ABSTRACT

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The Effect of Exercise on the Antibody Response to Influenza Vaccine in Type 2 Diabetics
(Under the direction of Melinda Beck, Ph.D.)

Obesity is a growing health problem that is affecting over one third of the adult population in the United States. Obesity increases an individual’s risk for diseases such as hypertension, cardiovascular disease, and type 2 diabetes. The prevalence of type 2 diabetes is growing in a trend similar to obesity in the United States. Obesity and type 2 diabetes has been shown to be immunosuppressive and increase an individual’s susceptibility to influenza infection. Increased physical activity and exercise has been shown to decrease the risks for complications associated with obesity and type 2 diabetes as well as increasing immune function. In the present pilot study we wanted to look at the differences in antibody response between obese non-diabetics and obese diabetics based on exercise and physical activity. To do this we collected serum from obese non-diabetics and obese diabetics pre- and post-vaccination of the influenza vaccine and used an ELISA assay to determine the response of the four subclasses Immunoglobulin G antibodies to the influenza vaccine pre- and post-vaccination of influenza vaccine. We found that vaccination for influenza resulted in a significant change in IgG1 and IgG3 serum titer. We found the amount of exercise did not result in significant changes in serum titer for any of the four IgG subclasses. Future studies should be conducted to determine defined relationship between exercise and immune response to influenza in obese and type 2 diabetics.
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LIST OF ABBREVIATIONS

U.S. United States
BMI body mass index
CDC Centers for Disease Control and Prevention
GLUT4 glucose transporter type 4
HbA1c hemoglobin A1c
H hemagglutinin
N neuraminidase
WHO World Health Organization
FDA Food and Drug Administration
TIV trivalent inactive vaccines
LAIV live attenuated cold-adapted influenza vaccines
IgG Immunoglobulin G
RAPA rapid assessment of physical activity
xg times the acceleration due to gravity
ELISA enzyme-linked immunosorbent assay
PBS phosphate buffered saline
PBSt phosphate buffered saline with Tween®
TMB tetramethylbenzidine
T2D type 2 diabetic
1.1 Obesity

Obesity is a health condition associated with excessive accumulation of adipose tissue. Obesity has become a growing health concern in the United States and around the world. In the U.S., the percentage of obese individuals has dramatically increased in the past two decades to approximately 35.7% among the adult population. Obesity is typically caused by an individual’s behavioral choices such as eating high calorie diets and lacking physical activity. Obesity is related to many health problems since the condition increases one’s risks for diseases such as type 2 diabetes, hypertension, heart disease, etc. In addition to these conditions, obesity has been shown to weaken immune responses.

There are many methods of determining whether an individual is obese such as measuring skinfold thickness, or waist circumference, and techniques such as bioelectric impedance and underwater weighing. The least expensive and most common way of determining obesity is by calculating an individual’s body mass index (BMI). BMI is calculated by dividing an individual’s weight in kilograms by their height squared in meters. According to the Centers for Disease Control and Prevention (CDC), an adult with a BMI lower than 18.5 is classified as underweight, an adult with a BMI between 18.5 and 24.9 is classified as healthy weight, an adult with a BMI between 25.0 and 29.9 is classified as overweight, and an adult with a BMI 30.0 and above is classified as obese. The main flaw with using BMI to determine obesity is that it correlates with body fat but does not actually measure the amount of body fat. Since muscle has a higher density than
adipose tissue, an individual with a high amount of muscle mass could have a high BMI despite having a low amount of body fat.

1.2 Diabetes Mellitus

Approximately 11.3% of adults in the United States have diabetes. The disease is associated with high blood glucose levels known as hyperglycemia. Complications from hyperglycemia include heart disease, stroke, kidney disease, and neuropathy. The hyperglycemia can be attributed to problems with insulin, one of the hormones involved in regulating blood glucose.

