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Acute exercise enhancement of pneumococcal vaccination response: A randomised controlled trial of weaker and stronger immune response

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Abstract

Acute exercise at the time of vaccination can enhance subsequent immune responses. However, the potential benefit of this effect will be its efficacy in boosting poor responses, and thus protection in at-risk populations. The current study tested the effect of exercise on the response to either a full- or half-dose Pneumococcal (Pn) vaccination to elicit stronger and weaker responses. Subjects were 133 young healthy adults, randomized to one of four groups: Exercise or control task, receiving a full- or half-dose Pn vaccination. Prior to vaccination, exercise groups completed a 15min arm and shoulder exercise task, control groups rested quietly. Antibody levels to 11 Pn strains were evaluated at baseline and 1- month. Across all participants, exercise groups showed significantly greater increase in antibody levels than control groups. When doses were compared, it emerged that those who exercised had significantly larger responses than those who rested in the half-dose group, but in the full-dose groups responses were similar. This data indicates the effectiveness of exercise as a vaccine adjuvant, particularly in weaker responses. Thus, given the potential public health benefits of no-cost behavioural intervention to enhance response to vaccination, testing in at-risk populations should be pursued.

Keywords

pneumococcal vaccination; acute exercise; antibody; behavioural adjuvant

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INTRODUCTION

The public health importance of vaccination success in reducing morbidity and mortality due to infectious disease is difficult to overestimate. Development of each vaccine is a huge undertaking and finding ways to gain the most benefit from available formulations is of great importance. Several populations suffer from immunocompromised conditions be it via disease or the accompaniment to normal aging; reduction in immune function, not only raises risk of infectious disease, but also risk of vaccine failure [1-3].

Enhancing responses to vaccines is achieved through adjuvants. The traditional exogenous adjuvant of material added to the vaccine formulation remains important, but development is beset by problems of associated side-effects. Recently, novel techniques which enhance the response have been suggested, including electroporation delivery of DNA vaccines [4], and illumination of vaccine site with a laser [5]. However, the development and safety of these techniques remain to be established. A novel alternative is a very short (15-30 minute) bout of exercise. This low side-effect, low-cost behavioural adjuvant uses a brief bout of acute exercise immediately prior to vaccination to enhance the immune response [6]. Evidence from several studies in young healthy adults indicates that acute exercise can enhance both the antibody and cell-mediated responses to vaccine antigens [7-9]. It appears that exercise-induced enhancement is most apparent in strains with weaker control responses, a finding that indicates a possible ceiling effect with stronger responses showing no further enhancement [10]. However, this has yet to be directly tested, thus, the current study manipulated response strength through full or half dose vaccine administration, and the effects of exercise compared. We hypothesised that the effect of exercise would be greater in the reduced dose group.

Vaccine adjuvants usually improve the vaccine response by stimulating the innate immune system, which provides for the rapid first line of defence against infection. Among the many effects of the innate response are a rapid burst of inflammatory cytokines and mobilization and activation of antigen-presenting cells, which prepare the immune system for subsequent development of specific adaptive immune responses to the vaccine [11, 12]. These characteristics of the activation of the innate immune system bear great similarity to the response elicited by acute exercise and can be assessed in the circulation. In particular, the cytokines interleukin-6 (IL-6) and granulocyte-macrophage colony stimulation factor (GM-CSF) have been identified for key roles in the immune response to vaccination. GM-CSF has potent dendritic cell stimulation activity and has been shown to be effective in enhancing vaccine antibody responses when local expression at site of antigen interaction is achieved through co-delivery [13, 14]. IL-6 is known for its key role in the inflammatory response to stress and acute exercise, but has also been shown to play a role in vaccine responses. For example, IL-6 levels have previously been shown to be higher in those classified as 'high antibody responders' to a live virus strain of *Francisella tularensis* [15], and circulating IL-6 levels have been found to be predictive of enhanced vaccine responses after acute exercise or psychological stress [9]. Other aspects of exercise-induced inflammation can be indirectly quantified by indices of oedema and muscle pain [16], which have also been previously associated with immune responses after eccentric exercise [8]. The current study included markers of the inflammatory response to exercise to assess the association with magnitude of antibody to vaccination.

