# Immune Response in Highly Active Young Men to the 2014/15 Seasonal Influenza Vaccine

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<tr>
<th>Journal:</th>
<th>Applied Physiology, Nutrition, and Metabolism</th>
</tr>
</thead>
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<tr>
<td>Manuscript ID</td>
<td>apnm-2017-0683.R2</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>04-Feb-2018</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Stewart, Andrew; University of Calgary Vanderkooi, Otto; University of Calgary Reimer, Raylene; University of Calgary Doyle-Baker, Patricia; University of Calgary</td>
</tr>
<tr>
<td>Keyword:</td>
<td>Influenza, Vaccination, Young Men, Adiposity, physical activity &lt; exercise</td>
</tr>
<tr>
<td>Is the invited manuscript for consideration in a Special Issue?:</td>
<td>N/A</td>
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Immune Response in Highly Active Young Men to the 2014/15 Seasonal Influenza Vaccine

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Abstract

During the 2009 H1N1 pandemic, individuals with obesity were disproportionately affected by H1N1 with increased levels of mortality and morbidity. This led to questions regarding the potential impact of lifestyle on the effectiveness of immunization. Currently, the research is limited on influenza vaccination and the associated changes in immune response with body composition and physical activity. The purpose of this pilot study was to investigate the potential role of adiposity and physical activity in the immune response elicited by the 2014/15 seasonal trivalent influenza vaccine (TIV). A prospective cohort study examining the 2014/15 seasonal TIV was conducted by collecting baseline and 4-week post vaccination fasting blood samples from 45 male Albertans between the ages of 18 and 35 years of age. Percent body fat (%BF) was assessed through Dual X-Ray Absorptiometry imagining and physical activity through self-reported survey scores. While no differences in median %BF were associated with seroconversion rates in participants, the median physical activity score was higher among those that did not seroconvert to the vaccine. Significant differences were found for the A/Texas strain (p<0.01) and a similar trend of lower magnitude observed for the remaining two influenza strains. These results suggest that higher physical activity levels may influence immune response to vaccination and that assessing factors beyond those commonly used can be of value when identifying vaccine response in the population.

Keywords: Influenza, Vaccination, Young Men, Adiposity, Physical Activity
1. Introduction

Canadian hospitalization and mortality rates associated with influenza indicate that the demographic composition of those most impacted by the virus can change over time (Alberta Health, 2015). For example, during the 2012/13 and 2013/14 seasons the rates reported for the young and elderly were observed to be much higher than any other age group. In comparison, during the 2009 H1N1 pandemic, differences in this pattern were observed with higher than usual proportions of young and middle aged adults being impacted (Alberta Health, 2014). Differences in these rates have also been found to be associated with other factors such as adiposity (Louie et al., 2011, Van Kerkhove et al., 2011), and lifestyle. For example, the volume of physical activity has been found to influence both risk of upper respiratory tract infections as well as antibody production to influenza vaccine (Kohut et al., 2002; Nieman, 1994).

After conducting a survey of published literature and noting that there was an absence of 'gold-standard' (RCT) data, we looked for an evidence base and this consisted mainly of observational studies comparing mortality in self-selected groups of diseases (Loubet et al., 2016). We did find that the currently available evidence highlighted adiposity and lifestyle as emerging factors in the field of immune response and influenza vaccination (Middleman et al., 2010; Milner & Beck, 2012; Sheridan et al., 2012, Talbot et al., 2012). To add to this discussion, we sought to determine the immune response of young men (aged 18-35) to the 2014/15 seasonal influenza vaccine. To further add to the literature our goals were to investigate if a trend could be found between adiposity and immune response to vaccine in this population. Finally, we wanted to investigate if a trend existed between physical activity level and immune response to vaccine. We hypothesized that seroconversion and seroprotection would be observed for most participants
but increases in adiposity and low levels of physical activity would reduce the effectiveness of vaccination.

2. Methods

2.1 Study Subjects:

The inclusion criteria for the AIM (Adiposity, Influenza in Men) study were sex (male), and age (18-35 yr.). Exclusion criteria included a body mass index (BMI) less than 18.5 as well as any contraindications for vaccination recommended by the National Center for Immunization and Respiratory Diseases (2015). The Print and online advertising was conducted in and around Calgary, Alberta to recruit participants with access to the testing facilities at the University of Calgary. To facilitate engagement of this age group, targeted advertisements were placed on popular social media platforms which included Facebook.com as well as Reddit.com. A pre-screening survey was conducted online to determine eligibility. Recruitment began on October 22nd and ended on December 22nd 2014. A total of 131 individuals responded via the online survey. Of these 131 (age range 18-35 yrs.; median = 22), 125 were contacted prior to the December 22nd deadline (the point at which vaccine was no longer available in clinics). Seventy-six participants attended the baseline session and 45 attended the post vaccination blood draw (Figure 1).

