The influenza virus can cause a disproportionate increase in serious illness and deaths in individuals 65 yr of age and older (24). The most effective way of avoiding influenza is through influenza vaccination. However, the vaccine is ineffective in about 25% of the older population (14,24). Numerous reasons may account for the lower influenza vaccine response in older adults including past vaccination history, natural exposure to influenza viruses, living situation, dietary factors, and immunosenescence (3,14). Hence, studying the role of exogenous and endogenous adjuvants to increase the efficacy of the vaccine in older populations is important.

It has been shown that high levels of chronic stress (psychological or physical) are detrimental to immune system function (6,12). Chronic stress has been shown to reduce influenza vaccine antibody titers in all age groups (20,22). However, there is evidence that acute stress can be immunoenhancing (4,8,21,23). The immunoenhancement has primarily been shown with acute psychological and/or physical stressors in animal models (7,8).

An acute psychological stressor and an acute physical stressor in close proximity to the administration of influenza vaccination induced higher antibody titers for A/Panama strain in women compared to an unstressed control group (10). Edwards et al. (10) reported greater antibody responses 4 and 20 wk after vaccination in young women compared to young men after an acute bout of 45-min aerobic exercise before vaccination, suggesting a sex-specific effect of stress on antibody responses to vaccination. Acute eccentric arm exercise also produced a sex-specific response after influenza vaccination, with women showing a greater antibody response than men (8). Furthermore, Edwards et al. (11) showed that acute eccentric arm exercise produced a sex-specific enhancement of the antibody response to a half-dose influenza vaccination, whereas exercise intensity did not affect the response. Their study also reported that exercise only affected
low-immunogenic antigens. Conversely, Campbell et al. (5) noted that acute eccentric exercise in young healthy adults did not further augment the antibody titer in response to influenza vaccination. Thus, acute exercise may enhance the antibody response to influenza vaccination in a sex-specific manner in young adults, while primarily affecting low-immunogenic antigens.

Most of the studies with acute exercise bout have focused on young healthy adults who typically have a robust antibody response to influenza vaccination. Recently, Long et al. (19) reported that an acute moderate-intensity aerobic bout (45 min of brisk walking with an uphill and downhill component) before pneumonia (Pneumovax II) and half-dose influenza vaccination (Fluarix) did not affect or augment the antibody response to either influenza or pneumonia vaccine in middle-aged adults. Because a reduced dose of influenza vaccination usually elicits an appropriate immune response in young healthy individuals (19), these data suggest that age may affect how acute exercise influences the antibody response to influenza vaccination. Although several studies have shown that exercise training improved antibody responses to influenza vaccination in older adults (16,17,24), to our knowledge, no data exist on the effect of acute exercise on the potential for sex-specific immune responses after influenza vaccination in older (above the age 65 yr) adults.

Previous work has provided support for interleukin-6 (IL-6) as a potential contributor to the immune enhancement after an acute exercise (10). It has also been previously reported that higher levels of IL-6 at the time of vaccination may be associated with greater antibody response to vaccination (18,19). The IL-6 response after vaccination has been used as a biomarker of subsequent vaccine efficacy, as it has been previously shown in humans that antibody responders to Francisella tularensis have a higher level of IL-6 compared to nonresponders (10,18). Because IL-6 was one of the primary cytokines to be elevated, it was hypothesized to be involved with the downstream response. Hence, in the present study, we evaluated the association between inflammatory cytokine (C-reactive protein [CRP] and IL-6) levels and antibody titers.

Therefore, the primary aim of this study was to evaluate the effect of acute moderate aerobic exercise immediately before administration of the influenza vaccine on antibody titers and seroprotective responses in older adults. A second exploratory aim was to investigate whether acute exercise immediately before influenza vaccination produces different effects on antibody response in older men versus older women.