Pneumococcal disease remains a major cause of mortality worldwide, even with several licensed vaccines available. The polysaccharide pneumococcal vaccine (PPV) Pneumovax 23 is recommended for adults and children > 2 years, conferring immunity against 23 bacterial strains. PPV is estimated to have a protective efficacy of only 50-70%, a number which is further reduced in immunocompromised groups [17-20]. The limitations of

responses to PPV indicate it might benefit from exercise-induced enhancement and was chosen for use in the current study.

MATERIALS & METHODS

Participants

One hundred and thirty-three healthy young adults (75 women) were recruited, of which 129 (72 women) completed the study (see CONSORT flow diagram). Exclusion criteria included regular smoking (reported current smoking >1 occasion / month), a history of immune or cardiovascular disease, current acute infection or illness, current psychiatric treatment or diagnosis or history of psychosis, pregnancy, current use of medication (except birth control), and a history of vaccine-related allergies or side effects. None of the participants had previously received the pneumococcal polysaccharide vaccine in adulthood, however it is expected that the majority of participants will have received the conjugate pneumococcal vaccine during childhood. In addition, none of the participants had performed any regular resistance training in the past six months. All participants were instructed to abstain from vigorous exercise for at least 24 h, alcohol for at least 12 h, and food or caffeine for at least 3 h prior to each session. All participants provided written informed consent, the project was approved by the University of California, San Diego (UCSD) Human Subjects Committee. All participants were paid US\$100 for completion of the study.

Procedure

After consent, participants were randomised to receive either a full-dose (0.5ml) or reduced-dose (0.25ml) PPV and to perform either an exercise or control task, by computerised random allocation with equal numbers for each condition (by sex). Final group sizes were: full dose + exercise (N=30), full dose + control (N=35), half-dose + exercise (N=35) and half-dose + control (N=33). See Figure 1 for participant flow. Laboratory personnel running the assays were blind to condition. Groups did not differ on stressful life events exposure, perceived stress and health behaviours (data not presented here); factors which have been associated with variation in vaccination response [21].

In the first session, participants had their height and weight measured and, following a 20 min rest period, baseline blood samples were taken from an antecubital vein in the dominant arm for baseline antibody and cytokine determination. Next, they completed the exercise task or remained quietly resting for 20 min (control group). Immediately after task completion, a nurse administered the PPV, either full (0.5ml) or half (0.25ml) dose dependent on group (Lot# 12464, Pneumovax 23, Merck, West Point, PA, USA) via intramuscular injection into the deltoid muscle of the non-dominant arm using a 1 inch needle. Participants rested for 30 min after which a final blood sample was drawn for assessment of cytokine response to vaccination and task. A follow-up visit at 28 days post-vaccination included a blood sample for antibody determination.

Exercise Task

The exercise task used elastic resistance bands, and was performed in sets of 30 seconds of exercise, followed by 30 seconds rest. Participants performed three exercise movements sequentially; lateral raise, upright row and chest press. Each movement was performed with instruction and encouragement to perform “as many as you can”. Participants alternated movements and performed each 5 times, completing 15 minutes of exercise. Resistance band strength was adjusted to remain challenging while still possible to maintain exercise for 30 seconds.

Heart rate, perceived exertion, pain and limb circumference

To monitor exercise intensity, heart rate and perceived exertion were recorded during the exercise task. Heart rate was recorded after each exercise set using Polar FS2c Heart Rate Monitors (Polar Electro, Lake Success, NY, USA), and participants provided a rating of perceived exertion immediately after each of the 15 exercise sets, using Borg's 15-point scale [22]. Muscle soreness was assessed using a visual analogue scale with anchors of 0 (no pain) and 100 (worst possible pain) to indicate pain in the arms [23]. Participants rated their pain twice immediately after task completion; once with arms resting by their side and the other while they replicated the movements of the exercise task without resistance bands. Change in upper and forearm limb circumferences from pre-task to immediately post-task were used to gauge the amount of swelling in the exercised limbs. Marked sites were used and measurements were taken with the arm relaxed by the participants' side on the non-dominant arm at point of flexed maximal circumference of the bicep (upper arm) and 5 cm below the elbow crease (forearm).

Blood sampling

Blood was collected in a 10 ml vacutainer containing potassium ethylene diaminetetraacetic acid (K3EDTA) at baseline and 30 min post-vaccination. Tubes were stored on ice until centrifugation (3000 rpm, 10 min, 7 °C) and plasma was stored at -80°C for later assessment of interleukin-6 (IL-6) and granulocyte macrophage colony-stimulating factor (GM-CSF). Blood was collected into a plain vacutainer at baseline, and 28 day follow-up, allowed to clot and serum was stored at -20°C for later antibody determination.

Assays

Pneumovax 23 contains 23 pneumococcal (Pn) serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F) of these, Luminex technology was used to assess twelve Pn IgG antibody serotypes (types 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). Assessment and selection of these specific Pn serotypes were based on clinical observations linking these common serotypes to invasive disease in Europe [24, 25]. Full details of this assay have been reported elsewhere [26, 27]. *Streptococcus pneumoniae* (pneumococcal; Pn) capsular polysaccharides were obtained from the American Type Culture Collection (ATCC; Virginia, USA). In accordance with the WHO protocol for detection of Pn antibody, Pn 22F was used as an absorbant to remove cross-reactive antibodies and not as an analyte in the assay [28]. Polysaccharides were reconstituted in sterile water at 5 mg/ml and were conjugated to poly-L-lysine (PLL; Sigma Aldrich, Dorset, UK) using cyanuric chloride and were purified using G25 PD-10 Sephadex desalting columns (GE Healthcare, Chalfont St Giles, UK). A two-step carbodiimide reaction was used to conjugate antigens to carboxylated microspheres (BioRad, Hertfordshire, UK) specific for different bead regions on a Luminex instrument (except Pn 22F). Beads were activated using 5 mg/ml EDC and 5 mg/ml sulphy-NHS (both Sigma) and the conjugation reaction allowed to proceed for 3 hours at room temperature. After washing with PBS, beads were resuspended in PBS 0.1% BSA, 0.05% sodium azide and the concentration of beads labelled with individual antigens was determined using a hemocytometer. Labelled beads were stored at 4°C. Of the 12 antigens, was added to each well of a 96 well filter plate (Millipore, Watford, UK).

Beads were washed with PBS 0.05% Tween 20 and washes removed using a vacuum pump. Serum samples were diluted 1:4000 in PBS 1% BSA, 0.05% Tween 20 containing 5 µg/ml pneumococcal cell wall polysaccharide (CWPS; Statens Serum Institute, Copenhagen, Denmark) and 5 µg/ml PnPS 22F to absorb non-specific antibodies. Beads were resuspended in 25 µl diluted test serum and incubated for 1 hour in the dark at room temperature at 500 rpm on an orbital shaker. Beads were then washed and incubated with

1:200 mouse anti-human IgG phycoerythrin (PE)-conjugate (Southern Biotech, Cambridge, UK) for a further 30 minutes prior to washing, resuspending in 125 μ l PBS 0.05% Tween 20 and acquisition of data using a Luminex-100 instrument (Bio-plex Systems, BioRad Laboratories, California, USA) using low 'RP1' (low PMT – photomultiplier tube) target setting. Acquisition software (BioPlex Software Manager (version 4), BioRads, Labs, CA, USA) was used to generate serotype antibody concentrations from a 5 parameter logistic curve fit. Serum Pn IgG levels are reported in μ g/ml.

Plasma IL-6 and GM-CSF were measured using high-sensitivity ELISA (Quantikine HS Human IL-6 and GM-CSF ELISAs, R&D Systems, Minneapolis, MN, USA) according to manufacturer's instructions. The reported sensitivity of the assay was less than 0.039 pg/ml for IL-6 and less than 0.26pg/ml for GM-CSF, with recorded intra-assay and inter-assay variation both <10%.

Statistical analysis

Initial examination of the strain specific antibody levels revealed that one strain showed significant baseline differences between control and exercise groups and was thus removed from the analyses, given the importance of baseline levels on antibody response. Outlier data points were identified and winsorised at the 95th percentile [29] and antibody concentrations log10 transformed due to skewed distribution. In order to control for Type I error inflation associated with multiple outcomes, we calculated averaged antibody levels (AvAB) in line with previous Pn vaccination research [30]. Initially AvAB was used as the single outcome in a repeated measures ANOVA, first testing 2 Time (baseline, 28 days) X 2 Group (exercise, control), across vaccine doses, followed by separate analyses for each dose. Significant results were followed by analysis of individual strains. Associations between predictor variables and antibody concentration were assessed using a series of hierarchical linear regressions, including demographic variables. A 5% significance level was adopted throughout. Occasional missing data and statistical outlier removal are reflected in the reported degrees of freedom.