Insulin is secreted by the beta cells in the pancreas when the levels of glucose in the blood are high such as after a meal. Insulin signals cells such as adipocytes, skeletal muscle cells, and heart muscle cells to transfer GLUT4 transporters to the cell membrane to take in more glucose and reduce the amount of glucose in the blood. Insulin also inhibits glucagon, a hormone that is secreted when blood glucose levels are low to result in more glucose being secreted in the blood.

There are two forms of diabetes, Type 1 and Type 2 diabetes. Type 1 diabetes is caused by an autoimmune attack of the beta cells of the pancreas and results in the body failing to produce insulin. Type 2 diabetes is associated with insulin resistance that comes from a disruption in the insulin signaling pathway. One of the risk factors for Type 2 diabetes is being overweight or obese since it contributes to insulin resistance. Other risk factors include old age and genetics. Type 2 diabetes accounts for 90-95% of the diabetics in the United States. Type 2 diabetes can be treated or prevented from lifestyle choices such as a healthier diet or increased physical activity. Other treatments include bariatric surgery and drugs such as Metformin.
Type 2 diabetes can be diagnosed by three different types of blood tests which include a fasting plasma glucose test, an oral glucose tolerance test, and measuring the levels of hemoglobin A1c. The fasting plasma glucose test involves testing the levels of glucose in the blood of an individual that has fasted in at least eight hours. \(^6\) A level below 100 mg/dL is considered normal.\(^6\) An individual with a level between 100 and 125 mg/dL is classified as pre-diabetic and an individual with levels higher than 125 mg/dL is classified as diabetic.\(^6\) Pre-diabetics are individuals that have a higher than normal fasting blood glucose level but are not considered diabetic.\(^6\) The oral glucose tolerance test is similar to the fasting plasma glucose test but the individual drinks 75 grams of glucose dissolved in water after an eight hour fast and 2 hours before the test. \(^6\) The classifications for this test are pre-diabetics have a plasma glucose level between 140 and 199 mg/dL and diabetics have a level higher than 199 mg/dL.\(^6\) The hemoglobin A1c test does not involve fasting and represents blood glucose levels over the past three months.\(^6\) Glucose covalently binds to the protein hemoglobin in red blood cells to form hemoglobin A1c and red bloods cells have an average lifespan of three months. A level between 5.7 and 6.4% is classified as pre-diabetic and a level higher than 6.4% is classified as diabetic.\(^6\)

1.3 Influenza Virus

The CDC estimates that between 5 to 20% of people in the U.S. get infected with influenza (flu) and more than 200,000 people are treated in the hospital for complications related to the flu.\(^7\) The spread of flu is typically at its peak during flu season which is between the fall and spring seasons in the U.S. Some common symptoms for the flu include fever, cough, sore throat, runny nose, muscle aches, headaches, and fatigue.\(^8\)
Some individuals that are more vulnerable to the flu include older adults, children, pregnant women, and people with compromised immune systems. The flu is caused by infection with influenza virus. Individuals can spread the flu from approximately 6 feet away in droplets that come from talking, coughing, or sneezing. The viruses in these droplets can be inhaled into another person to infect their respiratory tract through the nose, throat, and lungs. Adults can infect others about a day before flu symptoms appear and symptoms can arrive one to four days after the virus enters a host.

There are three different types of influenza viruses which are A, B, and C. Influenza A and B are the two types most associated with epidemics. The viral genome of Influenza A is composed of 8 RNA segments. Influenza A is classified into different subtypes based on two proteins on the viral surface known as hemagglutinin (H) and neuraminidase (N). There are 18 hemagglutinin subtypes that are classified from H1 to H18 and 11 neuraminidase subtypes that are classified from N1 to N11.

Influenza viruses can change through processes known as antigenic drift and antigenic shift. In antigenic drift, there are mutations in the genome of the virus that can result in changes in the surface H protein such that antibodies do not recognize the altered H protein. In antigenic shift, a new viral strain forms from two different viral strains infecting a host cell and combining their segmented genetic material to form a new viral strain.