METHODS

Subjects

Fifty-nine healthy volunteers (men and women) between the age of 55 and 75 yr (mean ± SD = 67 ± 4.4 yr) were recruited. Twenty-three men and 32 women completed the study, whereas 1 man and 3 women dropped out of the study after the initial visit. The reasons for dropping out were change in medication (1 man) and change of mind (3 women). All subjects were free from cardiovascular or respiratory disease, and none smoked. A stress test was performed on visit 1 to exclude any participant with evidence of stress-induced ischemia. Participants with diagnosed uncontrolled hypertension, stroke, or myocardial infarction within the 6 months before the study were excluded. Participants were excluded if they had metabolic disease (diabetes mellitus), inflammatory diseases (rheumatoid arthritis and systemic lupus erythematosus), or bleeding disorder; were taking medications known to affect inflammation (aspirin); and had a history of smoking (any form). Participants who had suffered from common cold or influenza and bacterial or viral infection or upper respiratory tract infection 2 months preceding testing were also excluded. Participants who had already received influenza vaccination for the season or who were taking thyroid medication or allergy medication on a regular basis were excluded as well. Participants were also excluded if they were taking over-the-counter pain/anti-inflammatory medication during the course of the study. All subjects were recruited from the local community and provided written informed consent before participation. This study was approved by the Institutional Review Board of the University of Illinois at Urbana-Champaign.

Study Design

In a randomized, counterbalanced, crossover design, subjects were randomized to an acute exercise or to a no-exercise group. Each subject was then administered either influenza or a sham (sterile saline) vaccination in a counterbalanced order on the first intervention day. The other injection was administered on the second intervention day. The intervention days were 2 wk apart to ensure that an acute inflammatory response to the first injection would have subsided and not influence the inflammatory response to the second injection. Subjects were asked not consume caffeine or alcohol or to exercise for 12 h before testing. All the participants were asked to fast for at least 10 h. All the measurements for every visit were performed between 6:30 and 9:30 a.m. to control for any diurnal variation. Subjects rested in the supine position for a period of 10 min in a temperature-controlled room before testing.

Visit Details

On visit 1, all the included subjects were asked to perform a maximal aerobic capacity test (\(\text{VO}_2\text{peak}\)) in the presence of a physician using a modified Balke treadmill protocol. Speed was maintained at 3.0 mph throughout the test while the grade increases by 2% every 2 min. The starting grade on the treadmill was 2%.

On a separate day at least 7 d after the maximal exercise test, participants received influenza vaccination for the years 2010 and 2011 (in a 0.5-mL prefilled syringe) in the deltoid muscle (shoulder) of their nondominant arm.
Subjects randomized to the exercise group performed a 40-min moderate-intensity aerobic exercise bout at an intensity of 55%–65% of their maximum HR immediately preceding the vaccination and sham injection. Those randomized to the no-exercise group did not perform any physical activity preceding the vaccination and sham injection. The participant and the researchers were blinded to when they received the vaccine or sham injection.

On visits at 24 and 48 h (inflammatory marker analyses) after each injection and 4-wk visits after influenza vaccine (efficacy marker), the participants underwent blood draws. The efficacy of the vaccine was measured at the 4-wk time point because this is the time of peak antibody response as shown in previous studies (10,19,24).

**Anthropometrics.** On visit 1, height and weight were measured using a stadiometer (to the nearest 0.5 cm) and a beam balance platform scale, respectively. Body mass index was calculated as weight (kg) divided by height (m) squared.

**Brachial blood pressure.** After 10 min in the supine position on visit 1, resting systolic and diastolic blood pressure (BP) were measured in the supine position using an automated oscillometric cuff (HEM-907 XL; Omron Corporation, Japan). All BP measurements were made in duplicate, and the average of the two values was recorded.

**Blood analysis.** After an overnight fast, blood samples were collected using a butterfly needle inserted into the antecubital vein. Samples were collected into 10-mL tubes containing EDTA (anticoagulant and chelating agent). Samples were separated by centrifuging at 1100 g for 15 min at 4°C and were stored at −80°C until analysis. Serum concentrations of CRP and IL-6 were measured to assess systemic inflammation. High-sensitivity Quantikine enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, and Abnova, Taipei City, Taiwan) were used to measure serum IL-6 and CRP, respectively. The inter- and intra-assay variability for IL-6 and CRP, respectively, were 5.2% and 3.4%, while that for CRP is 3.3% and 4.4%, respectively.

**Antibody responses.** Influenza antibody titers were measured using a hemagglutination inhibition test. This study was performed during two influenza seasons; the Fluarix vaccine for 2010–2011 contained three viral strains, namely, A/Perth/16/2009 (H3N2 strain), A/California/7/2009 (H1N1 strain), and B/Brisbane/60/2008 (B-Brisbane), and the Fluarix vaccine for 2011–2012 contained A/Victoria/210/2009 (H3N2 strain; an A/Perth/16/2009-like virus), A/California/7/2009 (H1N1 strain), and B/Brisbane/60/2008 (B-Brisbane) strain. Paired pre- and postimmunization duplicate sera samples from each individual were tested by the Clinical Virology Lab at Hackensack Medical Center, NJ. Geometric means for serum antibody titer were calculated and reported as log_{2} reciprocal titers.

**Seroconversion.** A hemagglutination inhibition titer of ≥40 was considered a seroprotective response (13,24).

**Statistical analysis.** All data are reported as mean ± SEM. *A priori* significance was set at *P* < 0.05. Normality of distribution was assessed using Shapiro–Wilk test. If the data were not normally distributed, outcome measures were logarithmically transformed, that is, antibody data for all strains, CRP, and IL-6. A three-factor (time, exercise, and treatment) repeated-measures ANOVA was performed for all the continuous variables to look at the effect of exercise. A three-factor (time, exercise, and sex) mixed-design ANOVA was performed for all the continuous variables to look at the effect of exercise in different groups. Because the baseline antibody titers for the H1N1 strain were lower in women in the exercise group, we performed a two (exercise vs control) by two (men vs women) ANCOVA on the H1N1 antibody titers at the 4-wk time point, using baseline values as the covariate.

Delta values for CRP and IL-6 were calculated and used for analysis. Post–sham injection blood was used only in this analysis to show that there was an inflammatory response after vaccine. Pearson correlation coefficients were used to assess relationships between inflammatory cytokines and antibody titers. Data analysis was carried out using Statistical Package for the Social Sciences (version 18; SPSS, Inc., Chicago, IL).

**RESULTS**

Subjects’ characteristics are presented in Table 1. There were no significant differences in the mean age, height, weight, or VO_{2peak} between the exercise and the control groups.

The antibody titers for all the strains increased significantly 4 wk after vaccination in response to the vaccine (Fig. 1). Exercise and vaccine effect: There was a significant increase in the levels of antibody titers 4 wk after vaccination in both exercise and control groups (Fig. 1). There was no significant interaction between exercise and control groups for any of the strains. Exercise and sex difference for H1N1: There was a significant time-by-sex-by-exercise group interaction (*F*{1,50} = 5.09, *P* = 0.02) for the H1N1 strain (Fig. 2A). H1N1 antibody titers increased significantly after 4 wk in all the groups except women in the control group. There was a significant difference in the prevaccine antibody titers for H1N1 in women. However, after covarying for baseline (prevaccine titers) values of H1N1, there was still a significant interaction between exercise and sex, showing that the women in the exercise group increased their H1N1 antibody titers significantly more than the women in the control group (*F*{1,49} = 5.38, *P* = 0.02). Exercise and sex effect for H3N2: There were no differences (*F*{1,49} = 3.49, *P* = 0.06) in the

<table>
<thead>
<tr>
<th>TABLE 1. Descriptive variables for exercise and control groups.</th>
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<tbody>
<tr>
<td>Exercise (n = 28)</td>
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<td>-------------------</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>BMI (kg m^{−2})</td>
</tr>
<tr>
<td>VO_{2peak} (mL kg^{−1} min^{−1})</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

There were no statistically significant differences between groups.

BMI, body mass index.
levels of antibody titers 4 wk after vaccination for H3N2 between the exercise group and the control group (Fig. 2B). There were also no sex-associated differences. There were no differences in the number of seroprotected individuals between the exercise and control groups (Table 2).

There was a significant group \((F_{1,54} = 6.35, P = 0.01)\) and time \((F_{1,54} = 15.64, P < 0.01)\) main effect for delta IL-6 and a significant group effect for delta CRP \((F_{1,53} = 11.27, P = 0.001)\), between vaccine and sham condition (Fig. 3).

There was a significant correlation \((r = 0.44)\) between delta IL-6 levels 24 h after vaccination and antibody titers after 4 wk for the H1N1 strain in the exercise group. The correlation was not significant in the control group \((r = 0.04)\).

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**DISCUSSION**

To our knowledge, this is the first study to evaluate the effect of acute aerobic exercise before a recommended full dose of influenza vaccine on the vaccine-induced anti-influenza antibody responses in older adults. In the present study, we did not find any evidence of immune enhancement as a result of acute moderate aerobic exercise in older men, but acute exercise provided a vaccine-induced immune enhancement for the H1N1 strain in older women.

Studies have shown that acute stress can be immune enhancing as shown in animal models (4,8). The immune enhancement of an acute stressor as shown in the mice model is related to the duration of the stressor, the type of the stressor, and the temporal relationship between the stressor and the challenge (8). Close proximity of the acute stressor before the antigen exposure seems to boost the immune response (8). Previous findings suggest that a 45-min bout of moderate-intensity cycling exercise before influenza vaccination augments the antibody response in young individuals (10). In addition to a moderate-intensity cycling exercise, eccentric exercise has shown to augment the antibody response to the influenza vaccine in young individuals (9). In a study similar to ours, Long et al. (19) reported that 45 min of brisk walking before pneumococcal vaccine and a half dose of influenza vaccine did not affect antibody response to either vaccine groups. Although the overall seroprotective response was unaltered by exercise in our present study and acute moderate exercise before the influenza vaccination did not provide immune enhancement for men, there was a selective vaccine-induced immune enhancement for the H1N1 strain in women.

There may be several possible explanations for this lack of overall enhanced seroprotection. It is possible that the intensity and mode of the acute exercise have affected the findings. Our choice of moderate aerobic exercise was partly based on previous data using cycle ergometry at 55% of predicted maximum workload where they reported that the antibody titers for A/Panama strain were higher in women for both a mental stressor and exercise stressor group as compared to the control group (10). Although all the participants were healthy, not all the participants were regular exercisers, and hence, an acute, intense, and prolonged exercise bout may not have been achievable in terms of cardiopulmonary physiology. Hence, we selected a moderate aerobic exercise bout between 55% and 65% of the heart rate maximum for 40 min on treadmill based on the American College of Sports Medicine (ACSM) guidelines (1). However, this intensity of the exercise may not have been a strong enough stimulus to augment vaccine efficacy. Although Long et al. had noted in their study that, walking is a habitual exercise for most individuals (19), the consideration to use an acute, intense, and prolonged exercise as a potential adjuvant might yield different effects.

Keylock et al. (15) have previously shown that older individuals with higher cardiovascular fitness show higher
antibody responses to the influenza vaccine. Because our sample had included both sedentary and physically active older individuals, the antibody response may have been different between groups. Hence, we compared the antibody response based on fitness categories constructed from the VO\textsubscript{2peak} data generated from the exercise tests (poor, fair, and good). As our sample size was not large enough, we combined the percentiles, based on the ACSM guidelines. However, there were no significant differences in the antibody responses when the groups were differentiated based on cardiovascular fitness. Hence, cardiovascular fitness may not explain the overall lack of antibody augmentation after vaccination in the exercise group.

Campbell et al. (5) have previously shown that there was no immunoenhancement in young healthy individuals with a relatively healthy immune system. The authors suggested that the immune response to the influenza vaccine could be maximal, and hence, there would be limited room for further immunoenhancement by exercise (e.g., a "ceiling effect"). Similarly, although our study focused on older individuals, our inclusion criteria were set specifically for healthy individuals, and hence, this may explain the lack of exercise-induced immune enhancement after vaccination in the exercise group.

TABLE 2. Percentage of individuals seroprotected (e.g., induced hemagglutination inhibition titer \textgreek{g} 40:1) by vaccination expressed as a percentage for the strains of H1N1, H3N2, and B-strain.

<table>
<thead>
<tr>
<th></th>
<th>Exercise (n = 27)</th>
<th>Control (n = 28)</th>
<th>Total (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1N1</td>
<td>44.00</td>
<td>56.00</td>
<td>45.45</td>
</tr>
<tr>
<td>H3N2</td>
<td>47.37</td>
<td>52.63</td>
<td>69.09</td>
</tr>
<tr>
<td>B-Brisbane\textsuperscript{a}</td>
<td>25.00</td>
<td>75.00</td>
<td>7.27</td>
</tr>
</tbody>
</table>

Values are mean percentages. There were no statistically significant differences between groups for H1N1 and H3N2.

aOnly 4 participants out of 55 were seroprotected for B-Brisbane, and hence, we have not provided a statistical analysis for it.

lack of overall enhancement of seroprotection. However, considering the relatively large number of individuals who did not receive seroprotection in the present study, this may be an unlikely explanation.

