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Full-length Article

Exercise after influenza or COVID-19 vaccination increases serum antibody without an increase in side effects

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ABSTRACT

Vaccination is an effective public health measure, yet vaccine efficacy varies across different populations. Adjuvants improve vaccine efficacy but often increase reactogenicity. An unconventional behavioral "adjuvant" is physical exercise at the time of vaccination. Here, in separate experiments, we examined the effect of 90-minute light- to moderate-intensity cycle ergometer or outdoor walk/jog aerobic exercise performed once after immunization on serum antibody response to three different vaccines (2009 pandemic influenza H1N1, seasonal influenza, and COVID-19). Exercise took place after influenza vaccination or after the first dose of Pfizer-BioNTech COVID-19 vaccine. A mouse model of influenza A immunization was used to examine the effect of exercise on antibody response and the role of IFNα as a potential mechanism by treating mice with anti-IFNα antibody. The results show that 90 min of exercise consistently increased serum antibody to each vaccine four weeks post-immunization, and IFNα may partially contribute to the exercise-related benefit. Exercise did not increase side effects after the COVID-19 vaccination. These findings suggest that adults who exercise regularly may increase antibody response to influenza or COVID-19 vaccine by performing a single session of light- to moderate-intensity exercise post-immunization.

1. Introduction

Physical exercise performed near the time of immunization may increase antibody response to vaccination. Several studies have reported such findings, demonstrating that exercise preceding immunization improved antibody response (Edwards et al. 2012; Edwards et al. 2006; Edwards, et al. 2007; Ranadive et al. 2014). One explanation given for these results is that exercise may act as an acute stressor. There are examples in the literature that demonstrate that acute stress may increase antibody response when applied before immunization (Edwards, et al. 2008; Edwards et al. 2006; Silberman et al. 2003). It has also been suggested that eccentric exercise produces a local inflammatory response, potentially resulting in greater antigen-presenting cell activation. In some studies, eccentric exercise before vaccination increased antibody response (Edwards, et al. 2007). As another possibility, it is recognized that an increase in serum IL-6 accompanies exercise (Reihmane and Dela, 2014; Vasconcelos and Salla, 2018), and the exercise-associated change in IL-6 may be another mechanism by which exercise may enhance antibody response (Edwards et al., 2006). Studies show the administration of IL-6 at the time of influenza immunization increases IgG, mediated indirectly by $CD4^+$ T cells (Dienz, et al. 2009). IL-6 has a role in T follicular helper (TFH) $CD4^+$ T cell differentiation (Choi, et al. 2013; Eto, et al. 2011), permitting germinal center TFH cells to receive continued T-cell receptor signaling (Papillion, et al. 2019). Therefore, evidence supports the possibility that increased IL-6 at the time of exercise could contribute to exercise-induced increases in serum antibody. However, a consistent association between IL and 6, exercise, and antibody response to vaccine has not been observed, and as a result, the mechanisms to explain the association between exercise and vaccine response remain speculative.

The current research on exercise and vaccination shows promising findings in several studies (Edwards, et al. 2006; Edwards, et al. 2007; Edwards, et al. 2008; Ranadive, et al. 2014), but significant challenges remain. For example, the results across studies are inconsistent, as some studies demonstrate no benefit of exercise (Bohn-Goldbaum, et al. 2020; Bohn-Goldbaum, et al. 2019; Bruunsgaard, et al. 1997; Campbell, et al. 2010; Long, et al. 2012). Others report an exercise-related response to one antigen in a vaccine but no effect on the response to other antigens

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contained in the same vaccine (Edwards, et al. 2006; Ranadive, et al. 2014). Ideally, an effective adjuvant would be expected to demonstrate a consistent enhancement across all antigens in a vaccine. The theory that eccentric exercise might elicit an inflammatory response to boost antibody has not held up consistently, given the findings showing no benefit of eccentric exercise or a difference only for one sex (Campbell, et al. 2010; Edwards, et al. 2007). Given the inconsistencies across studies, it has been suggested that an effect of exercise may be observed only under conditions in which antigen dose is low, or participants tend towards a reduced antibody response (Edwards, et al. 2012). This interpretation of existing findings implies that exercise effects are small in magnitude compared to the large immune stimulus from a vaccine, and therefore may be difficult to detect.

In order to advance the concept of exercise as a vaccine "behavioral adjuvant," with translational public health relevance, it is essential to define the exercise parameters that consistently result in enhanced serum antibody. It is also critical to identify the vaccine platforms and pathogens for which an effect of exercise may be present. In this study, we evaluated the effect of standardized aerobic exercise administered after immunization in contrast to other studies that focused on exercise prior to vaccine. In the stress and immunity literature, a stressor applied after immunization has been shown to enhance antibody response to vaccination (Karp, et al. 2000; Wood, et al. 1993). Therefore, we evaluated the effect of exercise on antibody response when exercise was administered after immunization rather than before.

Additional rationale for selecting 90 min of exercise was based partly on unpublished findings demonstrating that 90 min of exercise results in a significant increase in the type I interferon, interferon-alpha (IFNα) production by plasmacytoid dendritic cells upon activation (Supplement Table S1). Type I IFNs promote dendritic cell activation (Montoya, et al. 2002), increase antibody production, promote class switching (Le Bon, et al. 2001), and may have a direct stimulatory effect on B cells and T cells (Le Bon, et al. 2006), Adjuvants that induce type I IFN potently increase antibody response to vaccination (Junkins, et al. 2018). Therefore, in addition to studies with human participants, a rodent model was included. In the rodent experiments, anti-IFNα antibody was used to block IFNα at the time of immunization to evaluate whether IFNα may be one mechanism contributing to the exercise-induced enhancement of antibody response. We also evaluated 45 min of exercise in some experiments involving young and aged adults as aged adults may be more readily able to complete 45 min of light-intensity exercise instead of 90 min. We examined the role of exercise in response to a "novel" H1N1 antigen with vaccine response to 2009 H1N1 Pandemic (H1N1pdm09) virus and the response to a trivalent seasonal influenza virus in which neither type of influenza A would be considered novel. Finally, in early studies with human participants, we examined the influence of exercise on antibody response to an mRNA-based vaccine, PfizerBioNTech *BNT162b2,* against the disease caused by the novel coronavirus SARS-CoV-2 (COVID-19), using the same exercise approach that we found to be effective in influenza experiments. As the influenza vaccine platforms consisted of split-virus preparations and the PfizerBioNTech *BNT162b2* vaccine was based on an mRNA platform, we compared the effect of exercise across different vaccine platforms. Exercise has been proposed as a potential "behavioral" adjuvant for COVID-19 immunization (Valenzuela, et al. 2021), and the experiments conducted here investigated that possibility.

2. Methods

For all experiments involving human subjects, participants were recruited by flyers posted in the local community as well as by email sent to university staff, students, or community organizations and businesses that schedule group vaccination clinics.