RESULTS

Demographics

Participants had a mean (SD) age of 22 (2.7) years, and BMI of 23.1 (3.8) kg/m². Seventy-five were women and 58 were men; 51.6% identified as Asian, 2.4% as Black or African American, 36.5% as Caucasian, 4% as Pacific Islander or Native Hawaiian, and 5.6% as two or more races. Groups did not differ in age ($p=.3$), BMI ($p=.9$) or ethnicity ($p=.3$).

Antibody levels

Figure 2 shows geometric mean AvAB responses from baseline to 28 days according to group. Baseline levels of AvAB were not different among the four groups ($p=.959$). A 2 Time (baseline, 28 days) by 2 Group (exercise, control) repeated measures ANOVA revealed a significant Time by Group interaction ($F(1,117)=7.70$, $p=.006$, $\eta^2=.062$) such that the exercise groups showed greater AvAB responses than control groups. Next, effects within individual strains were examined and significant effects for greater responses in the exercise groups were seen for 4 strains; Pn1 ($p=.034$), Pn3 ($p=.006$), Pn4 ($p=.050$), Pn9V ($p=.008$), and trends toward significant effects in Pn19A ($p=.062$) and Pn23F ($p=.076$).

Analyses were then repeated separately for each dose of vaccine. A significant Group by Time effect was found in the half dose conditions ($F(1,54)=7.54$, $p=.008$, $\eta^2=.123$), confirming the effect that those participants who exercised exhibited significantly greater responses than those who rested. Importantly there was no significant main effect for group

among those who received the full dose vaccine ($p=.189$). In the half dose conditions, significantly greater responses were found for exercise group in 5 individual strains; Pn1 ($p=.026$), Pn3 ($p=.029$), Pn4 ($p=.039$), Pn5 ($p=.047$) and Pn9V ($p=.031$), and a trend toward significant effects in Pn18C ($p=.077$). Table 2 shows the geometric mean antibody concentrations for each strain in each group. To attempt to illustrate potential clinical benefits of the boosted responses, Figure 3 represents additional protection (according to WHO recommendation that $0.35\mu\text{g/ml}$ is the minimum protective antibody concentrations for pneumococcal vaccinations) elicited by vaccination in each group alongside total strains showing protective levels at 28 days.

Exercise Task Responses

Plasma cytokines—Table 2 presents cytokine concentrations. A 2 Time (Baseline, 30 min post-vaccination) by 4 Group repeated measures ANOVA was used to analyze cytokine responses. IL-6 showed a significant Group by Time interaction ($F(3,113)=2.81$, $p=.043$, $\eta^2=.069$). Post-hoc tests found that, as expected, the full dose exercise group showed greater increases in IL-6 than the full dose control and half dose control group. GM-CSF did not show any significant Time effect ($p<.5$) or Group by Time interaction ($p<.5$).

Heart rate and perceived exertion—Heart rate and Borg score of perceived exertion increased though the exercise task as expected. Significant effects of Time were seen for heart rate ($F(14,43)=32.9$, $p<.001$, $\eta^2=.915$) and for perceived exertion ($F(4,47)=22.1$, $p<.001$, $\eta^2=.8668$). During the final set of 3 exercises heart rate averaged 145.2 bpm ($SD=18.4$), and perceived exertion was rated as an average of 15.9 ($SD=2.2$) where 15 = 'hard'. There were no differences among full or half dose groups in heart rate or perceived exertion during the final set of exercises (heart rate during final 3 sets, $p=.6$; perceived exertion during final 3 sets $p=.3$).

Arm circumference and Pain—A significant effect of group (Exercise vs. Control) was found for change in bicep ($p<.001$) and forearm ($p=.001$) circumference. Increases in arm circumference were found in the exercise groups; bicep circumference increased by 0.88 cm ($SD=0.68$), while forearm circumference increased by 0.32 cm ($SD=0.61$) but not control groups. No difference was found between full and half dose groups (bicep circumference change, $p=.7$; forearm circumference change $p=.5$), but men showed significantly greater increase in bicep circumference than women ($p=.008$). When describing the pain in their arms at rest and performing the movements of the exercise without resistance, exercise participants rated their pain significantly higher than control participants ($p<.001$) (exercise groups 27.2 ($SD=18.0$) at rest and 32.7 ($SD=23.2$) during movement, control groups 3.5 ($SD=5.0$) at rest and 4.4 ($SD=5.5$) during movement). There were no differences among full or half dose groups (bicep circumference change, $p=.9$; forearm circumference change $p=.8$).

Predictors of Antibody Response

To explore the potential mechanisms underlying the acute exercise induced enhancement of vaccine response, a series of hierarchical linear regressions were performed. The association between circulating cytokine concentrations and subsequent antibody response was assessed using AvAB at 28 days as the outcome variable and AvAB level at baseline was entered at step 1. Step 2 then included the potential predictor variable, IL-6 or GM-CSF at 30 min post-task and vaccination. Neither IL-6 nor GM-CSF were significant predictors of AvAB response, but GM-CSF levels showed a trend towards association ($B=.187$, $\beta=.127$, $t=1.81$, $p=.074$, $\Delta R^2=.016$). Examination of individual strains found responses to Pn3 ($p=.006$) and Pn9V ($p=.027$) significantly associated with GM-CSF levels and Pn4 ($p=.071$) and Pn19A ($p=.077$) showed a trend towards association. The final potential predictor was change in bicep circumference, which was expressed as a percentage increase to adjust for muscle size.

This analysis was performed only within the exercise group, given that no change would be expected for the control group. A trend towards association was again seen ($B=-.037$, $\beta=-.181$, $t=-1.89$, $p=.065$, $\Delta R^2=.033$), within individual strains this association was reflected in significant prediction of Pn9V ($p=.009$) and Pn19A ($p=.042$).

DISCUSSION

This study examined the effects of acute exercise at the time of vaccine administration on the subsequent antibody response to pneumococcal polysaccharide vaccine (PPV), investigating the effects on a stronger (full-dose) and weaker (half-dose) response. A simple, safe, cost-effective behavioural adjuvant to vaccination such as brief exercise is an attractive prospect, especially in populations who suffer high rates of vaccine failure through weak responses. We found that a simple, 15 minute exercise task using elastic bands, with alternating 30 seconds of exercise and 30 seconds of rest, enhanced the antibody responses to PPV in young healthy adults. Overall, exercise enhanced responses over the entire range of responses, but as hypothesised, enhancement effects of exercise were significant in the group who received a half-dose vaccine, while in those who received a full-dose vaccine, both groups showed similarly strong responses. These findings are consistent with recent work; in two separate but similar studies in young healthy adults, the response to a full dose influenza vaccine was not different between control and exercise groups, but a half dose vaccine (which elicited smaller responses) was enhanced in exercise groups compared to control [31, 32].

The design of the current study to include groups receiving a full or half dose aimed to elicit weaker and stronger responses. Administering a reduced dose vaccine has been previously explored with a view to potential dose sparing, in both younger and older adults [33, 34]. In the setting of young healthy adults, responses to a reduced dose are still expected to be robust, and provide protection from disease. Given that participants would not otherwise receive the vaccine it is likely that additional protection would still provide clinical benefit even though dose was reduced. Direct comparison of responses in the resting control groups between full and half dose recipients only revealed small differences (significant in only 1 strain, although all strains showed smaller responses in the half dose group). These findings are not entirely unexpected given previous work aimed at examining dose-sparing procedures find similarly little to no inferiority in response in healthy adults receiving half-dose vaccines [33, 35, 36], and further suggests the importance of examining the effects of acute exercise as an adjuvant in populations with known sub-optimal responses. The finding that exercise effects were only significant in the half-dose group when control responses were similar is likely due to greater individual variation in the generally less robust response. This setting has been identified as allowing the opportunity for small to medium sized effects to emerge [21], and such specificity has been reported in the context of chronic stress and vaccination response [37, 38].

The mechanisms through which exercise enhances immune responses are not well defined, but are hypothesised to be multi-factorial [6]. Documented effects of acute exercise that might contribute to an enhanced immune response include: leukocytosis and increased trafficking of immune cells to site of damage (e.g., vaccine administration) [39], increased cytokine and chemokine production, increased vascular permeability and blood flow, and acutely increased lymph drainage [40, 41]. Determining the most salient of these changes for vaccine response enhancement requires a detailed examination outside of the scope of the current study. The current design measured limb volume changes and circulating levels of the cytokines IL-6 and GM-CSF. Limb circumference increase after exercise likely reflects a combination of increased blood flow to the muscle, shortening of the myofibres and oedema [42], this index has previously been associated with the cell-mediated response to influenza

vaccination in a study of acute exercise-induced immunoenhancement [8]. IL-6 and GM-CSF are cytokines both implicated in responses to vaccination [9, 11, 13, 15] and are increased after acute exercise. Although there are limitations associated with measurement of systemic cytokine levels, when local concentrations would be preferable, the invasive nature of techniques that would be required prevented their acquisition in this study. Regression analyses indicated that in the current data, both GM-CSF levels after task and vaccination, and change in bicep circumference with exercise were predictive of antibody responses. These exploratory analyses add to evidence that several aspects of the response to acute exercise, including the cytokine milieu, deserve further investigation when considering the most effective exercise task for adjuvanting vaccines.

A successful vaccine is a product that will elicit strong and long lived responses and thus provide the greatest possible protection against disease. The path toward this aim is littered with obstacles including strain variety and mutation capability, potential adverse reactions and reproducibility of production. Yet, there are a large number of vaccines licensed which have been recently estimated to save more than 2.5 million deaths per year, unfortunately, this success is mired by the parallel estimate that 1.5 million people die annually from infectious disease for which there are currently available, but not universally delivered vaccines [43]. Added to the problem of universal availability, affordability and delivery is the problem that vaccine efficacy is variable and often reduced in at-risk populations. Acute exercise as an adjuvant offers an avenue which potentially could improve the protective efficacy of vaccination programs in at-risk, immunocompromised populations, or even offer a route for dose sparing or reduction in booster necessity. Critically, this behavioural approach avoids costly clinical trials, is itself cheap and easy to administer, is well understood and accepted by the public, and incurs no side-effects apart from mild muscle soreness. Thus, it appears timely to examine the possibility for use of acute exercise as an adjuvant in at-risk populations.

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Highlights

Acute exercise enhancement of pneumococcal vaccination response: A randomised controlled trial model of weaker and stronger immune response.

- A 15min exercise task immediately prior to vaccination enhanced antibody responses
- Half dose vs. Full dose design modelled weaker immune responses
- Half dose responses were greater after exercise than after resting
- Full dose responses were similar in exercise and resting groups

Figure 1.
CONSORT participant flow diagram

Figure 2.
Geometric mean (95%CI) averaged antibody titres in exercise and control groups who received a full or half dose vaccine.

Figure 3. Strains with ‘protective’ antibody levels at 28 days post-vaccination, additionally induced by vaccination, and total.

Demographic statistics, mean (SD). Psychological stress over previous month was measured by the 14-item Perceived Stress Scale [44], sleep habits were assessed using the Pittsburgh Sleep Quality Index [45] and habitual exercise using the Leisure Time Exercise Questionnaire [46].

Table 1

	All participants	Full dose + Exercise	Full dose + Control	Half dose + Exercise	Half dose + Control
Sex	75 F, 58 M	16 F, 14 M	20 F, 15 M	20 F, 15 M	19 F, 14 M
Age (years)	22.1 (2.7)	21.9 (2.4)	22.3 (3.0)	21.5 (2.6)	22.6 (3.1)
BMI (kg/m ²)	23.1 (3.8)	23.1 (3.8)	23.1 (4.7)	23.1 (3.8)	23.2 (2.9)
<18.5	4.5%	6.7%	8.6%	2.9%	0.0%
18.5-24.9	73.7%	70.0%	68.6%	71.4%	84.8%
25-29.9	16.5%	20.0%	14.3%	20.0%	12.1%
>30	5.3%	3.3%	8.6%	5.7%	3.0%
Perceived stress (PSS score)	13.6 (5.8)	14.1 (5.8)	13.0 (5.2)	14.0 (4.1)	13.3 (7.8)
Sleep (PSQI global score)	4.1 (2.2)	3.4 (2.2)	4.2 (2.0)	4.6 (2.1)	4.1 (2.4)
Exercise (occasions per week of moderate to vigorous exercise)	1.8 (0.7)	1.7 (0.6)	1.9 (0.8)	1.8 (0.7)	1.8 (0.8)

Table 2

IL-6 and GM-CSF concentrations, mean (SD), at baseline and 30 minutes after task completion and vaccination.