2.2 Study Design:

This study was conducted as a prospective cohort with rolling recruitment of participants. Data collection occurred at two time points, one baseline session prior to vaccination and four weeks post vaccination. At the baseline session, demographic information was collected through self-
report surveys, a DXA Scan, and an 8-12 hour fasting blood draw. Participants were asked to attend either an Alberta Health Services vaccination clinic or visit the University of Calgary's Student Wellness centre to receive the 2014/15 trivalent influenza vaccine. The vaccine contained A/California/7/2009 (H1N1) pdm09-like virus, A/Texas/50/2012 (H3N2)-like virus and B/Massachusetts/2/2012-like virus (Yamagata lineage) produced by GlaxoSmithKline under the Fluviral® name. After receiving confirmation from participants that they received the vaccine they were scheduled for a four-week post vaccination blood draw.

2.3 Sample Collection

Whole blood was collected from participants using 3.5ml (13x75mm) BD Vacutainer® Serum Separator Tubes. Upon collection, tubes were inverted five times and allowed to sit at room temperature for 30 minutes to allow clotting to occur. Tubes were then centrifuged at 1300g at a temperature of 5º Celsius. Serum was aliquoted into Eppendorf tubes and kept in storage at a constant temperature of -80º Celsius. Serum samples were packaged in Styrofoam coolers with the sufficient quantity of dry ice to keep samples frozen for 48 hours. These coolers were either personally transported to analysis facilities within Calgary or shipped via expedited courier to ensure that samples remained frozen.

2.4 Influenza Hemagglutination Antibody Inhibition (HAI) Assay

HA Antibody Inhibition assays for each influenza strain were performed in duplicate by the Canadian Centre for Vaccinology (Halifax, NS Canada) following testing protocols put forth by the WHO (World Health Organization (Global), 2011). To quantify the HA antibody titer present in serum dilutions of influenza viral particles were introduced to wells containing erythrocytes
and serum. The antibody titer was determined to be the dilution in which serum antibodies no longer neutralize virus, allowing Turkey erythrocytes to bind together in a lattice by the virus (World Health Organization (Global), 2011).

2.5 DXA Scanning

Body composition was assessed using a Hologic Discovery QDR Series AX (Bedford, Massachusetts) Dual X-ray Absorptiometry scanner following standard manufacturer protocols.

2.6 Physical Activity Questionnaire

The Godin Shepard Leisure Time Physical Activity Questionnaire was used to generate a self-report score for physical activity. This questionnaire asked participants to report the duration and frequency of high, moderate and low physical activity that they participated over an average week. This information was then used to generate a Physical Activity Score using the formula developed by Godin and Shepard (1997).

2.7 Statistical Methods

The primary outcome measure for this study was seroconversion. Seroconversion was defined as an at least four-fold increase in antibody titer pre-to post vaccination. Using this measure, participants either seroconverted or failed to seroconvert for each of the three influenza strains included in the seasonal vaccine. Seroprotection was a secondary outcome measure for this study and was defined as an antibody titre of at least 160. The independent variables were %BF and Physical Activity Score. Single variable comparisons were identified as a more appropriate
measure over multivariate models due to a lower than anticipated return rate (59%). Normality was assessed by plotting continuous variables and visual inspection of these plots. The Shapiro-Wilk's test was used to assess the assumption of equal distributions between groups. The assumption of equal variance between groups was tested using an F test. Two-group Mann-Whitney U tests were completed comparing non-seroconverters and seroconverters across all outcome measures for each of the three vaccine influenza strains. A decision was made not to use an adjusted p-value due to the exploratory nature of these analysis. Statistical analysis was completed using R version 3.2.2 (2015-08-14) "Fire Safety" Copyright (C) 2015 The R Foundation for Statistical Computing. This software was run on an i686-pc-linux-gnu (32-bit) platform.

3. Results and Discussion

3.1 Characteristics of the study subjects

The baseline characteristics of all participants including dropout and return are described in Table 1. The median age of returning participants was 23 with an age range of 18-35 years. The sample was slightly skewed towards the inclusion of younger participants but the entire age range was represented in the final sample. Weight, BMI, Body Fat (%BF) and Physical Activity Score were not significantly different between dropouts and return participants.

