretion in response to S, M, and N peptides and was significantly higher at 80% $\dot{V}O_{2\max}$ when compared to resting values for all peptides ($p = 0.002$, $p = 0.0003$, $p = 0.002$, respectively) (Fig. 4C). Synthetic immunity displayed a predictably robust IFN- γ response to S peptides from rest to 80% $\dot{V}O_{2\max}$ ($p = 0.01$), with little to no response to M and N peptides (Fig. 4C).

Overall, these results indicate that acute exercise mobilizes SARS-CoV-2 specific T-cells in vaccinated individuals that are highly responsive to antigen stimulation, and that responses to peptides not included in current mRNA vaccines (M and N) are only elicited in those with hybrid immunity. In addition, acute exercise transiently increases the concentration of nAbs in individuals with hybrid immunity, but this response was not observed in those with synthetic immunity.

3.4. TCR- β sequencing of exercise-responsive SARS-CoV-2 specific T-cells reveals preferential mobilization of MHC-I-restricted clones

Next-generation TCR- β deep sequencing (Adaptive Biotechnologies; ImmunoSEQ T-MAP COVID Immune-CODE database) was utilized to determine the specificity of SARS-CoV-2 specific T-cell clones mobilized during exercise. Two individuals from the vaccine-only cohort (synthetic immunity) and 2 individuals with natural immunity from our previous study (prior to vaccination)²¹ were used for comparison in this descriptive analysis. Complementarity-determining region 3 length motif analysis allowed for the determination of antigen specificity for TCR sequences obtained. Determination of MHC class I or II, utilizing the VDJdb, demonstrated that a percentage increase in MHC-I-restricted T-cells occurred from rest to 80% $\dot{V}O_{2\max}$, with a percentage decrease in