	Full dose + Exercise	Full dose + Control	Half dose + Exercise	Half dose + Control
IL-6 baseline (pg/ml)	0.67 (0.4)	0.68 (0.4)	0.71 (0.6)	0.59 (0.4)
IL-6 +30 min (pg/ml)	0.88 (0.5)	0.69 (0.5)	0.90 (0.7)	0.62 (0.5)
GM-CSF baseline (pg/ml)	0.58 (0.1)	0.67 (0.3)	0.68 (0.3)	0.64 (0.4)
GM-CSF +30 min (pg/ml)	0.72 (0.4)	0.55 (0.2)	0.66 (0.3)	0.64 (0.3)

Pn strain geometric mean antibody concentration (95%CI). *All indicates significant difference between Exercise and Control groups over both full and half doses, *Half indicates significant difference between Exercise and Control groups receiving half dose vaccine.

Table 3

Strain	Full:Exercise		Full:Control		Half:Exercise		Half:Control	
	Baseline	28Days	Baseline	28Days	Baseline	28Days	Baseline	28Days
1	0.81 (0.16-4.09)	20.88 (3.29-132.71)	0.95 (0.19-4.71)	17.52 (2.52-121.95)	0.94 (0.20-4.40)	18.44 (2.39-142.12)	0.92 (0.17-5.06)	7.87 (1.54-40.19) *All *Half
3	0.34 (0.07-1.65)	2.53 (0.23-27.72)	0.23 (0.05-0.99)	0.90 (0.14-5.88)	0.23 (0.04-1.19)	1.87 (0.23-15.27)	0.31 (0.09-1.06)	0.91 (0.15-5.40) *All *Half
4	0.15 (0.06-.39)	0.69 (0.19-2.54)	0.20 (0.08-0.52)	0.81 (0.28-2.37)	0.15 (0.05-0.43)	0.77 (0.25-2.37)	0.21 (0.07-0.58)	0.61 (0.21-1.83) *All *Half
5	0.53 (0.14-1.93)	9.99 (2.03-49.03)	0.27 (0.07-0.99)	4.91 (0.80-30.26)	0.45 (0.07-3.04)	10.77 (1.20-96.38)	0.40 (0.08-1.97)	4.15 (0.61-28.30) *Half
6B	0.23 (0.03-1.64)	2.31 (0.18-29.03)	0.16 (0.03-0.97)	1.25 (0.19-8.24)	0.18 (0.03-0.94)	2.21 (0.21-22.91)	0.14 (0.02-1.13)	1.11 (0.12-10.71)
7F	0.49 (0.18-1.38)	3.08 (0.81-11.67)	0.48 (0.17-1.35)	2.97 (0.76-11.57)	0.52 (0.15-1.75)	3.28 (0.75-14.31)	0.43 (0.12-1.57)	2.76 (0.90-8.47)
9V	0.11 (0.03-0.43)	1.69 (0.18-15.65)	0.13 (0.03-0.54)	1.03 (0.13-8.21)	0.09 (0.02-0.57)	1.35 (0.16-11.21)	0.11 (0.02-0.67)	0.65 (0.11-3.73) *All *Half
14	0.24 (0.04-1.50)	2.67 (0.33-21.53)	0.18 (0.02-1.34)	2.05 (0.28-14.88)	0.15 (0.02-1.42)	1.56 (0.16-14.96)	0.13 (0.03-0.64)	1.73 (0.21-14.21)
18C	0.29 (0.04-2.08)	5.01 (0.55-45.38)	0.36 (0.05-2.51)	6.84 (0.70-67.31)	0.20 (0.03-1.11)	6.34 (1.07-37.56)	0.62 (0.10-3.98)	9.05 (1.08-75.63)
19A	0.80 (0.19-3.34)	6.45 (2.10-19.83)	0.96 (0.23-4.06)	4.85 (1.07-22.03)	0.60 (0.19-1.61)	3.86 (1.12-13.30)	0.74 (0.21-2.58)	3.66 (1.03-12.97)
23F	0.10 (0.01-0.76)	1.54 (0.13-18.56)	0.15 (0.03-0.89)	1.14 (0.13-9.85)	0.16 (0.02-1.10)	1.09 (0.11-10.55)	0.15 (0.03-0.86)	0.99 (0.11-9.26)