3.2 Vaccination History

The majority (66%) of participants self-reported that they had not received an influenza vaccination over the past five years (n = 27). The remaining participants indicated that they had
received between 1 and 6 vaccinations (the sixth vaccination being the 2009 pandemic immunization). A breakdown of participant’s five-year influenza vaccination history is represented in Figure 2.

3.3 Immune Response

The immune responses of participants to the 2014/2015 vaccine was observed to be different for each of the three component strains and is summarized in Figure 3. Pre-Vaccination Antibody levels for both the California and Texas influenza A strains were low with median values of 40 and 20 respectively. The Massachusetts influenza B strain was observed to have a noticeably higher median antibody titer of 160. Post Antibody Titers were highest for the influenza B/Massachusetts strain with a median of 640. This was followed by A/California with a median value of 320. The lowest median antibody titers were observed for A/Texas with a median value of 160. Table 2. shows the percent of the participants that seroconverted which ranged between 57 and 72%. The greatest seroprotection occurred for the A/California and B/Massachusetts strains with observed increases of 32 and 54%, respectively.

3.4 Body Fat Percent

No significant differences were found when comparing seroconversion rates for the three strains to participant %BF. Furthermore, no distinct trend emerged in the values of median %BF which is summarized in Table 3 for seroconverters and non-seroconverters for each vaccine strain.

3.5 Physical Activity
Participants who did not seroconvert reported higher Physical Activity Scores. Significant differences were found for the A/Texas strain (p<0.01) with a mean Physical Activity Score for non-seroconverters of 81 versus 46 in the seroconverting group. A similar trend of lower magnitude observed for A/California and B/Massachusetts (62 vs 53, p=0.25 and 47 vs 64, p=0.92 respectively). These results are summarized in Table 4, which contains the median Physical Activity Scores for seroconverters and non-seroconverters.

3.6 Recruited Sample

The study participants’ %BF range was 9.4-31.5 with a median of 15.9%. Currently the best resource that reports %BF in the North American population is the National Health and Nutrition Examination Survey (NHANES). In American males from a similar age group (20-39) a mean %BF of 26.1 was recorded (Li et al., 2009). Mean %BF in the sample recruited for this study was 17.5. This suggests that despite our interest in recruiting a representative sample of males in this age group, the sample represents a subset of the North American population with lower adiposity.

While participants (n=76) and dropouts (n=31) were not found to be significantly different in BMI (p=0.30), this study experienced a dropout rate of 41%. Due to the availability of vaccine, recruitment for this study occurred over a three-month period beginning at the end of October through to the end of December 2014. This is the only time of the year where Calgarians would have easy access to the influenza vaccine through Alberta Public Health clinics. A reality of this tight timeline is that it resulted in most of the data collection occurring during the lead up to the
holiday (Christmas) season. It is likely that the holiday season and the early morning fasting blood draws (6:00 am-7:30 am) were contributing factors to dropout.

3.7 Interpretation of Results

Most participants reported not having been vaccinated with an influenza vaccine in the past five years (Figure 2). This is in line with data collected by Statistics Canada that showed Canadians aged 18-34 were the least likely to receive influenza vaccine (Gionet, 2015). Comparing the two influenza A strains with the influenza B strain, antibody levels pre-vaccination for the influenza A strains were lower than for influenza B (Figure 3). This could in part be attributed to difference in attributes between influenza A and B strains. Antigenic evolution in influenza B strains occurs at a lower rate than for influenza A (Ferguson et al., 2003). Low antigenic evolution rates result in individuals being exposed to influenza B strains more like previous strains (Ferguson et al., 2003). A second contributor to this trend may be that in the previous American influenza season (2013-2014) influenza A activity peaked earlier in the year than influenza B (CDC, 2015). As HA antibody titers have been observed to decline over time (Hsu et al. 2014), exposure to influenza strains closer to this study’s baseline may in part explain the trends seen. The proportion of study participants post vaccination that were seroprotected was 93% or greater for each of the three influenza strains (Table 3). In the literature, in a sample of 20 males aged 18-62, Xie et al. (2015) reported seroprotection rates of 67% for A/California, 83% for A/Texas and 77% for B/Massachusetts. While these values appear to be lower, it is not by a wide margin (after considering the small sample sizes for each) suggesting that the two cohorts responded similarly to vaccination.
3.8 Immune Response and Body Composition

No trends were found between seroconversion rates and participants’ %BF within this study (Table 3). This result differed from our hypothesis (i.e., that differences in immune response would occur with changes in body composition) and this could have occurred due to several reasons. It may be that for the 2014/15 vaccine no trend occurred, or this trend could not be detected in the age group and sample chosen for this study. Both Talbot et al., (2012) and Sheridan et al., (2012) associated increases in HA antibody one-month post vaccination with increases in adiposity. However, these trends were not observed for all constituents of the vaccine indicating that any association may be variable by specific vaccinated strain.