1.4 Influenza Vaccine

The World Health Organization (WHO) recommends specific viral strains for inclusion in influenza vaccines and then individual countries make their own decisions.
based on the recommendations. In the US, the Food and Drug Administration (FDA) determines the influenza strains that will be included in the vaccine. The vaccine contains the strains of viruses that are predicted to be the most common during the upcoming flu season. The vaccine typically is trivalent with three viral strains but for the 2013-2014 flu season, a quadrivalent vaccine with four viral strains was made available.\textsuperscript{12} The trivalent vaccine contains two Influenza A strains and one B strain while the quadrivalent vaccine contains two A strains and two B strains.\textsuperscript{12} Trivalent inactive vaccine (TIV) and live attenuated influenza vaccine (LAIV) are the two methods of vaccination.\textsuperscript{12} TIV is injected into the muscle and contains inactivated virus which is enriched in the H and N for antigen recognition. TIV is recommended for individuals older than 6 months.\textsuperscript{12} LAIV is a nasal spray administered into the nostril and contains live and weakened viral strains. This type of vaccine is recommended for individuals between the ages of 2 and 49.\textsuperscript{12}

1.5 Immunoglobulin G

The innate immune system and the adaptive immune system are the two major components of the immune system. The innate immune system protects the body from foreign agents with physical barriers such as skin, and mucosal membranes as well as phagocytic cells. The adaptive immune system relies on encountering a foreign antigen and developing a memory against that specific antigen to have a more rapid and powerful response to another infection of the antigen. The second exposure to a specific antigen is referred to as the secondary response. Vaccines are designed to generate this memory for the adaptive immune system to be protected from a possible infection. The lymphocytes types that are mainly involved in adaptive immunity are T and B cells.\textsuperscript{15} After the initial
encounter with an antigen, B cells differentiate into plasma cells that secrete antibodies that can bind to the antigen and prevent infection.\textsuperscript{15}

Antibodies include IgG, IgM, IgD, and IgE. IgG is the most common antibody found in the blood.\textsuperscript{15} IgG antibodies are more active during a secondary response.\textsuperscript{15} IgG antibodies can be further classified into four different subclasses from IgG1 to IgG4. The number of the subclass correlates to each subclass’s relative abundance in the blood with IgG1 as the most abundant and IgG4 as the least abundant. IgG1 and IgG3 are more associated with reacting with viruses such as influenza.\textsuperscript{15} IgG2 is associated with reacting with pathogens with polysaccharide antigens such as bacteria like \textit{Streptococcus pneumonia}.\textsuperscript{15} IgG4 is associated with repeated exposures to an antigen.\textsuperscript{15}

\textbf{1.6 Obesity and Influenza Vaccination}

Studies have shown that obesity is immunosuppressive and decreases the antibody response to tetanus toxoid and the hepatitis B vaccine.\textsuperscript{2} Diet-induced obese mice have a greater mortality to flu infection and weakened innate immune responses.\textsuperscript{2} An ongoing prospective observational study at the University of North Carolina Family Medicine Center examined the effect of BMI on the immune response to flu vaccine in human adults.\textsuperscript{2} Based on data from the first two years, it was determined that obese and healthy weight individuals increased their IgG antibodies specific for the vaccine one month after vaccination. However, one year after vaccination, the obese had a greater decline in antibody titers when compared with the healthy weight population.\textsuperscript{2} The decline in antibody titer in the obese from the study demonstrates that the obesity may have impair the immune response to the influenza vaccine.

\textbf{1.7 Diabetes, Obesity, and Exercise}
As highlighted in the previous section, the immunosuppressive nature of obesity can put obese individuals at a higher risk for infection and possibly reduce the effectiveness of vaccination. The immunity impairment may be due to leptin resistance, inflammation, metabolic changes, or other unknown mechanisms.\textsuperscript{2} Diabetes has also been shown to impair immunity. The impaired immunity can be due solely to diabetes or the common characteristics shared by diabetics such as old age and obesity.