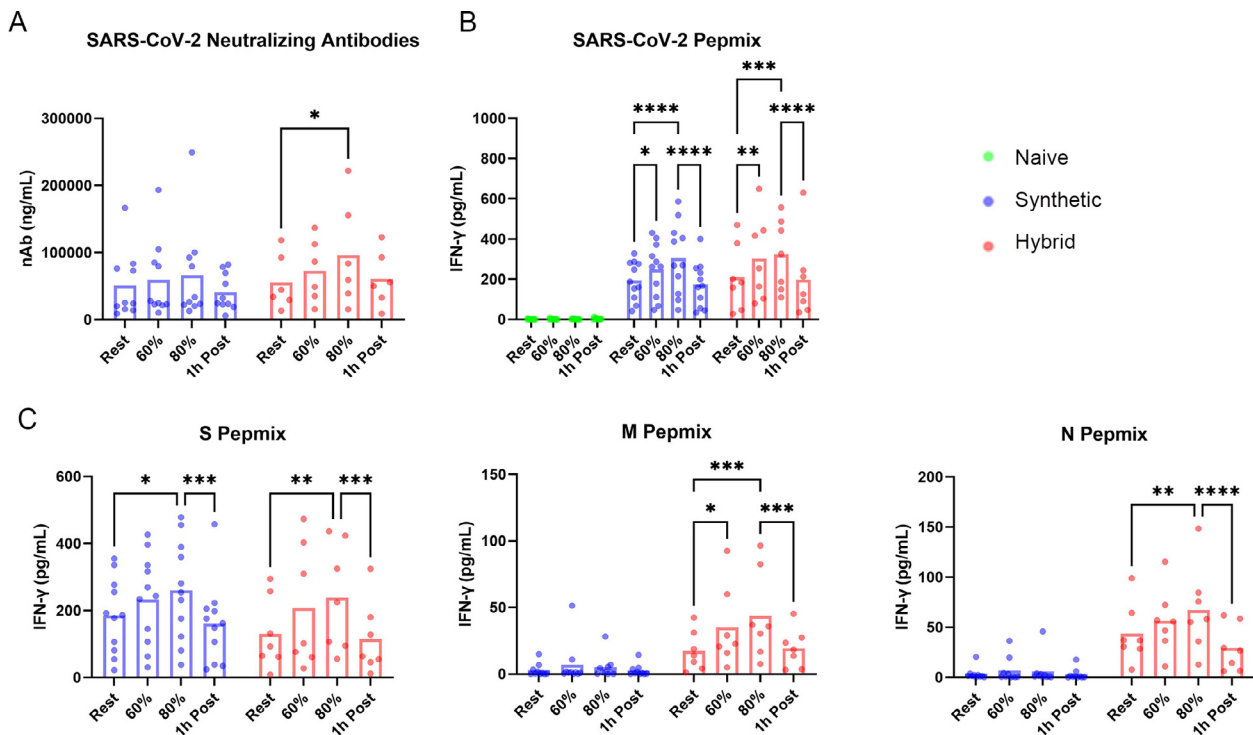


Fig. 4. Recent COVID-19 vaccination elicits transient increases in neutralizing antibody titers and an intensity-dependent increase in T-cell function. Humoral and cellular immune measures are displayed across time as neutralizing antibody titers and IFN- γ responses in whole blood. (A) nAb concentrations across exercise intensities and through recovery in individuals with synthetic and hybrid immunity; (B) Whole blood IFN- γ responses to a SARS-CoV-2 pepmix containing all major structural proteins (S, M, N); (C) Whole blood IFN- γ responses to each individual component of the SARS-CoV-2 pepmix, demonstrating the specificity of vaccination. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. COVID-19 = coronavirus disease 2019; IFN- γ = interferon gamma; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

MHC-II–restricted T-cells seen in both individuals within the synthetic group (Fig. 5A); however, the total number of MHC-I– and MHC-II–restricted clones increased in response to exercise. This is demonstrative of the larger mobilization effect observed in CD8⁺ T-cells during exercise relative to CD4⁺ T-cells (Fig. 2). Accompanying the mobilization of T-cells during exercise, there was an increase in the number of hits for clones specific to SARS-CoV-2 epitopes from rest to 80% $\dot{V}O_{2\max}$ in 2 individuals with synthetic immunity (Fig. 5B). Furthermore, specific clones that were heavily mobilized during exercise tended to egress the blood compartment by 1 h post-exercise (Fig. 5C). Finally, comparison of epitope specificity of SARS-CoV-2 specific T-cell clones is displayed between individuals with synthetic immunity and those with natural immunity (Fig. 5D). Overall, acute exercise did not alter the proportion of SARS-CoV-2 clones being mobilized to circulation, therefore indicating the diversity is maintained even within vaccinated individuals, which was previously observed in a naturally infected cohort.²¹

4. Discussion

Increased levels of physical activity have been associated with a lower risk of hospitalization, disease severity, and mortality in individuals with COVID-19,^{11,13,25} although the mechanisms associated with the protective anti-viral effects of exercise in the context of COVID-19 are still elusive. Building upon our previous findings in individuals with natural immunity,²⁰ we sought to determine whether acute exercise enhances cellular and humoral immune responses against SARS-CoV-2 after COVID-19 vaccination. We report here

that acute exercise promotes the mobilization and egress of functional SARS-CoV-2 VSTs after vaccination regardless of infection history and transiently increases nAb titers after vaccination in individuals who possess hybrid immunity.

Seropositivity for neutralizing IgG has been demonstrated to be 90% or higher 6–8 months after infection,^{26,27} while vaccination with 2-dose mRNA vaccines elicits strong nAb responses that may surpass concentrations seen after infection.²⁸ However, nAb concentrations and memory B-cell frequencies may wane more quickly in individuals with synthetic immunity.²⁸ While additional instances of antigen exposure elicit accretion in nAb titers,^{20,28,29} acute exercise has been shown to transiently enhance IgG titers to SARS-CoV-2.²⁰ We replicate these findings here, as there was an intensity-dependent increase in nAb titers during a 20-min cycling bout. In addition, this transient increase in circulating nAbs was greatest in those possessing hybrid immunity, which has been recently found to maintain significantly higher nAb titers against ancestral variants.^{28,30,31} It is possible, therefore, that each exercise bout will facilitate nAb transportation to increase the likelihood of antibody–virus contact.²⁰ While high circulating nAb titers provide significant protective immunity,²⁸ it should be noted that they are not present in many cases.^{28,32} The importance of humoral immunity cannot be understated, however epidemiological and animal data indicate that the T-cell-mediated branch of adaptive immunity is paramount for efficient clearance of infection.²⁸

Memory CD8⁺ T-cells elicited by mRNA vaccines recognize diverse S epitopes,^{33–35} typically possess effector memory phenotypes, express IFN- γ , and are capable of proliferation.^{36,37} There is less research on adaptive responses with hybrid immunity, but it