3.9 Seroconversion and Physical Activity Score

Within the literature, the impact of physical activity on vaccine and antigen response has been studied primarily in older adults. While no formal meta-analysis of this population could be identified at the time of writing, studies conducted in 2002 (Kohut et al.), 2004 (Smith et al.), and 2007 (Yang et al.) have found that moderate physical activity in this group improves the development of immunity. Few studies have been conducted on the link between physical activity and vaccine response in young adults, the most notable being a study published in 2010 which attempted to study the efficacy of an acute eccentric exercise intervention on immune response to influenza vaccination in a group of 156 healthy participants (Campbell et al.) and a study of 60 young healthy adults that investigated the effects of acute exercise prior to influenza vaccination (Edwards et al., 2006). The results of these two studies conflicted with the 2010 paper finding no significance difference in vaccine response between the exercise and control groups and the 2006 paper finding a positive effect for the influenza A/Panama strain. It must
however, be noted that both studies focused on acute exercise interventions and as such, it is difficult to draw parallels to the cohort of individuals included in this work. Physical Activity Scores were high in the group recruited for this study with most participants reporting levels of activity far beyond the interventions used in previous literature. Godin states that a Physical Activity Score of greater than 24 provides “substantial [health] benefits” (Godin, 2011). The median Physical Activity Score of all returning participants was 47 (Table 1) indicating that overall, that they were highly active. The higher median Physical Activity Scores seen in non-seroconverters (Table 4) suggest that they did not have as robust an immune response to influenza vaccination. This result again differs from our hypothesis that increased physical activity would lead to improved response to vaccination. These observations reflect the “j-shape” curve found in studies focusing on exercise and immune response (Nieman, 1994; Heath et al., 1991) with moderate levels of physical activity being beneficial to immune response to vaccine and high levels exerting a negative effect. More recently, work completed by Cooper et al. (2007) was found to agree with Nieman’s observations, suggesting that the differentiation of immune cell populations that are exposed to dysregulated inflammatory response to physical activity may exert a negative effect on immune function. Currently, there is limited literature available on the immunosuppressive effects of high levels of physical activity on vaccine response. In the case of Tetanus booster vaccine, high levels of activity have been found to result in a reduction in antibody production (Kapasi et al., 2003). If a parallel can be drawn between Tetanus and Influenza vaccines then this analogous finding may provide the necessary justification for further research in this area.

3.10 Strengths and Limitations
Participants in this study are representative of a young and healthy group and the findings are
generalizable to a larger group of similar aged and active males in Alberta, Canada. A
methodological strength in this study was the use of the popular social networking sites Facebook.com and Reddit.com. Using minimal resources these sites allowed for the efficient engagement of a subset of the population that may not have been accessible through more traditional recruitment strategies. While the recruitment strategy was effective, 41% of participants failed to return for the final blood draw. Three attempts were made to contact and book participants before they were discontinued from the study. As the holiday season approached, participant communications became increasingly more sporadic making it difficult to book participants within the short time-line. One approach to addressing this for future study is to privately source the vaccine and administer it an earlier date. Finally, the findings of this study are limited to some extent due to reliance on the self-reporting physical activity measure. The chosen measure has been validated (Gionet and Godin, 1989) and is in common usage but inherent in self-report is the potential for recall bias to occur (Sallis and Saelens, 2000). Given additional time and resources a more scientifically rigorous confirmation would have been ideal.

3.11 Conclusions and Future Directions for Research

The most interesting result of this study was the trend observed towards lower vaccine response in young men who are very physically active. Balanced levels of moderate to vigorous physical activity are important for the maintenance of health and therefore further research needs to be completed to confirm and characterize this relationship. The potential influence of physical activity volume, intensity and type must also be considered. From a public health perspective, if individuals who are more physically active experience less protection, then steps will need to be
taken to consider more than just age and disease when classifying influenza “high risk” groups. The inclusion of fitness level alongside adiposity and physical activity would better address the complexity of their biological interface. Given these results, an expanded prospective cohort incorporating direct measures of fitness (i.e. aerobic capacity) would be needed to address the existing gap in the literature. For this study, an in depth cytokine analysis of inflammatory mediators was not possible. Future work in this area should consider inflammatory cytokine levels as potential modifiers of immune response to vaccination.

These results highlight several challenges that currently exist in the field of vaccine assessment. Individuals who fall into the “lower risk” category for influenza are underrepresented in vaccine research and subsequently this group become vulnerable and more likely to not develop herd immunity. This is recognized in the literature as Canadians within this age group have some of the lowest vaccination rates in the population (Gionet, 2015). Low vaccination rates coupled with a lack of understanding of the factors that influence immune response increases the potential vulnerability of this subset of the Canadian population (Lautermilch et al., 2016). These findings provide support for the argument that this age group warrants further study. An improved understanding of the impact of lifestyle on vaccine effectiveness has important implications when considering the current and future health of the population.

**Acknowledgements**

Traineeship support for this project was provided through the Canadian Institutes for Health Research.
The authors have no conflicts of interest to report.
References


https://doi.org/10.1080/14760584.2016.1188696


Xie, H., Wan, X.F., Ye, Z., Plant, E.P., Zhao, Y., Xu, Y., ... and Zoueva, O. 2015. H3N2 Mismatch

### Table 1. Baseline Characteristics of Participants (n=76)

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Table 2. Seroconversion and Seroprotection Rates baseline to post vaccination (4 weeks)

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<tr>
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<td>72</td>
<td>221</td>
<td>0.88</td>
<td>66</td>
<td>98</td>
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<td>63</td>
<td>156</td>
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<td>39</td>
<td>93</td>
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<tr>
<td>B\Massachusetts</td>
<td>57</td>
<td>277</td>
<td>0.72</td>
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Table 3. Non-Seroconverters and Seroconverters by %BF and Participant Number

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<th>Median %BF, IQR (n)</th>
<th>p-value</th>
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<td>Seroconverters</td>
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<td>16.3, 13.6-26.2 (31)</td>
</tr>
<tr>
<td>A\Texas</td>
<td>17.8, 14.9-26.4 (17)</td>
<td>15.7, 13.3-17.2 (28)</td>
</tr>
<tr>
<td>B\Massachusetts</td>
<td>15.9, 13.6-20 (23)</td>
<td>16.0, 13.3-18.6 (23)</td>
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Table 4. Non-Seroconverters and Seroconverters by Physical Activity (PA) and Participant Number

<table>
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<th>Median PA, IQR (n)</th>
<th>p-value</th>
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<tr>
<td></td>
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<td>Seroconverters</td>
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<tr>
<td>A\California</td>
<td>62, 31.5-108 (16)</td>
<td>53, 31-81 (31)</td>
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<td>A\Texas</td>
<td>81, 47-102 (21)</td>
<td>46, 27-72 (26)</td>
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<tr>
<td>B\Massachusetts</td>
<td>47, 31-96 (23)</td>
<td>64, 43-81 (23)</td>
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Figure 1: Participant Flow Diagram

Figure 1 Legend: Detailed study participant flow diagram. Of the 136 eligible participants identified prior to closure of enrollment 76 attended the baseline data collection session and 47 completed the study by attending the final data collection session. Assay results were obtained for all samples, except for an out of range result for one of the 47 B/Massachusetts HAI assays run.

Figure 2: Participant’s (n=47; age18-35 yr.) Self-Reported Number of Influenza Vaccinations in the Past 5 Years

Figure 2 Legend: Self-Reported vaccination history of participants who completed the study. The number of participants is plotted along the y-axis and the number of Influenza Vaccinations in the past 5 years is reported on the x-axis. One participant reported receiving 6 vaccinations over the 5 year period as they received the 2009 H1N1 (pandemic) vaccine and 2009 seasonal influenza vaccine in the same year. The majority of participants did not report receiving an influenza vaccine in the past 5 years (n=27).

Figure 3: Participant Median Antibody Titers over 4 weeks for the Trivalent Vaccine 2014/15 (n=47)

Figure 3 Legend: Hemagglutinin Antibody Titer’s obtained through Hemagglutinin Antibody Inhibition Assay Pre-Vaccination and 4 Weeks Post Vaccination for each of the 3 influenza strains included in the 2014/15 seasonal influenza vaccine. Pre and Post vaccination titers were
found to be highest for the B-Massachusetts strain. Note: one B/Massachusetts assay was returned with an inconclusive result (out of range) and as such was excluded from analysis.
Respondents to Pre-Screening Recruitment Survey (n=136)

Excluded (n=11)
- Female (n=1)
- Declined to participate (n=1)
- Could not be contacted prior to close of study recruitment (n=9)

Respondents contacted and booked for baseline data collection (n=125)

- Did not attend baseline collection (n=49)
- Lost to follow up after 3 attempts to rebook

- Did not attend final collection (n=29)
- Lost to follow up after 3 attempts to rebook

Included in Statistical Analysis (n=47)
*1 participant sample inconclusive for B/Mass Titer