Exercise and increased physical activity has been known as a behavioral change to treat and reverse the effects of obesity and diabetes. Exercise can decrease body fat and increase insulin sensitivity. Exercise has also been shown to reduce inflammation that can cause immunosuppression.\textsuperscript{13} The CDC recommends an equal mix of moderately intense to vigorous aerobic exercise per week and muscle strengthening exercise on 2 or more days a week for adults.\textsuperscript{14}

This project was designed to determine if there are differences in IgG subclass levels post influenza vaccination between obese individuals with and without diabetes who exercise compared with those who do not exercise. We hypothesized that exercising diabetics will have a greater response to influenza vaccination as compared to diabetics that do not exercise.
CHAPTER 2
SPECIFIC AIMS AND HYPOTHESES

Specific Aim One: To determine if vaccination for influenza increases the antibody titer for IgG1, IgG2, IgG3, and IgG4 in the study subjects.

Hypothesis: The titer will increase for all four antibodies in all of the study subjects.

Specific Aim Two: To determine if exercise exhibits a unique difference in antibody response to influenza vaccine in Type 2 diabetic subjects as compared to obese non-diabetic subjects.

Hypothesis: There will be a greater antibody response to influenza vaccine in high exercising diabetics than non-exercising diabetics. There will be similar results for the obese non-diabetic subjects.
CHAPTER 3

METHODS

3.1 Study Design:

The subjects in the study were taken from volunteers in an ongoing prospective observational study at the University of North Carolina Family Medicine Center which is an outpatient primary care facility located in Chapel Hill, NC. The larger study enrolled participants that were at least 18 years of age and were scheduled to receive the seasonal flu vaccine for the 2013-2014 flu season. All participants were excluded if they had the following criteria: immunosuppression, self-reported use of immunomodulator or immunosuppressive drugs, acute febrile illness, history of hypersensitivity to any influenza vaccine components, history of Guillian-Barre syndrome, or use of theophylline preparations or Warfarin.

After enrollment, informed consent was obtained from all of the participants. The height and weight for all the participants were measured and a baseline blood sample was taken from each participants. The participants were then injected with one dose of the 2013-2014 TIV (0.5 mL Fluzone (Sanofi Pasteur, Swiftwater, PA, USA) containing A/California/7/2009 (H1N1)pdm09-like virus, A/Texas/50/2012 (H3N2) and B/Massachusetts/2/2012-like virus) in the deltoid muscle. All participants were given a Rapid Assessment of Physical Activity (RAPA) survey that they filled out at the center. Participants returned 28-35 days later to the center to obtain a post-vaccination blood sample. The serum from these blood samples were isolated and preserved as described below.
In this study we selected serum samples from 107 participants. The participants were separated into six groups: non-exercising obese, non-exercising diabetics, inadequate exercising obese, inadequate exercising diabetics, high exercising obese, and high exercising diabetics. All of the participants reported their diabetes status orally after enrollment and were confirmed from their medical records. All of the obese participants in the study were participants that did not report diabetes (and confirmed from the medical record) and had a BMI classified as obese. The diabetic participants were participants that had reported having type 2 diabetes and had a BMI classified as obese. The high exercising obese category includes diabetics with a BMI that is classified as overweight to increase the sample size of the group. Exercise status was classified based on the participants’ response to the RAPA survey shown in Figure 1. Non-exercising participants were classified as such if they answered “no” to question 3 through question 7, answered “yes” to either question 1 or question 2, and answered “no” to the question corresponding to “strength.” High exercising participants were classified as such if they met the CDC’s recommendation for physical activity which includes both activities to increase muscle strength and aerobic activity every week. To fit in this category, participants would have to answer “yes” for the question corresponding to “strength” and “yes” for either question 6 or question 7. Participants that did not fit into non-exercising or high exercising categories are classified in the inadequate exercising category. The individual that answered the survey in Figure 1 would be classified in the inadequate exercising group.
**Figure 1.** The RAPA survey given to all of the participants in the ongoing prospective observational study at the University of North Carolina Family Medicine Center to assess the amount of physical activity the participants conducted.
### Table 1. Demographics of the study population