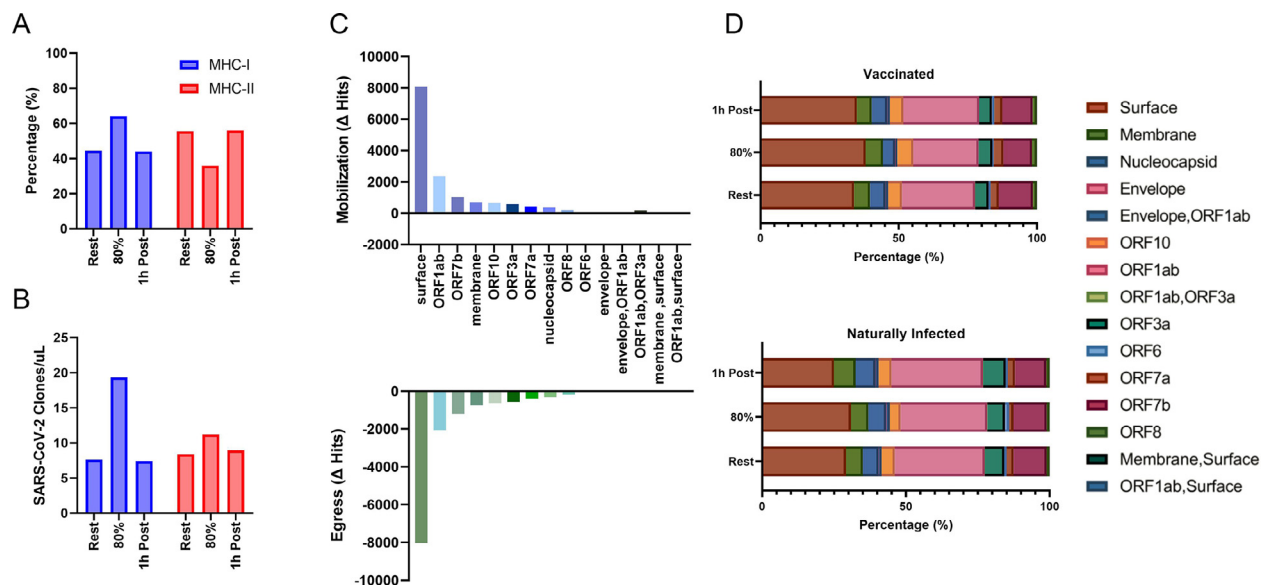


Fig. 5. TCR- β specificity in vaccinated and naturally infected individuals during exercise and recovery Deep TCR- β sequencing. Performed on T-cells present in the blood at rest, during exercise, and 1 h post-exercise in vaccinated ($n=2$) and naturally infected ($n=2$) individuals. (A) Percentage of MHC-I and MHC-II restricted T-cells across time in 2 individuals with synthetic immunity; (B) Number of SARS-CoV-2 specific clones across time in 2 individuals with synthetic immunity; (C) Changes in the number of hits for SARS-CoV-2 specific clones during mobilization (rest to exercise) and egress (exercise to 1 h post) alongside epitope specificity in 2 individuals with synthetic immunity; (D) Percentage of epitope dominance across time between vaccinated ($n=2$) and naturally infected ($n=2$) individuals. MHC = major histocompatibility complex; ORF = open reading frame; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TCR = T-cell receptor.

has been established that T-cell repertoire diversity is maintained,³⁸ and some studies have shown improved responses compared to infection or vaccination alone.³⁵ Regardless, each subsequent exposure or vaccination further matures the T-cell compartment towards effector phenotypes.^{28,35} Acute bouts of dynamic exercise demonstrate enhanced CD8⁺ T-cell responses against various viral pathogens (e.g., cytomegalovirus, Epstein–Barr virus, adenovirus).^{16,39,40} Although T-cell responses to SARS-CoV-2 are predominated by CD4⁺ VSTs,³⁵ we present here that exercise preferentially mobilizes SARS-CoV-2 specific CD8⁺ VSTs. Moreover, we observed decreases in the exercise-induced mobilization of naive and CM T-cells shortly after vaccination, suggesting the maturation and expansion of an antigen-specific SARS-CoV-2 T-cell repertoire. This effect was observed in those who were virus-naïve relative to those who were previously infected; we speculate that vaccination in virus-naïve individuals elicited the differentiation of naive T-cells, while vaccination expanded the memory compartment in those who were previously infected. Expectedly, individuals with synthetic immunity exhibited robust IFN- γ secretion in response to S pepmixes during exercise, while those with hybrid immunity had robust responses to S, M, and N pepmixes.

Overall, there have been more unique SARS-CoV-2 T-cell epitopes identified for CD8⁺ T-cells than for CD4⁺ T-cells.⁴¹ Within the main structural antigens (S, M, and N), CD4⁺ T-cells have more pronounced immunodominant regions while CD8⁺ T-cell responses are more evenly distributed.⁴¹ Indeed, details on TCR specificity and responses to specific epitopes are well-documented;⁴¹ however, we present here the exercise-mobilization response of SARS-CoV-2 clones between synthetic and natural immunity in a small number of individuals. Although clones specific to surface and ORF1ab displayed the greatest mobilization, there was consistency in epitope recognition across all 3 timepoints regardless of synthetic or natural immunity. Clones that were mobilized to the greatest extent displayed the greatest egress, suggesting these clones may possess higher migratory potential and are more readily redistributed to local sites of need. Moreover, there was a large increase in the percentage and total number of MHC-I (e.g., CD8⁺ T-cells) restricted clones during exercise regardless of how SARS-CoV-2 immunity was acquired. It is plausible, therefore, that the exercise-induced mobilization of diverse antigen-specific clones enhances the potential for viral resolution and general immunosurveillance.³⁵

The evolution and emergence of SARS-CoV-2 variants is a critical area of investigation and one that is not specifically addressed in this study. However, Tarke et al.³³ have demonstrated that vaccination elicits memory and reactivity against all currently existing variants. In addition, our previous work in infected individuals demonstrated that exercise mobilized VSTs collected during the ancestral strain outbreak respond to both alpha and delta variants.²¹ The impact of SARS-CoV-2 mutations on memory CD4⁺ and CD8⁺ T-cells is limited; however, predictive models demonstrate that, while newer variants (e.g., omicron) have fewer conserved epitopes in multiple proteins, the majority of both CD4⁺ and CD8⁺ T-cell epitopes are unaffected by mutations.⁴¹ It is possible that the

regular mobilization and exchange of SARS-CoV-2 VSTs to peripheral tissues with every bout of exercise may be beneficial in this regard; the broad recognition of thousands of epitopes suggests T-cells may be well-poised to recognize developing variants of concern as exercise enhances the immunosurveillance of T-cells.¹

We acknowledge that the small sample size within our hybrid immunity group may limit the scope of our findings. However, the recruitment of virus-naïve individuals is currently impractical with the global spread of SARS-CoV-2 and large-scale administration of vaccines. While our investigation did not specifically address evolving variants, our work still details the mobilization of diverse antigen-specific T-cells with effector phenotype and function. Subsequent studies are necessary to understand whether exercise training influences the durability of T-cell and humoral immune responses to SARS-CoV-2. The results demonstrated within the TCR- β sequencing analyses are only representative of 2 individuals who were naturally infected and 2 who obtained synthetic immunity. Moreover, we were not able to compare TCR-sequencing for individuals in the hybrid group, although our data corroborates previous findings,³⁵ further detailing the mobilization of MHC I (i.e., CD8⁺ T-cells) restricted SARS-CoV-2 clones. Future work should investigate, in more detail, the specific clones mobilized and egressed with exercise in those with synthetic, natural, and hybrid immunity. Finally, there is a need to determine whether repeated bouts of physical activity resolve symptoms of long COVID, which may be influenced by T-cell and nAb exercise responses reported here.

5. Conclusion

This is the first study to show that a single bout of exercise preferentially mobilizes MHC-I–restricted SARS-CoV-2 VSTs in vaccinated individuals and elevates circulating nAbs after COVID-19 vaccination in previously infected individuals. We have also demonstrated that exercise-mobilized VSTs are highly responsive to SARS-CoV-2 peptides *ex vivo*. We contend that the elevations in VST responsiveness and nAb titers could be compounded over time in those regularly engaging in physical activity and could putatively provide enhanced immunosurveillance and protection against SARS-CoV-2. Future studies should investigate the potential for chronic exercise to stimulate the persistence of SARS-CoV-2 VSTs and nAbs, further contributing to long-lasting immunity and protection from re-exposure or variant mutations.

Authors' contributions

KAS and TMZ collected and analyzed the data, performed the statistical analysis, and drafted the manuscript; FLB and HB collected the data; CRP, SCB, MPG, and EK contributed to the design of the study; RJS designed the study and supervised all aspects of manuscript preparation. All authors aided in revision of the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

References

1. Simpson RJ, Kunz H, Agha N, Graff R. Exercise and the regulation of immune functions. *Prog Mol Biol Transl Sci* 2015;**135**:355–80.
2. Nieman DC. Exercise, infection, and immunity. *Int J Sports Med* 1994;**15** (Suppl. 3):S131–41.
3. Campbell JP, Riddell NE, Burns VE, et al. Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. *Brain Behav Immun* 2009;**23**:767–75.
4. Bigley AB, Rezvani K, Pistillo M, et al. Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells. Part II: Impact of latent cytomegalovirus infection and catecholamine sensitivity. *Brain Behav Immun* 2015;**49**:59–65.
5. Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system. *J Sport Health Sci* 2019;**8**:201–17.
6. Davis JM, Kohut ML, Colbert LH, Jackson DA, Ghaffar A, Mayer EP. Exercise, alveolar macrophage function, and susceptibility to respiratory infection. *J Appl Physiol* (1985) 1997;**83**:1461–6.
7. Kohut ML, Boehm GW, Moynihan JA. Moderate exercise is associated with enhanced antigen-specific cytokine, but not IGM antibody production in aged mice. *Mech Ageing Dev* 2001;**122**:1135–50.
8. Lowder T, Padgett DA, Woods JA. Moderate exercise protects mice from death due to influenza virus. *Brain Behav Immun* 2005;**19**:377–80.
9. Simpson RJ, Katsanis E. The immunological case for staying active during the COVID-19 pandemic. *Brain Behav Immun* 2020;**87**:6–7.
10. da Silveira MP, da Silva Fagundes KK, Bizuti MR, et al. Physical exercise as a tool to help the immune system against COVID-19: An integrative review of the current literature. *Clin Exp Med* 2021;**21**:15–28.
11. de Souza FR, Motta-Santos D, dos Santos Soares D, et al. Association of physical activity levels and the prevalence of COVID-19-associated hospitalization. *J Sci Med Sport* 2021;**24**:913–8.
12. Udina C, Ars J, Morandi A, Vilaró J, Cáceres C, Inzitari M. Rehabilitation in adult post-COVID-19 patients in post-acute care with therapeutic exercise. *J Frailty Aging* 2021;**10**:297–300.
13. Sallis R, Young DR, Tartof SY, et al. Physical inactivity is associated with a higher risk for severe COVID-19 outcomes: A study in 48 440 adult patients. *Br J Sports Med* 2021;**55**:1099–105.
14. Steenkamp L, Saggars RT, Bandini R, et al. Small steps, strong shield: Directly measured, moderate physical activity in 65,361 adults is associated with significant protective effects from severe COVID-19 outcomes. *Br J Sports Med* 2022;**56**:568–76.
15. Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC, Kunz H. Exercise and the aging immune system. *Ageing Res Rev* 2012;**11**:404–20.
16. Spielmann G, Bollard CM, Kunz H, Hanley PJ, Simpson RJ. A single exercise bout enhances the manufacture of viral-specific T-cells from healthy donors: Implications for allogeneic adoptive transfer immunotherapy. *Sci Rep* 2016;**6**:25852. doi:10.1038/srep25852.
17. Kunz HE, Spielmann G, Agha NH, O'Connor DP, Bollard CM, Simpson RJ. A single exercise bout augments adenovirus-specific T-cell mobilization and function. *Physiol Behav* 2018;**194**:56–65.
18. Nieman DC, Nehlsen-Cannarella SL, Markoff PA, et al. The effects of moderate exercise training on natural killer cells and acute upper respiratory tract infections. *Int J Sports Med* 1990;**11**:467–73.
19. Chubak J, McTiernan A, Sorensen B, et al. Moderate-intensity exercise reduces the incidence of colds among postmenopausal women. *Am J Med* 2006;**119**:937–42.
20. Baker FL, Smith KA, Zúñiga TM, et al. Acute exercise increases immune responses to SARS CoV-2 in a previously infected man. *Brain Behav Immun Health* 2021;**18**:100343. doi:10.1016/j.bbih.2021.100343.
21. Baker FL, Zúñiga TM, Smith KA, et al. Exercise mobilizes diverse antigen specific T-cells and elevates neutralizing antibodies in humans with natural immunity to SARS CoV-2. *Brain Behav Immun Health* 2023;**28**:100600. doi:10.1016/j.bbih.2023.100600.
22. Balady GJ, Chaitman B, Driscoll D, et al. Recommendations for cardiovascular screening, staffing, and emergency policies at health/fitness facilities. *Circulation* 1998;**97**:2283–93.
23. Jackson AS, Blair SN, Mahar MT, Wier LT, Ross RM, Stuteville JE. Prediction of functional aerobic capacity without exercise testing. *Med Sci Sports Exerc* 1990;**22**:863–70.
24. Batatinha H, Baker FL, Smith KA, et al. Recent COVID-19 vaccination has minimal effects on the physiological responses to graded exercise in physically active healthy people. *J Appl Physiol* (1985) 2022;**132**:275–82.
25. Salgado-Aranda R, Pérez-Castellano N, Núñez-Gil I, et al. Influence of baseline physical activity as a modifying factor on COVID-19 mortality: A single-center, retrospective study. *Infect Dis Ther* 2021;**10**:801–14.
26. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 2021;**371**:eabf4063. doi:10.1126/science.abf4063.
27. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* 2021;**595**:426–31.
28. Sette A, Crotty S. Immunological memory to SARS-COV-2 infection and COVID-19 vaccines. *Immunol Rev* 2022;**310**:27–46.
29. Samanovic MI, Cornelius AR, Gray-Gaillard SL, et al. Robust immune responses are observed after one dose of BNT162b2 mRNA vaccine dose in SARS-CoV-2-experienced individuals. *Sci Transl Med* 2022;**14**:eabi8961. doi:10.1126/scitranslmed.abi8961.
30. Walls AC, Sprouse KR, Bowen JE, et al. SARS-CoV-2 breakthrough infections elicit potent, broad, and durable neutralizing antibody responses. *Cell* 2022;**185**:872–80. e3.
31. Goel RR, Painter MM, Apostolidis SA, et al. mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. *Science* 2021;**374**:abm0829. doi:10.1126/science.abm0829.
32. Sadarangani M, Marchant A, Kollmann TR. Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. *Nat Rev Immunol* 2021;**21**:475–84.
33. Tarke A, Sidney J, Methot N, et al. Impact of SARS-CoV-2 variants on the total CD4+ and CD8+ T cell reactivity in infected or vaccinated individuals. *Cell Rep Med* 2021;**2**:100355. doi:10.1016/j.xcrm.2021.100355.
34. Tarke A, Coelho CH, Zhang Z, et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. *Cell* 2022;**185**:847–59. e11.
35. Kedzierska K, Thomas PG. Count on us: T cells in SARS-CoV-2 infection and vaccination. *Cell Rep Med* 2022;**3**:100562. doi:10.1016/j.xcrm.2022.100562.
36. Oberhardt V, Luxemburger H, Kemming J, et al. Rapid and stable mobilization of CD8+ T cells by SARS-CoV-2 mRNA vaccine. *Nature* 2021;**597**:268–73.
37. Zhang Z, Mateus J, Coelho CH, et al. Humoral and cellular immune memory to four COVID-19 vaccines. *Cell* 2022;**185**:2434–51. e17.
38. Minervina AA, Pogorelyy MV, Kirk AM, et al. SARS-CoV-2 antigen exposure history shapes phenotypes and specificity of memory CD8+ T cells. *Nat Immunol* 2022;**23**:781–90.
39. Bigley AB, Spielmann G, Agha N, Simpson RJ. The effects of age and latent cytomegalovirus infection on NK-cell phenotype and exercise responsiveness in man. *Oxid Med Cell Longev* 2015;**2015**:979645. doi:10.1155/2015/979645.
40. Spielmann G, Bollard CM, Bigley AB, et al. The effects of age and latent cytomegalovirus infection on the redeployment of CD8+ T cell subsets in response to acute exercise in humans. *Brain Behav Immun* 2014;**39**:142–51.
41. Grifoni A, Sidney J, Vita R, et al. SARS-CoV-2 human T cell epitopes: Adaptive immune response against COVID-19. *Cell Host Microbe* 2022;**30**:1788. doi:10.1016/j.chom.2022.10.017.