

# Acute exercise does not improve immune response to HPV vaccination series in adolescents

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## 1. Introduction

Vaccines have been used for over 200 years to prevent communicable diseases and are now routinely used worldwide. Globally, average vaccination uptake is about 85%, with an estimated additional 1.5million deaths preventable through increased immunization rates [1]. The human papilloma virus (HPV) vaccine prevents infection from HPV, a virus causing cervical cancer, and other HPV-related diseases and cancers [2]; its introduction in 2006 marked a notable addition in the use of vaccinations: to prevent cancer as well as communicable diseases.

Cervical cancer is the fourth most frequent cancer in women and has a global mortality rate of 52% [3]. Countries that include the HPV vaccination in immunization programs have already halved their incidence of precancerous cervical abnormalities, whereas cervical cancer mortality rates are increasing in areas without routine HPV immunization [4]. In Australia, the introduction of a free, school-based national HPV vaccination program for adolescent girls in 2007, and extended to boys 2013, has resulted in an uptake rate for 3 doses of approximately 76–82% among 15 year-olds [5]. Until 2018, a 4-valent vaccine (Gardasil) targeting two high-risk HPV types, HPV16 and HPV18, which cause the majority of cervical cancers, was administered in a 3-dose schedule [5]. As of 2018, the vaccination program uses a nine-valent vaccine with a 2-dose schedule [5]. These approaches of gender-neutral vaccination and broader protection against high risk genotypes offer several benefits: wider health protection through better herd immunity and prevention of additional HPV-associated disease; and the lower number of doses potentially increases acceptability as adolescents express uneasiness about the vaccination experience [6]. Fewer doses also reduces resource-demand for program administration.

A large body of literature demonstrates that stress influences the immune system; furthermore, a series of studies provide evidence that acute exercise has potential to improve the immune response to

vaccines. Studies have shown increased antibody titers in women who exercised prior to influenza vaccination [7–9]; in their male counterparts, however, acute exercise either provided no additional immune response [7] or the impact was an enhanced cell-mediated response [8]. By contrast, two studies found no difference among those who exercised or rested, although there were differences in the type of exercise (aerobic and strength training), vaccines and age cohort used [10,11].

The effects of acute exercise on the immune response to vaccines given in series remain unknown. Many vaccines are given as part of a primary series to elicit adequate immunity to confer protection. Boosters may later be given to sustain protection. It is therefore possible that acute exercise can have a strong effect on responses to vaccines given as a primary dose or series, as suggested by the evidence for exercise effects against less immunogenic strains. If this is true, the implications for vaccination programs are considerable, including the reduction of a primary series from three to two doses. Thus, the aim of this study was to determine the effects of an acute exercise bout just prior to each injection in the HPV vaccine series on HPV16 and HPV18 antibody titers in adolescents.

## 2. Materials and methods

### 2.1. Study population

Participant recruitment, consent, exercises used and vaccination protocols have previously been described in detail [13]. In brief, 119 healthy students (11–13 years old) who were scheduled to receive 3 doses of the 4-valent HPV vaccine as part of the national school-based immunization program participated in the full study between 2015 and 2016. Of these, 66 consented to have blood drawn for antibody analysis.

Participants were randomly allocated to control or exercise groups; those allocated to the control group proceeded according to the usual

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vaccination process outlined in the Australian immunization handbook [14], while the participants allocated to the exercise group completed 15 min of upper body resistance exercise using elastic resistance bands before receiving their vaccination, following the protocol of Edwards et al. [12]. This exercise protocol has been shown to decrease adverse events in adolescents [20] and increase pneumococcal antibody response in young adults [12]. The HPV vaccination (Gardasil) schedule required three doses (at zero, two and six months), and at each dose the participants repeated the procedure, remaining in the allocated group throughout.

## 2.2. Biological sample collection

Blood samples were obtained by venepuncture at the antecubital fossa on the day of (directly following) the third vaccination dose at six months (third dose) and again at 7.5 months (follow-up). Samples were allowed to clot for at least 60 min before centrifugation (3000 rpm, 10 min, 7 °C). Serum was then aliquoted and stored at -80 °C until prepared and assayed according to the method of Pastrana et al. [15]. In brief, samples were serially diluted and incubated with pseudovirions before neutralization assays were performed to calculate the IC50 (the serum dilution at which point antibody binding is at half of maximum). Samples with high variability (n = 2 controls) or in which IC50 could not be obtained at highest dilution (n = 3 controls, 1 exercise) were excluded.

## 2.3. Statistical analysis

Data was analyzed using IBM SPSS (v24) using a p-value of < 0.05 for significance. Participant demographics were analyzed with t-test for scalar variables and chi-square for categorical variables. Antibody titers were log-transformed due to skewed distribution. Because blood samples for the final time point could not be obtained at one school due to a scheduling conflict, titers were compared between group at each timepoint using t-tests and the change between timepoints was then compared using one-way repeated measures ANOVA and Wilks' lambda post-hoc.

## 2.4. Ethical considerations

The study was approved by the University of Sydney Human Research Ethics Committee (Protocol number: 2015/090).

## 3. Results

Antibody samples were obtained from 45 of the 119 in the original study. Of the 65 students consenting to have blood drawn for antibody analysis, 19 did not receive the full vaccination course due to absences and a venous sample could not be obtained in one, resulting in a sample size of 45. Due to school holidays coinciding with follow-up timing, the follow-up sample size was reduced to 32.

Participant characteristics are shown in Table 1. The cohort providing blood samples did not differ from the larger full study sample for age, sex, height, weight or BMI values at the first measurement (data not shown). The exercise and control groups providing blood samples did not differ for age ( $p = 0.8$ : exercise =  $12.4 \pm 0.3$  (mean  $\pm$

**Table 1**  
Baseline characteristics.

	Exercise (n = 22)	Control (n = 23)
Male (%)	9 (40.9%)	8 (34.8%)
Age in years (mean $\pm$ SD)	12.4 $\pm$ 0.3	12.4 $\pm$ 0.2
Height in cm (mean $\pm$ SD)	154.2 $\pm$ 7.3	153.9 $\pm$ 8.6 (n = 22)
Weight in kg (mean $\pm$ SD)	47.7 $\pm$ 11.8	45.0 $\pm$ 9.0 (n = 22)

standard deviation), control =  $12.4 \pm 0.2$ ), gender ( $p = 0.8$ : 39% male), or BMI scores ( $p = 0.3$ : exercise =  $19.9 \pm 4.0$ , control =  $18.9 \pm 2.6$ ).

Antibody titers at third dose (N = 45) and follow-up (N = 32) were not different between exercise and control groups for either HPV16 antibody titers (at third dose,  $p = 1.0$ : exercise =  $8.2 \pm 1.2$ , control =  $8.2 \pm 1.0$ ; at follow-up,  $p = 0.6$ : exercise =  $10.2 \pm 0.9$ , control =  $10.0 \pm 1.3$ ) or HPV18 antibody titers (at third dose,  $p = 0.6$ : exercise =  $8.4 \pm 0.9$ , control =  $8.5 \pm 1.1$ ; at follow-up,  $p = 0.9$ : exercise =  $10.4 \pm 0.9$ , control =  $10.3 \pm 0.9$ ). Using a repeated measure ANOVA, antibody titers increased with time for both HPV16 and HPV18 ( $p < 0.001$  for each), as expected, shown in Fig. 1. There was no group  $\times$  time interaction ( $p > 0.28$ ), nor group  $\times$  time  $\times$  sex interaction ( $p > 0.42$ ; data not shown) for antibody titers to either strain. In HPV18 titers a significant time  $\times$  sex interaction was observed ( $p = 0.018$ ) with males showing greater increase from third dose to follow-up than females Fig. 2), but no sex  $\times$  time interaction was found for HPV16 titers ( $p = 0.231$ ).

## 4. Discussion

In this study, an acute bout of exercise did not improve the immune response after the third dose of an HPV vaccine series or at 42-day follow-up in adolescents. HPV16 and HPV18 titers were not different between exercise and control groups at the time of the third dose and increased similarly at 6 weeks' follow-up. Males showed higher HPV18 titers than females in response to the third dose, but there was no sex difference in HPV16 titers.