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Subjects</th>
<th>Sex Ratio (M:F)</th>
<th>Mean Age ± SD</th>
<th>Age Range</th>
<th>Race</th>
<th>Mean BMI ± SD</th>
<th>BMI Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Exercising Obese</td>
<td>15</td>
<td>3:12</td>
<td>56±9</td>
<td>41-69</td>
<td>9 white and 6 black</td>
<td>35.9±6</td>
<td>30.3-49.6</td>
</tr>
<tr>
<td>Non Exercising T2D</td>
<td>18</td>
<td>8:10</td>
<td>57±10</td>
<td>32-70</td>
<td>11 white and seven black</td>
<td>37.6±7</td>
<td>28.7-54.2</td>
</tr>
<tr>
<td>Inadequate Exercising Obese</td>
<td>27</td>
<td>5:22</td>
<td>50±10</td>
<td>32-71</td>
<td>18 white and 9 black</td>
<td>35.4±4</td>
<td>30.6-41.9</td>
</tr>
<tr>
<td>Inadequate Exercising T2D</td>
<td>28</td>
<td>10:18</td>
<td>59±7</td>
<td>43-71</td>
<td>14 white, 13 black, 1 Asian</td>
<td>38±7</td>
<td>30.0-55.6</td>
</tr>
<tr>
<td>High Exercising Obese</td>
<td>9</td>
<td>3:06</td>
<td>57±12</td>
<td>33-71</td>
<td>5 white, 3 black, 1 Hispanic</td>
<td>35±4</td>
<td>29.9-42.1</td>
</tr>
<tr>
<td>High Exercising T2D</td>
<td>10</td>
<td>5:05</td>
<td>62±9</td>
<td>46-73</td>
<td>4 white and 6 black</td>
<td>32.5±5</td>
<td>26.6-41.8</td>
</tr>
</tbody>
</table>
3.2 Serum Collection

Pre- and post-vaccine blood draws were collected into a vacutainer tube and allowed to clot for 30-60 minutes at room temperature and then refrigerated at 4°C. The blood was then centrifuged in the tubes at 800 xg for 10 minutes in a IEC Centra MP4R. After the centrifuge step, 0.5 mL of the serum was aliquoted into the small 1.2 mL serum tubes. These tubes were stored in a freezer at -80°C.

3.3 Enzyme-Linked Immunosorbent Assay (ELISA)

To quantify the four different IgG antibodies, ELISA was used with the 2013-2014 seasonal TIV as the antigen. The vaccine was diluted in a carbonate coating buffer to be absorbed in 96-well microtitration ELISA plates. Four different ELISA plates were used for the four different antibodies. For three of the plates that would quantify IgG1, IgG2, and IgG3, the vaccine was diluted in a 1:160 ratio in the coating buffer. For the fourth plate that would quantify IgG4, the vaccine was diluted in a 1:80 in the coating buffer. All of the plates were filled with 50 μL of the diluted vaccine solution in each well. The plates were refrigerated overnight at 4°C.

After refrigeration, the plates were discarded of the vaccine solution and the wells were filled with 200 μL of block buffer to block the remaining protein-binding sites in the well. The block buffer was composed of coating buffer and 3% of nonfat dry milk. The plates were then incubated in 37°C for at least one hour.