2.1. Influenza vaccine research participants

A total of 20 participants were enrolled in the monovalent Influenza A/California/7/09 H1N1 vaccine experiment, and 16 were included in the final analysis (see Supplement Fig. 1 CONSORT diagram). A total of 28 participants were enrolled in the trivalent seasonal influenza vaccine experiment, and 26 were included in the final analysis (see Supplement Fig. 2 CONSORT diagram). Individuals were excluded if they were: taking medications for psychological disorders or medications that altered immune variables of interest (e.g., oral corticosteroids); had any medical condition that may directly impact immune outcomes, including autoimmune disorders; or were unable to perform the prescribed exercise safely. Participants were included if they had been exercising regularly for at least the previous six months and met the criteria set forth for moderate-intensity exercise in accordance with American College of Sports Medicine Guidelines (American College of Sports Medicine, 2018). In the first experiment, participants were immunized with a monovalent vaccine of a novel strain (single antigen A/California/7/09 Influenza H1N1, pdm09), hereafter referred to as "monovalent". In a second experiment, participants were vaccinated with the trivalent influenza vaccine ((Influenza A/California/7/09, A/Perth /16/2009, B/Brisbane/60/2008), termed "seasonal" vaccine. All participants received the current influenza Vaccine Information Statement and were asked to report any concerning side effects to study personnel. The Institutional Review Board approved all procedures at Iowa State University.

2.2. COVID-19 vaccine research participants

A total of 36 individuals that received the Pfizer BNT162b2 (Pfizer-BioNTech COVID-19 Vaccine) between March 2021-June 2021 were enrolled in the study (see Supplement Fig. 3 CONSORT diagram). Data from eight participants who were possibly previously infected with SARS-CoV-2 based on higher pre-immunization antibody levels and a larger magnitude of change in response to the first vaccine was reported but not considered part of the primary analysis. Participants were included in the study if they regularly participated in moderate or vigorous-intensity exercise two or more times a week, with, on average, at least one session lasting 50 min or longer. Participation in the study followed the American College of Sports Medicine recommendations for preparticipation health screening (Riebe, et al. 2015). Individuals were excluded if they had an immune condition that would be expected to impact the variables of interest or if they were taking a medication that significantly alters immune response. Individuals who were not pregnant, who planned to receive the COVID-19 vaccine, and who were willing to donate blood were included in the study.

2.3. Psychosocial surveys for influenza experiments

All participants in the influenza vaccine experiments completed several psychosocial surveys to determine whether there was an association between antibody response to the vaccine and psychosocial factors. All participants completed the following surveys: Perceived Stress Scale (PSS) (Cohen, et al. 1983), Sense of Coherence (SOC) (Antonovsky, 1993), and Profile of Mood States (POMS) (McNair, et al. 1992).

2.4. Blood Collection, Vaccination, and timeline

Blood was taken from an antecubital vein (30 ml) in subjects just before immunization with either the monovalent or seasonal influenza vaccine. Blood was collected at two weeks and four weeks post-immunization.

A pre-immunization blood sample was collected within the week preceding the COVID-19 vaccination. After the first Pfizer BioNTech COVID-19 vaccine was administered, subjects returned two weeks later

for blood collection. The second dose of vaccine was given three weeks after the first vaccine dose. An additional blood sample was collected one week following the second Pfizer BioNTech COVID-19 vaccine (1 week post dose 2). All participants received side effect report forms listing the side effects described on the Pfizer BioNTech emergency use authorization. Side effects were recorded every 24 h for the first 72 h after each vaccine by placing a check next to each side effect if present. A score of 1 was given each day a given side effect was recorded by the participant. Therefore, scores ranged from 0 (not present on any day) to 3 (present on each of the three days following the vaccine) for each side effect listed on the emergency use authorization form.

2.5. Exercise conditions

In the experiment in which monovalent H1N1 vaccine was administered, subjects were randomly assigned to a light- to moderate-intensity exercise group (90 min) or no exercise control group. If randomized to exercise, participants began the exercise session within 30 min of receiving the single antigen vaccine. If assigned to control, the participants started a sedentary period within 30 min of vaccination. The sedentary period consisted of sitting while watching videos for 90 min. Adults randomized to exercise performed 90 min of exercise on a cycle ergometer at 60–70% of estimated maximal heart rate (HR max) with the range calculated as [(220 – age) × 0.6] to [(220-age) × 0.7], typically corresponding to an intensity perceived as light to somewhat hard based on the Rating of Perceived Exertion (RPE) Borg 6–20 scale (Borg, 1982). After a 10-minute warm-up period, the cycling rate and workload were adjusted to maintain heart rate in the appropriate range. Heart rate and RPE (Borg 6–20 scale) were assessed every 10 min, and water was available for the participant.

In the experiment in which seasonal vaccine was administered, young (age 18–33) subjects were randomly assigned to one of three groups: no exercise, 45 min of exercise, or 90 min of exercise, whereas aged subjects (age 62–87) were randomly assigned to one of two groups: no exercise, or 45 min of exercise. The exercise or rest period commenced within 30 min after receiving the seasonal influenza vaccine. The same exercise intensity was adhered to throughout exercise (60–70% of age-estimated HR max on a cycle ergometer). Heart rate and RPE were assessed every 10 min. Participants assigned to the no exercise condition remained sedentary for 90 min post-vaccine, seated watching videos.

In the experiments involving the COVID-19 vaccine, participants were randomly assigned to either 90 min of exercise or instructed to go about their daily routine but avoid exercise on the day of the first vaccination. All participants were asked to avoid exercise on the day the second vaccine dose was given. Exercise took place outdoors at the location where participants were vaccinated to limit SARS-CoV-2 infection risk for participants and study personnel. Study personnel identified a safe walking/jogging route, and routes were designed to monitor heart rate and RPE approximately every 10 min. An exercise heart rate zone of approximately 120–140 beats per minute was targeted as this range was consistent with the average heart rate range performed at the target zone of 60–70% of HR max in the influenza vaccine studies. As exercise took place outdoors to minimize risk of SARS-CoV-2 infection, it was not possible to precisely control heart rates as compared to the influenza vaccine studies in which a cycle ergometer was used to set a workload. Therefore, although target heart rate was approximately equal to the target zone of 60–70% of HR max as in the influenza vaccine experiments, there was slightly more variability due to terrain conditions. The exercise intensity was also monitored by RPE and consistent with the influenza vaccine studies in which target RPE was light to somewhat hard. RPE was also used to adjust exercise intensity for any participants treated with beta-blocker medications.

2.6. Serum antibody Detection assays (Human Sera)

After collection, blood sat at room temperature for 45 min until clotted, was then centrifuged at 180 × g, and was frozen at −80 C^{o} until subsequent measurement of anti-influenza antibodies by ELISA. Samples from the same individual for all time points were run together on the same plate to minimize variability. Briefly, for A/California/7/09, plates were coated with 1 μg/ml hemagglutinin peptide in carbonate coating buffer, followed by overnight incubation. For A/Perth/16/2009, plates were coated with 256 hemagglutination (HA) units/ml, followed by overnight incubation. Plates were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 between each step. Dilutions of serum were added to the wells in duplicate with an optimal dilution of 1:200 for IgG based on preliminary experiments in which the optimal dilution yielding a difference from pre-immunization values was identified. After overnight incubation, AP-conjugated goat anti-human IgG (Southern Biotech) was added at 1:100 dilution. Phosphatase substrate (Sigma) was added, and optical density (OD) was assessed at 405 nm on a Fluostar plate reader. Data is shown as OD or fold change in OD from pre-immunization to post-immunization time points. The OD representing the level of detectability for each assay is indicated in the appropriate figure legend. This endpoint was calculated by a dilution series carried out in a subset of participants to determine the OD at the titer at which subsequent dilutions no longer detected a change in OD. The detectable limits were calculated separately for each vaccine antigen and in the appropriate group of participants. For experiments involving COVID-19 vaccination, antibody level was measured using GenScript SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA Detection Kit. The manufacturer's directions were followed. Briefly, serum was diluted 1:100 and the HRP-conjugated mouse anti-human IgG Fc was added to the plate to detect anti-RBD IgG antibody. Banked pre-pandemic serum was used as a negative control.

2.7. Mice, exercise conditions, vaccination, and IFNα antibody treatment

In all experiments, male BALB/c mice at 10 to 12 weeks of age (Charles River Labs) were used. First, we compared different durations of moderate-intensity exercise to determine the extent to which exercise duration altered antibody response to intramuscular injection of influenza vaccine. These experiments were replicated to confirm 90 min as the duration in which increased antibody to vaccination was observed. Next, we tested the hypothesis that IFNα mediates exercise-induced changes in serum antibody concentration by administering anti-IFNα antibody to a subset of mice. Vaccinated mice in both experiments received 50 μL of Binary ethyleneimine inactivated A/PR/8/34 virus (512 HA units) administered intramuscularly into the quadriceps. Saline control mice received 50 μL of saline and did not exercise. All mice were acclimated to the treadmill running for three days in the week preceding experiments by exposing mice to gradually increasing speeds on a motorized treadmill for 10–15 min. All studies were performed according to Institutional Animal Care and Use Committee guidelines at Iowa State University and within the guidelines set by the NIH for the care and use of laboratory animals.

In the first experiment, thirty-eight BALB/c mice were randomly assigned to one of four treatment groups (8–10 mice per group): no exercise, or moderate-intensity exercise for 45 min post-immunization, 90 min post-immunization, or 3 h post-immunization. In a subsequent experiment, we tested the role of IFNα as a mechanism by which exercise may influence antibody response to vaccine. Mice were randomly assigned to one of four groups (9–10 mice per group): no exercise, no exercise + anti-IFNα antibody, 90 min moderate-intensity exercise, 90 min moderate-intensity exercise + anti-IFNα antibody. The anti-IFNα treatment consisted of daily injection with 20 μg/mouse of rat IgG1 anti-mouse IFNα, clone RMMA-1 (PBL Interferon Source) diluted in saline containing 0.01% bovine serum albumin (BSA). Antibody administration began the day before vaccination (day −1) and continued until day

three post-immunization. Mice that did not receive anti-IFNα treatment were injected with 20 μg/mouse of an irrelevant antibody, rat IgG1, at the same dose and time of day as anti-IFNα treated mice. The 90-minute exercise duration was selected based upon results from the initial experiments, which demonstrated this exercise duration resulted in increased antibody response to vaccine.

Mice began their respective exercise protocols within 15 min following vaccination. Mice performed exercise on a treadmill at a speed of 15 m/min, which has been shown to be moderate intensity (Fernando, et al. 1993; Hoydal, et al. 2007). No-exercise mice were placed in housing cages affixed to the top of the treadmill to mimic stressors associated with the treadmill environment (noise, vibration) as closely as possible. All mice were acclimated to the treadmill environment on two separate occasions before immunization.

2.8. Serum antibody response to vaccine (mouse sera)

Blood was collected from all mice at two weeks post-immunization or four weeks post-immunization. Blood was allowed to clot at room temperature and then centrifuged at 180 × g for 15 min. Serum was collected and frozen at −80 °C until subsequent measurement of IgG, IgG1, and IgG2a anti-influenza antibodies by ELISA. Briefly, plates were coated overnight at 4°C with influenza virus A/PR/8/34 diluted in carbonate coating buffer (pH 9.6) at a 200 HA units/ml concentration for anti-influenza IgG, IgG1, and IgG2a. The wells were blocked with 0.1% BSA solution at 37 °C for one hour. Plates were washed three times with PBS containing 0.05% Tween 20 between each step. Diluted serum (1:50 for IgG and 1:5 for IgG1 or IgG2a) was added to the wells and incubated for 3 h at 37 °C. These dilutions were chosen based on preliminary assay optimization (data not shown). After incubation, AP-conjugated rat anti-mouse IgG, IgG1, or IgG2a were added (at 1:100 dilution for IgG and 1:10 for IgG1 and IgG2a), then plates were incubated overnight at 4 °C. Finally, phosphatase substrate (4-Nitrophenyl phosphate disodium salt hexahydrate, (Sigma) was added, and OD was assessed at 405 nm at 30 min (IgG) and 50 min (IgG1, IgG2a) using a Fluostar plate reader.

2.9. Statistical analysis

Statistics for all surveys, exercise data, and ELISA results in experiments involving human participants were calculated using SPSS statistical software (PASW/IBM Inc.) to perform ANOVAs. A mixed ANOVA (exercise treatment * time) was used to compare serum anti-influenza immunoglobulins (IgG) to the different exercise durations post-vaccination (either direct OD readings at respective serum dilutions or fold change in OD relative to pre-immunization level, as indicated in results). In experiments that included different age groups (seasonal Influenza vaccine), age and exercise were included in the model. In the experiment with a wide range of ages (COVID-19 vaccine), initial analyses examined the effect of exercise only. A secondary analysis was performed in which the top quartile for age was used to define a middle-aged population (ages 44–62) compared with participants considered young adults (ages 18–43). Pearson correlations were used to compare psychological survey outcomes and antibody response to the Influenza A/California/7/09 H1N1 antigen, combining the monovalent and seasonal vaccine results. All data are reported as mean ± standard error of the mean (SEM) unless otherwise indicated. In mouse experiments, a one-way ANOVA was used to evaluate the effect of exercise duration on antibody response followed by post-hoc analysis (Sidak). A two-way ANOVA (exercise group and anti-IFNα antibody treatment group) was used to compare antibody response in experiments to test the role of IFNα as a mechanism by which exercise may impact antibody response. Values of $p < 0.05$ were considered statistically significant, while values of $0.05 < p < 0.1$ were considered a statistical trend.