These results are unsurprising given the strong immunogenicity of the HPV vaccination and the healthy, young study population. Studies suggest acute exercise benefits immune system responses in vaccinations with weaker responses [12,16]. For example, pneumococcal antibody levels were higher in healthy young adults who exercised prior to half-dose vaccination compared to controls receiving the half-dose; whereas the effect was not seen in the exercise group receiving a full dose vaccination compared to the full-dose control group [12]. In this study, geometric means for antibodies at the third dosage timepoint were above 5000 for both groups and strains, whereas for girls 9–13 years old, Donken et al. report geometric means of 7640 and 1207 (HPV16 and HPV18, respectively) at 7mo [17]. Thus, as antibody levels are already high following the second dose, there appears little potential for exercise to have an adjuvant effect, and hence the lack of additional response with exercise is not unexpected. Similarly, given the population in this study were young, healthy adolescents, whose immune response to an alternative two-dose HPV vaccination schedule is comparable with that following three doses in young adult women [17], these results are also unsurprising. Interestingly, a recent study suggests adjuvant effects of other behavioral measures may be limited when seroprotection has already been met. Ayling et al. showed positive mood on the day of vaccination predicted antibody levels for an influenza strain for which the seroprotection threshold was not already reached but no such effect for two strains for which the older adults already had high seroprotection [18].

While we found a difference between sexes in HPV18 but not HPV16 antibody titers, these results must be interpreted with caution given the small sample size. Block et al. also reported sex-variations in response to HPV vaccinations, with HPV16 and HPV18 titers 1.2- to 1.3-fold higher in boys than girls [1][19]. Sex differences are often found in immune responses but inconsistencies in the direction of the difference among vaccine responses are commonplace. Further, some studies of exercise effects on vaccine immunogenicity have found sex differences [9,16] while others have not [10,11].

## 5. Conclusions

Future research should measure potential acute exercise benefits on

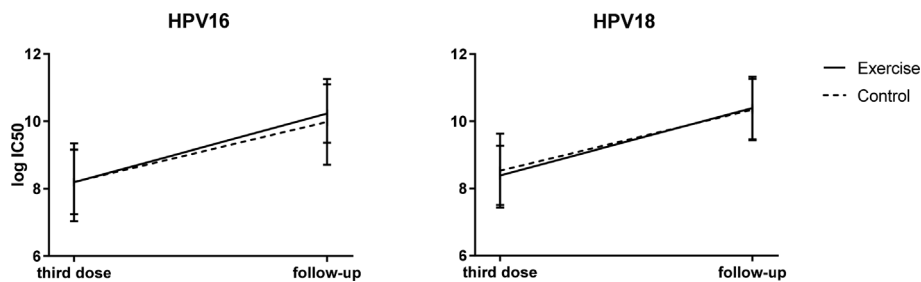


Fig. 1. Serum HPV16 and HPV18 antibody titer responses following HPV vaccination in adolescents.

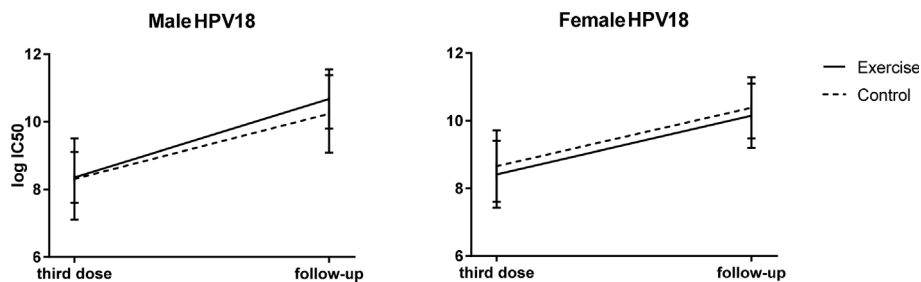


Fig. 2. The sex  $\times$  time interaction on serum HPV18 antibody titer response.

the immune response at the first and subsequent doses of serial vaccinations to better understand the response. Although our results did not indicate that acute exercise improves the immune response to the HPV vaccine series in adolescents on a population level, it is possible that it may do so for individuals (or antigens) who show weaker responses. Beyond the potential immunological benefits from including acute exercise as part of a vaccination program, exercise may offer benefits such as reduced pain. Two recent studies in adolescents demonstrated that girls who performed 15 min of upper body exercise prior to HPV vaccination experienced less tenderness post injection with trends also for less days of feeling ill or having reduced appetite [20] and less pain during vaccination [13]. Thus, acute exercise may still merit consideration in the vaccination protocol for adolescents despite the lack of added immunogenicity demonstrated in this study.

#### Conflicts of interest

The authors have no conflicts of interest to declare.

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#### Declaration of interests

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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