The next step involved thawing the serum samples and diluting in dilution buffer. The dilution buffer was composed of PBS solution and 3% nonfat dry milk. Fifteen pre- and post-vaccination serum samples were used for each four plate set. An internal control sera and a blank sample were included in each set. The blank sample did not contain any
serum. Each thawed serum samples were individually diluted into four different solutions. One solution had the serum diluted in a 1:6400 ratio, another solution had the serum diluted in a 1:100 ratio, another solution had the serum diluted in a 1:400 ratio, and the last solution had the serum diluted in a 1:200 ratio.

After the incubation, the plates were washed three times with PBSt solution using an ELISA plate washer. All of the plates were filled with 50 μL triplicates of each sample. The IgG1 plate was filled with the 1:6400 dilution solution, the IgG2 plate was filled with the 1:100 dilution solution, the IgG3 plate was filled with the 1:400 dilution solution, and the IgG4 plate was filled with the 1:200 dilution solution. The plates were then incubated in 37°C for 2 hours. The antibodies in the serum would react and bind to the vaccine antigen in the wells.

The next step involved detecting the bound antibodies with a corresponding horseradish peroxidase-conjugated goat anti-human IgG subtype (Abcam, Cambridge, MA, USA). Anti-human IgG1, IgG2, and IgG3 were diluted in dilution buffer in a 1:1000 ratio. Anti-human IgG4 was diluted in dilution buffer in a 1:500 ratio. After the incubation, the plates were washed three times with PBSt using the ELISA plate washer. The anti-human IgG solutions were added to the plate corresponding to specific antibody subtype. Each well was added with 50 μL of the solution. The plates were incubated in 37°C for 1 hour.

After the incubation, the plates were washed three times with PBSt using the ELISA plate washer. Immediately after the plate washing, every well was filled with 100 μL of TMB substrate solution mix which was composed of an equal mix of TMB solution and peroxide solution. The addition of the TMB substrate solution would form a
blue solution of varying intensities based on the samples. The plates were incubated at room temperature for 30 minutes. The wells were then added with 100 μL of 2M sulfuric acid to stop the reaction with the TMB substrate solution and turn the blue solution into a yellow solution. The color intensity of each well was measured by absorbance at 450 nm using an ELISA plate reader. The color intensity would correspond with the antibody titer in the well.

3.4 Statistical Analysis:

The ELISA data was assessed using GraphPad Prism®. Differences in antibody titer were assessed using the ANOVA test. A p-value <0.05 was considered significant.
CHAPTER 4

RESULTS

4.1 Demographics of the Study Population

The participants in the study were classified into six groups: non-exercising obese, non-exercising type 2 diabetics, inadequate exercising obese, inadequate exercising type 2 diabetics, high exercising obese, and high exercising type 2 diabetics. The demographics of the participants are listed in Table 1.

4.2 Antibody Response

For IgG1, there was a significant change in antibody titer for all groups from pre- to post-vaccination with influenza vaccine. There was not a significant change in antibody titer based on the different groups. Among the obese, higher exercise correlated with increased expression of IgG1 after vaccination. For the non-exercising obese, $24\% \pm 70$ was the percent change. In the inadequate exercising obese, there was a $30\% \pm 40$ percent change, while the percent change was $35\% \pm 30$ among the high exercising obese. The percentage of obese with at least a $30\%$ percent change in IgG1 among the non-exercising, inadequate exercising, and high exercising were $53\%$, $57\%$, and $50\%$ respectively showing no clear trend (Figure 7). Between non-exercising and high exercising diabetics, exercise correlated with a higher expression of IgG1 with the percent change being $21\% \pm 60$ and $40\% \pm 60$ for the respective groups. However, inadequate exercising diabetics showed a $-7.1\% \pm 60$ percent change in IgG1 antibody titer from pre- to post-vaccination. The percentage of diabetics with at least a $30\%$ percent change in IgG1 among the non-exercising, inadequate exercising, and high exercising were $65\%$, $26\%$, and $57\%$ respectively showing no clear trend (Figure 7).
Figure 2. The mean percent change in IgG1 antibody titer from pre- to post-vaccination among the different groups.