It has been shown that acute exercise before an influenza vaccine augmented the antibody responses in young women compared to young men in the A/Panama strain (10). However, other exercise training studies (16,17) have not reported any sex differences after vaccination. The results from the present study show that acute moderate-intensity aerobic exercise has no differential effects between older men and women in terms of the antibody titer of the H3N2 strain (Fig. 1) of the influenza vaccine. Interestingly, there was a significant exercise effect in women as compared to that in older men and women in terms of the antibody titer of the H3N2 strain (Fig. 1) of the influenza vaccine.
men for the H1N1 strain of the influenza vaccine (Fig. 2A). Although this is a different strain of influenza vaccine compared to that previously reported by Edwards et al. with significant changes, consistent with previous literature, we show the strongest augmented response in the group with the strain (H1N1) that had a lower control response. In other words, women in the exercise group who had lower prevaccination antibody titers showed the strongest augmented response after vaccination. Edwards et al. have previously argued that exercise appears to augment the antibody response to vaccination only in individuals who are low responders. Although our results support these findings, we have to be careful in interpreting our data because postvaccine antibody titers were similar in women in the exercise group and in the no-exercise group. This may suggest that the exercise effect was primarily driven by the low prevaccination antibody titers values in women in the exercise group. However, the significant effect was still present after covarying for baseline values, suggesting that exercise may indeed enhance vaccine-induced H1N1 antibody titers in older women. A subsequent study is warranted to investigate the mechanistic perspective of the augmented antibody titers to influenza vaccination in men versus women.

Although the exact mechanisms for the vaccine-induced immune enhancement effects of acute exercise are elusive, IL-6 has been noted as one of the potential biomarkers predicting immune enhancement in response to acute stress (8,10). IL-6 is among the first cytokines to be released and elevated after exercise, thus it may help regulate the immune response (10). As previously reported in humans, the antibody responders to F. tularensis had a higher level of IL-6 compared to the nonresponders (10,18). It may be speculated that the enhancement of antibody titer could be related to the levels of IL-6. Hence, in the present study, we evaluated the correlation between the change in IL-6 levels and antibody titers. Although we did not note any exercise effect for the delta IL-6 values 24 and 48 h after exercise, we found that there was a significant correlation \( r = 0.44 \) between delta IL-6 levels 24 h after vaccination and antibody titers 4 wk after vaccination in the exercise group for the H1N1 strain. There was no significant correlation in the control group \( r = 0.04 \). On further evaluation, we found that the delta IL-6 for women in the exercise group \( n = 16 \) had a trend to being correlated to H1N1 titers \( P = 0.073 \), with \( r = 0.459 \), whereas for the men \( n = 10 \) did not approach significance \( P = 0.656 \), with \( r = 0.162 \). This seems to replicate some of the results reported by Edwards et al. However, based on the results of the present study, we are unable to evaluate if elevated IL-6 is the key factor responsible for the immune enhancement of the antibody titer.

Un fortunately, because of the smaller sample size, we were unable to perform a regression modeling for mediation analysis to implicate the role of IL-6. The other potential factor are the glucocorticoids as implicated by Dhabhar and McEwen in their animal model. They have suggested that introducing or elevating glucocorticoids in close proximity to the antigen exposure may play an immune-enhancing role. Thus, we may be able to speculate that elevation of glucocorticoids after an acute bout of aerobic exercise could have been one of the reasons. However, as we did not measure the glucocorticoids in the present study, we are unable to implicate its role.

**Limitations.** In the present study, we did not perform a detailed immunological assessment; thus, we are unable to address key mechanisms and modulations of the immune system after an acute moderate-intensity aerobic exercise. We did not experimentally control for the prevaccination antibody titers. The present study included only healthy older adults; thus, our data may not be representative of the population of older adults at large. We also did not control for the training status of the participants, and hence, the cohort includes older individuals who are sedentary as well as physically active. Although the older adults demonstrated a significant increase in the inflammatory markers at 24 and 48 h after vaccination, we did not have a young control group to compare the inflammatory response to the influenza vaccine.

**CONCLUSIONS**

The present study suggests that acute moderate-intensity aerobic exercise may not be immunostimulatory in healthy older men but may provide selective immune enhancement in older women. The association between IL-6 and post-4 wk antibody titers may be indicative of a potential mechanism between acute inflammation induced by the vaccine and subsequent antibody response; however, because of the correlational analysis, we can only speculate as to its importance. Seroprotection in response to the vaccine was unaltered by prior exercise, but the data reinforce the fact that there are a large number of older individuals who remain unprotected even after obtaining the recommended vaccine.

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