3. Results

3.1. Demographics and response to exercise

Age, exercise heart rate, and RPE data are shown in Supplement Table 2 (S2). There were no age differences between non-exercisers or exercise treatment groups within each separate vaccine experiment. Within the influenza seasonal vaccine experiment, heart rate among young participants was not different between the 45- or 90-minute exercise condition, but RPE in the 90-minute condition was slightly greater than the 45-minute condition.

3.2. Antibody response to monovalent or seasonal influenza vaccine, effect of exercise, and association with psychosocial factors

The serum IgG response to monovalent H1N1 vaccine increased as expected following vaccination (significant main effect of time), as shown in Fig. 1a. A significant treatment by time interaction suggested that the antibody response between the exercise group and no exercise group responded differently over time with a greater response in the exercise group (Fig. 1a). When results were calculated as fold change relative to pre-immunization, a trend towards greater fold change in antibody was observed in the exercise group relative to no exercise (main effect of exercise, Fig. 1b). Individual antibody data for monovalent vaccine is also shown in Supplement Figure S4a-S4c. Antibody response to seasonal influenza vaccine was compared in young or aged adults that exercised moderately for 45 min, 90 min (young only), or did not exercise. The results show that anti-H1N1 serum IgG increased in response to the vaccine as expected (significant effect of time, Fig. 2a). Over time, the change in antibody response differed between groups (significant time by exercise interaction), and the 90-minute exercise condition resulted in greater serum antibody than no exercise. However, antibody response in those assigned to the 45-minute exercise condition was not different than no exercise in young or aged participants (Fig. 2a and 2b, main effect of exercise, post-hoc analyses 90-min exercise > no-ex). When data was calculated as fold change relative to pre-immunization, the results were similar in that fold change of antibody level in response to 90 min of exercise was significantly greater than no-exercise or 45 min of exercise (Fig. 2c), (main effect of exercise, 90-min > no-exercise or 45-min ex in post-hoc analyses). There was no benefit in fold change in antibody level for the 45-minute exercise condition relative to no exercise for either young or aged adults. The results concerning anti-H3N2 antibody response as shown in Fig. 2d – 2f are similar to H1N1. Again, 90 min of exercise treatment resulted in greater antibody level (main effect of exercise, Fig. 2d), and as expected, antibody increased in response to vaccination (main effect of time, Fig. 2d and 2e). The 45-minute exercise condition did not improve antibody response in young or aged participants, and there was no effect of age on antibody response. Individual data for the response to seasonal vaccine are shown in Supplement Figures S5a-S5h.

Given that the same Influenza A/California/7/09 H1N1 antigen was present in the monovalent and seasonal vaccine experiments, we combined the psychosocial survey data from these two experiments to analyze associations between antibody and psychosocial factors. A negative association between antibody titer and perceived stress and a similar negative association between antibody level and total mood disturbance were noted, but the correlations did not meet statistical significance (Supplement Figures 8a-8c). A positive association between the Sense of Coherence score and antibody was observed, but again this relationship did not meet statistical significance.

3.3. Antibody response to Pfizer BioNTech COVID-19 vaccine and effect of exercise

Antibody to SARS-CoV-2 was not measured before enrollment. Therefore, we could not determine which participants may have

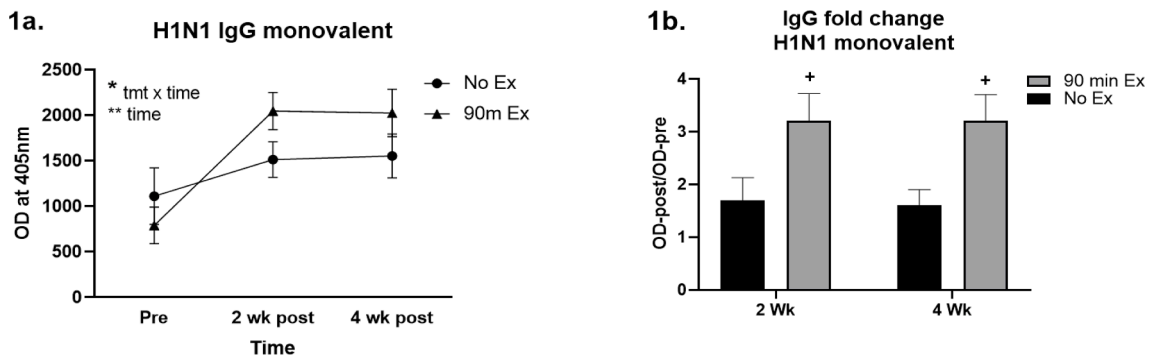

Fig. 1. *Serum IgG response to monovalent influenza H1N1 vaccine.* 1a. Serum anti-influenza IgG shown as optical density at 405 nm by ELISA assay from serum samples collected pre-immunization, two, or four-weeks post. Serum dilution series indicate assay level of detectability at OD = 203. A significant main effect of time (**p = 0.001, F = 15.07), and a significant time by treatment interaction between the no-exercise and 90-minute exercise condition (*p = 0.04, F = 3.68, df = 15, η^2partial = 0.235) were found. 1b. Calculated fold change in antibody response, with a trend to main effect of exercise treatment (+p = 0.059, F = 4.36, df = 15, η^2partial = 0.255).

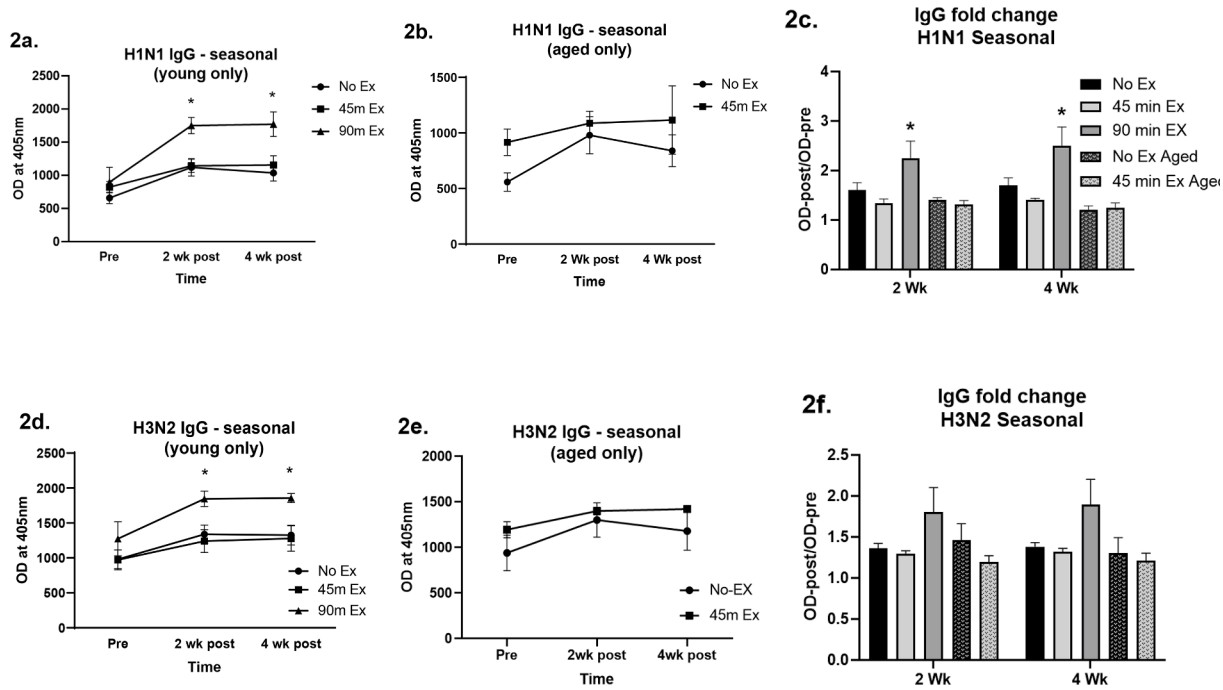

Fig. 2. *Serum IgG response to seasonal influenza vaccine.* 2a & 2b. Serum antibody level assessed as optical density at 405 nm by ELISA. Serum dilution series indicate assay level of detectability for H1N1 at OD = 224. A significant main effect of time was observed as antibody increased in response to vaccination (main effect of time, $p < 0.0001$, F = 111.59). 90-minute exercise increased antibody response (time * exercise interaction, $p < 0.001$, F = 10.5, df = 24, η^2partial = 0.489; main effect of exercise, $p = 0.027$, F = 4.379, df = 24, η^2partial = 0.277; post-hoc analysis (Sidak)) with no difference between no-ex and 45-min ex or 90-min ex and 45-min ex). No significant effect of age. 2c. Fold change in antibody level calculated relative to pre-immunization, * indicates greater fold change in 90-min exercise condition than either no-ex or 45-min exercise (main effect of exercise, $p = 0.005$, F = 7.13, df = 24, η^2partial = 0.387, post hoc analysis (Sidak) with no difference between no-ex and 45-min ex, and 90-min ex > 45-min ex ($p = 0.001$), 90-min ex > no-ex ($p = 0.005$). 2d & 2e. Serum antibody level assessed as optical density at 405 nm by ELISA. Serum dilution series indicate assay level of detectability for H3N2 at OD = 219. In the overall model, a significant main effect of time as antibody increased in response to vaccination (main effect of time, $p < 0.001$, F = 31.7, df = 25). There was a significant time by exercise interactions ($p = 0.021$, F = 3.2, df = 25, η^2partial = 0.235, and the 90-minute exercise was associated with increased antibody response (* main effect of exercise, $p = 0.014$, F = 5.217, df = 25, η^2partial = 0.332) post-hoc analysis (Sidak) with no difference between no-ex and 45-min ex, but 90-min was significantly greater than no ex ($p = 0.015$) and also>45-min ex, ($p = 0.04$). 2f. Fold change in antibody titer calculated relative to pre-immunization. A trend toward main effect of exercise was noted ($p = 0.07$, F = 2.93, df = 25, η^2partial = 0.219).

experienced asymptomatic infection before immunization or experienced an infection with COVID-19-like symptoms but did not have a confirmed positive COVID antigen test. Upon analysis of antibody level pre- and post-immunization, we noted that 8 of 36 participants were possibly infected based upon > 1.5 fold greater pre-immunization OD value as compared those assumed to be not previously infected, and a significant increase in antibody level after the first vaccine nearly equivalent to the antibody level observed after the second dose in those assumed to be not previously infected. Based on these criteria, the antibody level of "possibly infected" participants was significantly higher at the pre-immunization time point and two weeks after the initial vaccine (Fig. 3a). The finding that antibody titer in response to the first dose of vaccine is typically greater in previously infected as compared to naïve individuals is a common finding in the literature, whereas differences in antibody response between previously infected relative to naïve may or may not be present in response to the second

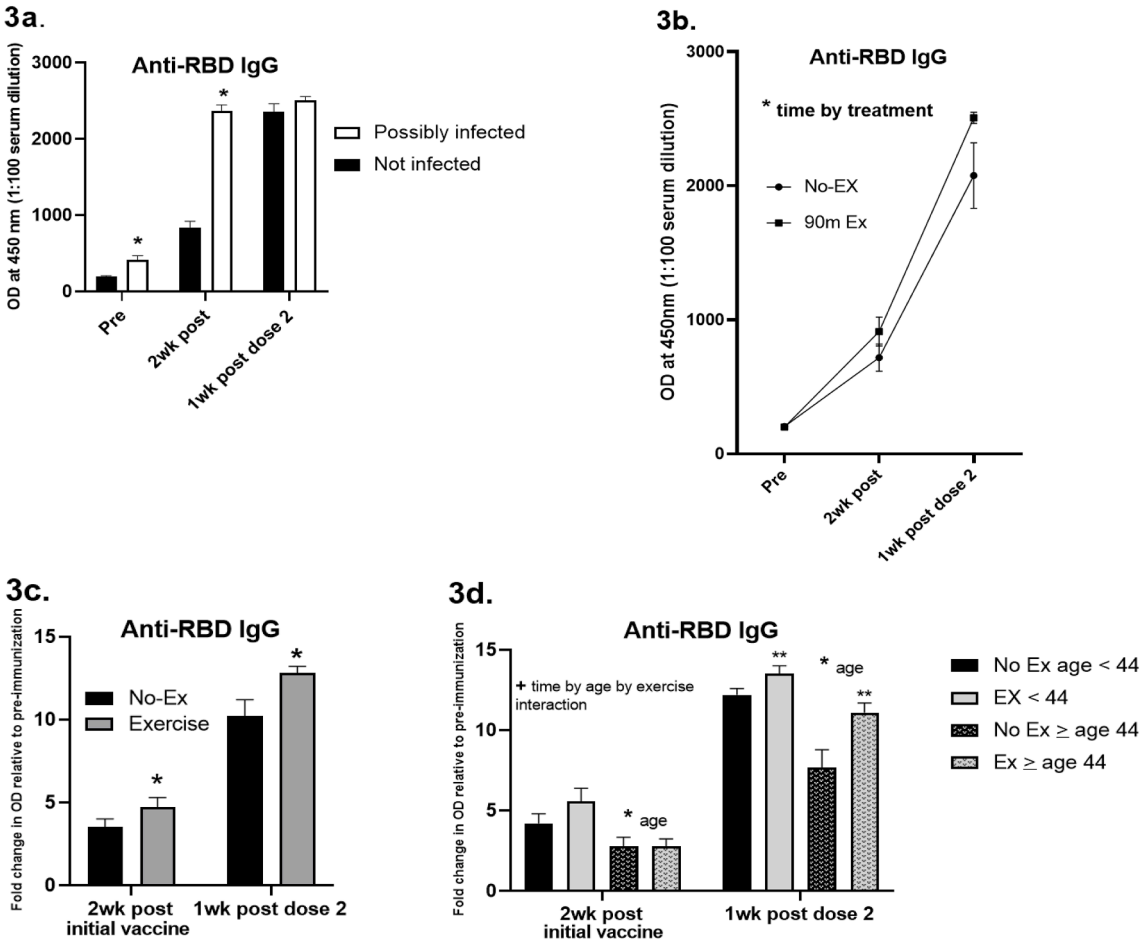

Fig. 3. *Serum anti-RBD IgG response to Pfizer BioNTech COVID-19 vaccine* 3a. SARS-CoV-2 Spike S1-RBD IgG from serum of participants categorized as "possibly infected" prior to immunization was significantly greater at pre-immunization and at 2 wk after the initial vaccine, but not different at 1 wk post-dose 2 (main effect of time, $p < 0.001$, F = 61.2, main effect of group, $p < 0.001$, F = 19.1, time by group interaction, p , F = 19.6; and follow up one-way ANOVA to identify time points of difference, * indicates difference at pre-immunization $p < 0.001$, F = 37.8; and * indicates difference at 2 wk post $p <$, 0.001, F = 46.2). 3b. SARS-CoV-2 Spike S1-RBD IgG as optical density (OD) in serum collected pre-immunization, 2 wk after initial vaccine or 1 wk after second vaccine dose (the second vaccine was administered 3 weeks after the first vaccine dose). * indicates significant treatment group by time interaction ($p = 0.039$, F = 3.50, df = 25, η^2partial = 0.137). A main effect of time ($p < 0.001$, F = 473.6) was observed and a trend to main effect of treatment ($p = 0.055$, F = 4.075, df = 25, η^2partial = 0.149). 3c. Fold change in SARS-CoV-2 Spike S1-RBD IgG relative to pre-immunization, (90 m exercise > no exercise *$p = 0.048$, F = 4.37, df = 25, η^2partial = 0.160; 1wk post dose 2 > 2wk post initial vaccine, $p < 0.001$, F = 200.4 df = 25). 3d. Fold change in SARS-CoV-2 Spike S1-RBD IgG relative to pre-immunization as compared by exercise, age, and time post-immunization. Antibody response in middle aged adults < young adults (main effect of age, $p = 0.003$, F = 11.3, df = 25, η^2partial = 0.350) and exercise treatment trended towards a difference based on age (exercise by age × antibody interaction, $p = 0.057$, F = 4.06 df = 25).

vaccine (Ali et al., 2021, Ebinger, et al. 2021, Fraley, et al., 2021, Geers, et al., 2021, Gobbi et al., 2021, Goel, et al., 2021, Krammer et al., 2021). However, the presence of antibody to nucleocapsid protein can further differentiate these populations, which was not measured in this study, and this is a limitation in the method we used to define "possibly infected." As the initial vaccination likely served as a second dose for possibly infected participants, these participants were removed from the primary data analysis because the fold change in antibody response differed significantly from participants who were assumed to be naive. The serum anti-RBD IgG antibody level assessed over time shows a significant time by treatment interaction (Fig. 3b) with a greater increase in the exercise group over time. When results are expressed as fold change in antibody relative to pre-immunization, exercise participants have significantly greater antibody (Fig. 3c). Individual data as fold change or OD are shown in Supplement Figures S6a-S6c. We also observed a significant negative correlation between age and antibody level at two weeks following the initial immunization and one week following the second vaccine dose when antibody was measured either as OD or fold change. The Pearson correlation between age and OD two weeks post-immunization was 0.534, $p = 0.006$; at one-week post-dose 2, the Pearson correlation between age and OD was 0.603, $p = 0.002$. Given the effect of age, the results of a secondary analysis in which age group was included as a factor (young adult as 18–43, middle-aged as 44–64) showed a significant interaction between age, exercise, and antibody level over time (pre, 2 wk post, 1 wk post-dose 2; $p = 0.008$, F = 5.38; graph not shown). When antibody was assessed as fold change relative to pre-immunization, a similar pattern was observed with a trend towards a significant interaction between age, exercise, and fold change in antibody (2 wk post, 1 wk post-dose 2; Fig. 3d). Middle-aged participants had significantly lower fold changes in antibody response compared to young (Fig. 3d). Participants recorded side effects for three days after receiving each vaccine. The side effect scores were not significantly different between 90-minute exercise participants compared to no exercise (Supplement, Table S3). Reported side effects on a percentage basis are also shown (Supplement, Table S4). Initial findings for the participants that were in the "possibly infected" category included only eight subjects, but this data is reported (n = 5 no exercise, n = 3 exercise) (Supplement Figure S9).

3.4. Antibody response to influenza A/PR/8/34 inactivated vaccine and effect of exercise duration in mouse model

A rodent model of influenza immunization was used to examine the effect of varying durations of exercise on antibody response to the vaccine. The results showed no effect of exercise on antibody level measured at two weeks post-immunization, but at four weeks after immunization, mice exercising for 90 min had significantly greater serum antibody level than no exercise or 180 min of exercise (Fig. 4a and individual data shown in Supplement Figure S7a). The IgG1 subclass of antibody was measured at four weeks post-immunization (Fig. 4b), and results showed a similar pattern to total IgG, although the overall effect of exercise met the criteria for a statistical trend ($p = 0.09$).

3.5. Requirement for IFNα at time of immunization for optimal antibody response

Separate experiments examined the potential role of IFNα as a mechanism that may contribute to the effects of exercise on antibody response. Mice treated with anti-IFNα antibody or control antibody at

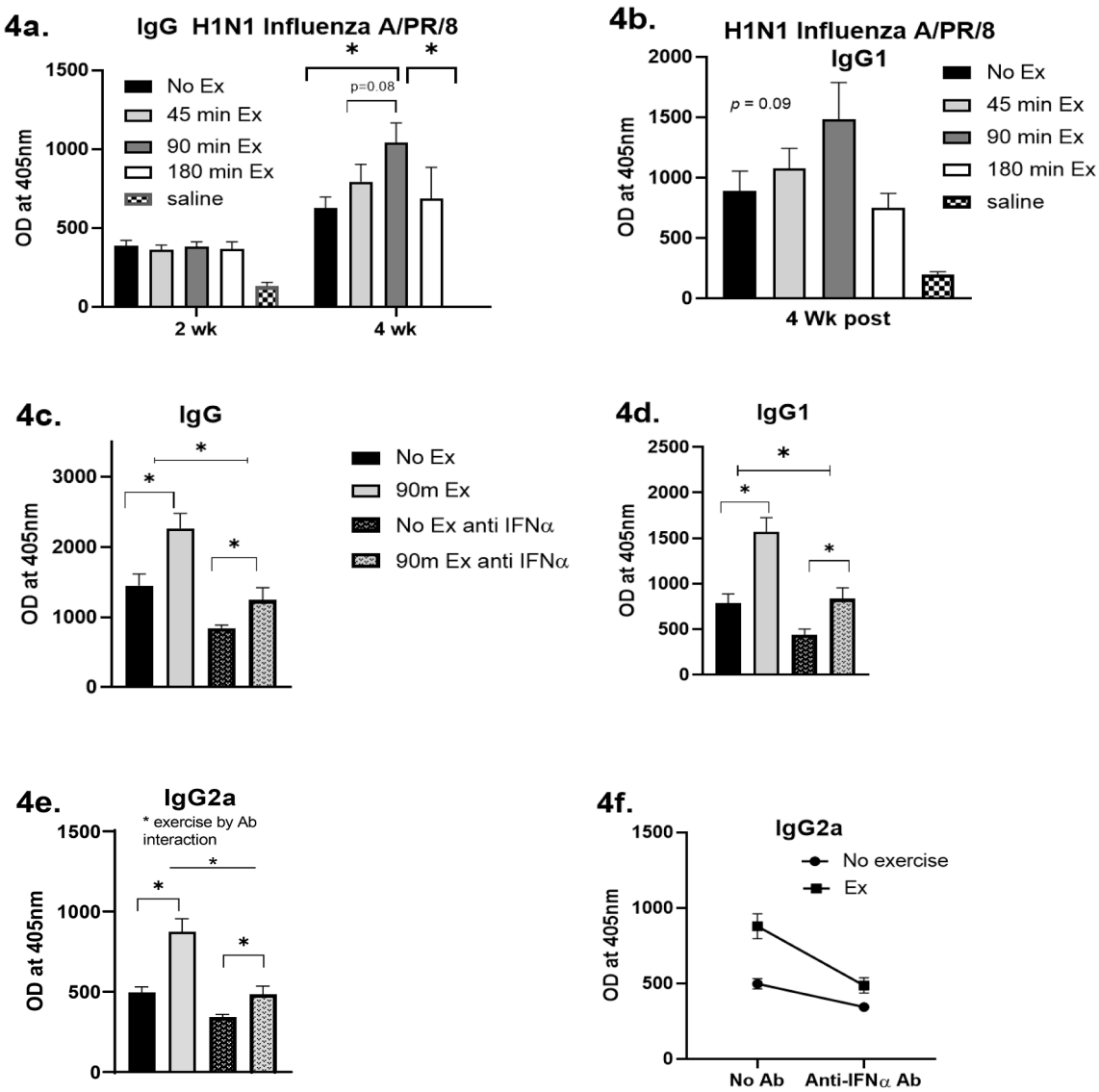

Fig. 4. *Mouse Serum anti-Influenza A/PR/8/34 IgG, IgG1, or IgG2a response to vaccination following varying durations of exercise, and the role of IFNα (mice).* 4a. Serum was collected at either 2 weeks or 4 weeks post-immunization (separate mice at 2 weeks and 4 weeks). No significant effect of exercise at 2 weeks. At 4 weeks of exercise, exercise treatment altered antibody response (overall significant effect of exercise, $p = 0.044$, F = 3.046, df = 34, η^2partial = 0.233) and post hoc analysis results indicated serum IgG in mice completing 90 min of exercise > no ex (*$p = 0.004$). 4b. Serum anti-influenza IgG1 assessed at 4 weeks post-immunization. There was a statistical trend (overall effect of exercise, $p = 0.09$, F = 2.92, df = 34, η^2partial = 0.168) towards a difference between groups. 4c. Total anti-influenza serum IgG was measured four-weeks post-immunization. Anti-IFNα antibody-treated mice had significantly less IgG but exercise increased IgG in both anti-IFNα treated mice and mice treated with irrelevant antibody (main effect of antibody, * $p < 0.001$, F = 28.3, df = 38, η^2partial = 0.447 ; main effect of exercise, $p = 0.002$, F = 11.5, df = 38, η^2partial = 0.247). There was no significant exercise group * anti-IFNα interaction, $p = 0.128$). 4d & 4e. Anti-influenza serum IgG1 (6b) or IgG2a (6c) were measured at four weeks post-immunization. Without IFNα, antibody response was significantly reduced for IgG1 (main effect of IFNα antibody treatment, $p < 0.001$, F = 24.5, df = 38, η^2partial = 0.447) and exercise treatment significantly increased IgG1 ($p < 0.001$, F = 23.5, df = 38, η^2partial = 0.402), and there was a trend towards an anti-IFNα antibody by exercise treatment interaction ($p = 0.06$). With respect to IgG2a there was a significant interaction between exercise treatment and anti-IFNα antibody (exercise * antibody interaction, $p = 0.014$, F = 6.63, df = 38, η^2partial = 0.159) implying the exercise effect was not consistent across antibody treatment. For IgG2a, effect of exercise ($p < 0.001$, F = 23.73, df = 38, η^2partial = 0.404) and IFNα antibody treatment, $p < 0.001$, F = 31.6, df = 38, η^2partial = 0.475). 4f. The significant interaction for IgG2a (from 4f) is shown in a line graph format.

the time of immunization completed either 90 min of exercise or no exercise. The results of serum anti-influenza IgG antibody, IgG1, and IgG2a were similar in that a lack of IFNα at the time of immunization significantly impaired antibody response (Fig. 4c –4f). Mice assigned to the 90-minute exercise intervention had higher IgG, IgG1, and IgG2a than no exercise mice, regardless of whether mice received anti-IFNα antibody treatment. However, there was a significant interaction with respect to IgG2a in which the anti-IFNα antibody treatment attenuated the effect of exercise (significant exercise by IFNα antibody treatment interaction, Fig. 4e and 4f), suggesting that IFNα may contribute to some extent to the effect of exercise on antibody class switching. Individual mouse data is shown in Supplement Figures S7b-S7d.

4. Discussion

The findings presented here demonstrate that 90 min of exercise after immunization increases antibody response several weeks later across several immunization models. To the best of our knowledge, these findings are the first to show that light- to moderate-intensity long-duration exercise enhances antibody response across several vaccine formulations, including COVID-19 vaccination. These results may have immediately translatable public health relevance as the exercise paradigm is straightforward to implement and does not require special equipment. The exercise intervention is feasible for people who exercise regularly at light intensities such as walking, and persons with a range of health characteristics were able to complete the exercise. For example, nearly half of the participants in the COVID-19 vaccination trial had a BMI in the overweight or obese category, and the distance covered in 90 min ranged from approximately four miles to over 10 miles, representing a variety of fitness levels as heart rate and relative perceived exertion level were maintained within a constant range. It will be essential to determine the length of time post-vaccination for which an exercise-associated increase in serum antibody may be present. Longer term antibody response will be assessed as these findings are an early report. It would also be helpful to define how the change in antibody translates to protection from infection. However, appropriate study designs to address that question typically require thousands of participants, and may not be feasible, in which case inference from antibody level and protection studies will be necessary. Serum antibody is recognized as an immune correlate of protection for influenza (Potter and Oxford, 1979), including IgG measured by ELISA (Trombetta, et al. 2018), and therefore we expect that the increase in antibody that we report would confer some benefit. The immune correlates of protection from COVID-19 vaccination are under investigation, but early evidence supports serum antibody as a potential correlate (Sadarangani, et al. 2021).

In comparing the results from our studies with the existing literature on exercise and vaccines, one limitation that arises is the wide variety of exercise approaches used. To our knowledge, there are no other studies that investigated the immunomodulatory effects of a 90-minute aerobic exercise session. However, of the studies that examined aerobic exercise rather than eccentric exercise, there were conflicting results. For example, one study showed no benefit in antibody response to either pneumococcal or influenza vaccine after 45 min of aerobic exercise (Long, et al. 2012). In other studies, mixed results were found in response to 45 min of dynamic exercise or aerobic exercise, with an increase in antibody response to one antigenic component of influenza vaccine, but not both influenza A antigens, and variable results by sex were present (Edwards, et al. 2006; Ranadive, et al. 2014). Although the timing of exercise in relation to vaccination was different in our experiments with exercise performed after immunization, we observed that 45 min of exercise was of insufficient duration to increase antibody response to either influenza A antigen (in young or aged adults) or in a mouse model of influenza A vaccination. Therefore, our findings align with the current literature reporting that aerobic exercise interventions of 45 min or less do not enhance the antibody response following influenza A vaccination.

Notably, there were no differences in the total number of side effects or duration of side effects in exercise compared to no exercise subjects in response to the vaccine with greater reactogenicity (COVID-19), indicating a potential benefit of exercise with no change in side effect profile. No adverse reactions to either of the influenza vaccines were reported. Two separate studies found decreased side effects of exercisers versus non-exercisers (Bohn-Goldbaum, et al. 2020; Lee, et al. 2018). We did not see a similar exercise-associated reduction of vaccine side effects, but exercise mode and duration differed, and COVID-19 vaccine reactogenicity may also differ, thereby limiting the ability to make direct comparisons.

The mechanisms by which exercise may increase antibody response to vaccines remain to be elucidated, although our results provide some initial support for a potential role of IFNα based on findings from the mouse model. The exercise-induced enhancement of IgG2a was attenuated in mice that received anti-IFNα antibody treatment, and a similar trend was apparent for IgG1. Type I IFN promotes class switching (Le Bon, et al. 2001), supported by our data showing that mice lacking IFNα at the time of immunization had significantly reduced IgG1 and IgG2a. IFNα also promotes dendritic cell costimulatory molecule expression and dendritic cell activation (Montoya, et al. 2002; Tough, 2004), germinal center formation (Le Bon, et al. 2001), and may have direct or indirect stimulatory effects on B cells and T cells (Le Bon, et al. 2006). Type I IFN, inducers of Type I IFN, or IFNα alone have adjuvant properties as demonstrated in an influenza model (Junkins, et al. 2018; Proietti, et al. 2002), may specifically stimulate IgG2c or IgA antibody in response to influenza vaccine (Ye, et al. 2019), and may serve as a mucosal adjuvant in porcine influenza vaccination (Liu, et al. 2019). Inducers of IFNα (imiquimod) delivered intradermally with influenza vaccine improved antibody response in young or older adults (Hung, et al. 2016; Hung, et al. 2014). Altogether, the evidence from these studies suggests that IFNα can have immunostimulatory effects and adjuvant activity for vaccines. Therefore, if exercise results in significant increases in IFNα, or the ability to produce greater IFNα upon stimulation, IFNα may be one mechanism by which exercise improves antibody response to vaccines. With 90 min of exercise, we had previously observed a significant increase in IFNα by human plasmacytoid dendritic cells (Table S1). We acknowledge that 90 min of exercise in a mouse is not directly translatable to humans, but the mouse model affords the opportunity to begin identifying potential mechanisms.

Considering other possible mechanisms, the lack of response to 45 min of aerobic exercise compared with enhanced antibody response to immunization with 90 min of exercise may provide some insight. Exercise duration and intensity influence the metabolic and neuroendocrine responses to exercise. Multiple aspects of the vaccine itself, including the dose of antigen, the inclusion of antigens, the delivery route, the vaccine platform (lipid nanoparticle encapsulated-mRNA compared to subunit/split virus preparation), may impact the kinetics and quality of antigen-presenting cell response and subsequent activation of T cell and B cell response (Bachmann and Jennings, 2010; Chappell, et al. 2014; Liang, et al. 2017; Zeng, et al. 2020). These factors may influence the immunomodulatory effects of exercise. The findings presented here provide initial insights into mechanisms to further explore in future studies. Our results suggest a possible partial role for IFNα and show that exercise benefits extend across different vaccine formulations but require 90 min of light- to moderate-intensity exercise instead of only 45 min. Our findings also cannot rule out or confirm a role for IL-6 as a potential mechanism by which exercise may contribute to enhanced antibody response. One might expect a dose response such that as exercise duration increases, IL-6 increases (Fischer, 2006). The data presented in the mouse model of vaccination in which 45, 90, and 180 min of exercise were compared do not support a potential dose–response effect of IL-6, but the experimental design did not specifically address IL-6.

A limitation of the findings from these experiments is the relatively small number of participants. However, the reproducibility of the results

across three different human vaccine experiments with varying vaccine formulations (inactivated, mRNA-based) that contain either novel antigens or antigens to which participants had previous exposure (seasonal influenza vaccine) lends credence to these results. Studies in a mouse model replicated the findings in human experiments. Although the conclusions of the COVID-19 vaccination are an early report with relatively limited sample size, analysis of long-term antibody response will be performed. Due to the potential public health relevance, these early findings with COVID-19 were reported. Larger scale trials should be undertaken that confirm these findings and examine the extent to which similar effects may occur after booster immunizations. From a public health perspective, it would be worthwhile to determine whether an exercise duration that falls between 45 and 90 min would confer some benefit as more adults may be able to complete an exercise session that is less than 90 min. Another limitation of the findings is that the mouse experiments involved only male mice, and therefore it remains possible that these findings may not apply to female mice. The human studies included males and females but with a limited number of participants, it was not possible to establish whether there were significant sex effects. In future studies, it will be important to establish whether sex by exercise interactions exist and influence any exercise-associated changes in the antibody response to vaccine.

In summary, our results are the first to demonstrate an exercise-induced enhancement of antibody response to COVID-19 immunization without an increase in reported side effects. Our findings also show longer-duration light- to moderate-intensity exercise increases antibody response across different vaccine formulations, and exercise-induced alterations of IFNα may partially contribute to this effect.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2022.02.005.

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