There were not any significant changes in IgG2 expression between the groups and from pre- to post-vaccination in all of the groups. For non-exercising obese, there was a -15% ± 80 percent change in IgG2 from pre- to post-vaccination. From inadequate exercising obese showed a 2.4% ± 40 percent change while high exercising obese showed a -32% ± 100 decrease. The percentage of obese with at least a 30% percent change in IgG2 among the non-exercising, inadequate exercising, and high exercising were 42%,
32%, and 17% respectively showing a decreasing trend (Figure 7). In non-exercising diabetics, there was a 12% ± 40 percent change in IgG2 expression. In inadequate exercising diabetics, there was a slight decrease in expression to 6.7% ± 120 and while in high exercising diabetics, the expression was 9.3 ± 30 percent change. The percentage of diabetics with at least a 30% percent change in IgG2 among the non-exercising, inadequate exercising, and high exercising were 30%, 22%, and 14% respectively showing a decreasing trend (Figure 7).
Figure 3. The mean percent change in IgG2 antibody titer from pre- to post-vaccination among the different groups.

For IgG3, there was a significant change in antibody expression in all groups from pre- to post-vaccination. The difference in expression between the groups was not significant. For the obese, exercise slightly correlated with a decrease in IgG3 expression. The percentage of obese with at least a 30% percent change in IgG3 among the non-exercising, inadequate exercising, and high exercising were 64%, 30%, and 50% respectively showing no clear trend (Figure 7). In the non-exercising obese category,
there was a 31% ± 50 percent change from pre- to post-vaccination. From inadequate exercising to high exercising diabetics, there was a 27% ± 40 percent change and an 18% ± 27 percent change respectively. The percentage of diabetics with at least a 30% percent change in IgG3 among the non-exercising, inadequate exercising, and high exercising were 55%, 45%, and 50% respectively showing no clear trend (Figure 7).

**Figure 4.** The mean percent change in IgG3 antibody titer from pre- to post-vaccination among the different groups.
For IgG4, there were not any significant differences between the groups and significant changes from vaccine treatment in all of the groups. For non-exercising obese, there was a -38% ± 60 percent change in IgG4 expression from pre- to post-vaccination. From inadequate exercising to high exercising obese, there was a slight increase from 9.0% ± 40 to 14% ± 40 respectively. The percentage of obese with at least a 30% percent change in IgG4 among the non-exercising, inadequate exercising, and high exercising were 8.3%, 36%, and 43% respectively showing an increasing trend (Figure 7). In non-exercising diabetics, there was a -46% ± 200 percent change in expression. In inadequate exercising diabetics, there was a -47% ± 80 percent change. In high exercising diabetics, the expression increased 7.7% ± 30 fold. The percentage of diabetics with at least a 30% percent change in IgG4 among the non-exercising, inadequate exercising, and high exercising were 19%, 13%, and 29% respectively showing no clear trend (Figure 7).
Figure 5. The mean percent change in IgG4 antibody titer from pre- to post-vaccination among the different groups.

All of the groups had varying changes in the different IgG antibodies from pre- to post-vaccination for flu. In the non-exercising obese group, there was a 24% percent change in IgG1, a -15% percent change in IgG2, a 31% percent change in IgG3, and -38% percent change in IgG4. In the non-exercising diabetic group, there was a 21% percent change in IgG1, a 12% percent change in IgG2, a -18% percent change in IgG3, and a -46% decrease in IgG4. In the inadequate exercising obese group, there was a 30%
percent change in IgG1, a 2.4% percent change in IgG2, a 27% percent change in IgG3, and a 9.0% percent change in IgG4. In the inadequate exercising diabetic group, there was a -7.1% percent change in IgG1, a 6.7% percent change in IgG2, a 26% percent change in IgG3, and a -47% percent change in IgG4. In the high exercising obese group, there was a 35% percent change in IgG1, a -32% percent change in IgG2, an 18% percent change in IgG3, and a 14% percent change in IgG4. In the high exercising diabetic group, there was a 40% percent change in IgG1, a 9.3% percent change in IgG2, a -36% percent change in IgG3, and a 7.7% percent change in IgG4.

Figure 6. Vaccine treatment induced significant expression in IgG1 and IgG3, but not in IgG2 and IgG4.
**Figure 7.** The percentage of each group that had a least a 30% percent change four each of the antibodies.
5.1 Immune Response to the Influenza Virus

Vaccination has been recommended as the most effective protection against influenza infection. Obesity and diabetes are immunosuppressive conditions so it is important for individuals with these conditions to be vaccinated to better protect themselves from the flu. It is important to measure how individuals with these conditions respond to the flu vaccine to determine if they are potentially protected from flu infection. In this study, we observed obese individuals that are either diabetic or non-diabetic and measured their differences in antibody response to influenza vaccination based on their level of exercise. We hypothesized that the participants that did the higher amounts of exercise would have an increased expression of all four IgG antibody subclasses after vaccination for the flu. The study came up with some unexpected results.

IgG1 antibodies react with viral antigens so it is expected that vaccine treatment would result in increased expression. The trend was seen in all groups except the inadequate exercising diabetic group. More exercise correlated with an increase in IgG1 expression in the obese suggesting that exercise can help immune response for the obese. The same trend was seen between non-exercising and high exercising diabetics similar to the obese which would suggest that exercise improves immune response in diabetics in the same way as obesity.

Exercise shows an increasing trend in IgG2 expression in the obese. In diabetics, exercise does not show a clear trend. The lack of trend could be due to IgG2 antibodies are associated more with bacterial antigens rather than viral antigens such as the flu.
For IgG3 antibodies, exercise correlates with decreased percentage changes in expression after vaccination in the obese. For diabetics, exercise does not show a clear trend in IgG3 expression. For all groups except non-exercising diabetics and high exercising diabetics, there was an increase in antibody expression for IgG3. The increase in expression is expected since IgG3 is another antibody associated with viral antigens such as the flu.

For the obese, exercise shows a slight increasing trend in IgG4 antibody expression. In the diabetic groups, exercise does not show trend in antibody expression. This could be due to IgG4 not being associated with viral antigens.

5.2 Limitations

This study had several limitations. One limitation was the use of ELISA for determining immune response. The use of ELISA is a limitation because the technique shows the amount of antibodies binding to the vaccine antigens but does not actually show where the antibodies bind to the virus and whether such binding would actually hinder viral activity. Another limitation was that diabetes was based on a previous medical diagnosis and we did not know the time of diagnosis meaning that individuals that were diagnosed with diabetes may no longer be considered diabetics by the time of the study. Not knowing the time of diagnosis also means that results could be different for diabetics that were diagnosed recently or diagnosed many years ago. Another limitation is that we did not determine specific types of medications that individuals could be taking at the time. Individuals could be taking medications that hinder or improve immunity and thus alter the antibody response due to the medications. Another limitation is that individuals were separated into exercise categories based on their results
on a survey meaning that their responses on the survey may not actually reflect actual exercise habits.

5.3 Future Studies

Future studies could separate the inadequate exercising groups into more groups that reflect exercise habits such as aerobic exercise or muscle strengthening exercise to observe whether the type of exercise for individuals that do not meet the CDC’s requirement for exercise can have a different effect on antibody expression. Another study can include more subjects to increase statistical power. Future studies could also include healthy weight and overweight individuals to see if all individuals experience differences in antibody expression. Future studies can look at type 1 diabetes to see if there are different results between type 1 and type 2 diabetes. Future studies can examine other aspects of the adaptive immunity outside of the antibodies such as T cell and cytokine expression.
References: