DOES RACE PLAY A ROLE IN THE ROBUSTNESS OF THE FLU VACCINE RESPONSE IN OBESE WOMEN?

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ABSTRACT

Obesity prevalence has increased drastically over the last couple of decades. Obesity increases an individual’s risk of health complications such as cardiovascular disease, hypertension, and other chronic conditions. Obesity has been related to an increased susceptibility to infection and complications from the influenza virus. In the present study, the immune responses to the influenza vaccine between races in obese women will be compared by determining the percent increases in immunoglobulin G subtype levels from pre to post vaccination. To accomplish this, serum was collected from study participants and tested for antibody levels pre and post vaccination using an indirect enzyme-linked immunosorbent assay. For IgG1 and IgG3, average percent increases pre to post vaccination displayed no difference between African American women and Caucasian women. IgG2 was not present in detectable levels in the population studied and was not able to be analyzed. For IgG4, the average percent increase pre to post vaccination displayed a statistically significant difference between African American women and Caucasian women. African Americans had a significantly lower average percent increase in IgG4 pre to post vaccination compared to Caucasians. This suggests that there may be lower levels of IgG4 in African American women in general compared to Caucasian women. Future studies should be conducted to further investigate these relationships.
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CHAPTER 1
Introduction

1.1 The Obesity Epidemic:

Obesity has become an increasing public health concern in the United States in recent years. There has been a dramatic increase in obesity in the United States from 1990 through 2010, increasing from a prevalence of 12% in 1990 to a prevalence of 35.7% in 2010\(^3\). Obesity is a term that describes a range of weights that is greater than what is generally considered healthy for a given height. This range of weight has been shown to increase the likelihood of certain diseases and other health problems, such as diabetes, hypertension and cancer. Each year, over 300,000 adults in the U.S. will die from obesity related causes\(^2\).

One method of measuring obesity is using BMI, or body mass index. BMI is used in adults to measure body fat based on height and weight using the formula; \(\text{BMI} = \frac{\text{weight in kilograms}}{\text{height in meters}}^2\). According to the National Institutes of Health, a healthy weight BMI is between 18.5-24.9 kg/m\(^2\), underweight is below 18.5 kg/m\(^2\), overweight is between 25.0-29.9 kg/m\(^2\), and obese individuals are 30.0 kg/m\(^2\) and above\(^5\). However, BMI should be used cautiously. BMI does not directly measure body fat and may identify some people as overweight even if they do not have excess body fat, such as athletes with high muscle mass\(^1\).

A variety of factors including behavior, environment, and genetic factors can cause an individual to be overweight or obese. Positive energy imbalance, or consuming more calories than expending through physical activity, contributes to fat gain. Furthermore, obesogenic environments such as food deserts or areas without sidewalks or parks can influence poor nutritional intake or lack of physical activity. These factors have been a target in public health prevention. Genes may also play a role in obesity and enhance the effects of an obesogenic environment\(^4\).

Obesity prevalence varies greatly in the United States depending on race, sex, and age. Specifically, the prevalence of obesity is significantly varied between racial and ethnic groups. Non-Hispanic Blacks have the highest age-
adjusted rates of obesity (49.5%) compared with Mexican Americans (40.4%), all Hispanics (39.1%) and non-Hispanic whites (34.3%)\textsuperscript{6}.

The prevalence of obesity in US adults is significantly higher between minority women and Caucasian women. In 1988-1994, the prevalence of obesity in Non-Hispanic White women was 22.9%, 38.4% in Non-Hispanic Black women, and 35.4% of Mexican American women. In 2010, these prevalence rates were significantly higher at 32.2% of Non-Hispanic White women, 58.5% of Non-Hispanic Black women, and 44.9% of Mexican American women\textsuperscript{7}.

1.2 Influenza Virus

Influenza is one of the most widely spread human infectious diseases and causes significant morbidity and mortality every year. There are three types of influenza virus, type A, B, and C. Human influenza A infects mostly humans and several types of animals, including birds, pigs, and horses. Type B influenza is normally found only in humans, and type C is mostly found in humans, but has also been found in pigs and dogs. Type A viruses typically cause influenza pandemics and are the most threatening in humans. Type A influenza viruses can be further classified based on two glycoproteins that exist on the surface of the virus called hemagglutinin (HA) and neuraminidase (NA). There are 17 known different versions of HA and 9 known different versions of NA and these versions are assigned a specific number, helping to name a particular viral strain\textsuperscript{8}.

The influenza virus is typically rounded, but can also be elongated or irregularly shaped. The glycoproteins HA or NA rest on the surface. The HA protein aids in the attachment of the virus to the cell, so that it can enter into a host cell and start the infection process. HA proteins are also recognized by the human immune system through antibodies that will signal for an immune response\textsuperscript{10}. The NA protein aids in helping the virus exit the host cell so that new viruses that were made inside the host cell can be released in order to infect more cells. The influenza virus is enveloped, segmented, single-stranded RNA
virus. Inside the virus, there are eight segments of RNA that encode 11 proteins related to viral replication\(^9\).

The potency of influenza virus lies in its ability to change, especially influenza virus A. This is why the flu vaccine must be changed from year to year. The influenza virus can change in two ways, through drift and shift. Antigenic drifting is a gradual, continuous change that results through mistakes that occur from gene replication, resulting in differences in the HA or NA proteins. Although the changes can be small, they may no longer be recognizable to the human immune system\(^12\). Antigenic shift, a much more unlikely occurrence, is an abrupt, major change in the virus that produces a new combination of the HA and NA proteins, which results in a pandemic. There have been 4 such flu pandemics, including the first flu pandemic of the 21\(^{st}\) century- pandemic H1N1\(^9\).

### 1.3 Influenza Virus Infection and Immune Response

Infection of the virus occurs from person to person when people infected with flu cough, sneeze, or talk and spread virus-laden droplets. The virus gains entry into the host through the respiratory tract and infects the host in their nose, throat, and lungs. Once the virus has gained entrance into the host, it can travel into the nucleus of the respiratory tract cells by binding their hemagglutinin glycoproteins to the sialic acid sugars on the epithelial cell surface, and then use their replication machinery in order to replicate and release viral particles to the surrounding cells, spreading the infection\(^11\).

Upon infection by the virus, the innate immune system, or the non-specific immune system, responds first to the viral antigens. Influenza A viral infection is sensed by infected cells via pattern-recognition receptors (PRRs) that recognize viral RNA, the main pathogen-associated marker pattern (PAMP) of influenza A viruses. PRRs include toll like receptors (TLRs) and retinoic acid inducible gene-1 (RIG-1). Single stranded viral RNA bind to TLR7 and double-stranded viral RNA binds to TLR3 and RIG-1, signaling receptor cascades to produce proinflammatory cytokines and type I interferons including IFN-\(\beta\) and IFN-\(\alpha\) that have strong antiviral activity exerted by inhibiting protein synthesis in host cells.
and limiting viral replication. These PRRs are located on cells of the innate immune system including macrophages and dendritic cells\textsuperscript{16}.

Antigen-presenting cells, specifically dendritic cells and macrophages, promote destruction of a virus by activating cytotoxic T-cells (CD8 T cells) or ingesting viruses through pinocytosis. In addition, they activate the adaptive immune response to create antibodies for the virus through class II MHC glycoproteins. The antigen-presenting cell processes antigen and presents it on class II MHC molecules that are recognized by CD4\textsuperscript{+} helper T cells in the lymph nodes. Virus-derived peptides in the host cell are also recognized by class I MHC glycoproteins. The peptides and the Class I MHC glycoprotein form a complex that activates the cloning and differentiation of CD8\textsuperscript{+} T cells to enhance viral clearance\textsuperscript{16}.

Helper T cells, once stimulated by the class II MHC antigen complex, make proteins that induce B cells to proliferate and cause its progeny to differentiate into antibody-secreting cells. Antigen-specific T-cell help is required for antibody responses\textsuperscript{14}. When a B cell encounters the kind of antigen that triggers it to become active, it gives rise to many large cells known as plasma cells, which produce antibodies. Antigens from influenza viruses stimulate the B cells to release a variety of immunoglobulins, including IgG subclasses\textsuperscript{14,17}.

1.4 Influenza Vaccine

Vaccinations are recommended to individuals in order to prevent viral infection and its spread. Vaccines help develop immunity to the influenza virus by imitating viral infection without causing illness\textsuperscript{15}. The influenza vaccine varies by season, depending on the prediction of circulating strains. For the 2013-2014 year, the seasonal trivalent vaccine was composed of two strains of type A viruses and one strain of type B. Because the flu vaccine is manufactured several months before flu activity begins, the strains that are used in the vaccine are predicted from the available evidence. If the prediction matches the
circulating strains, then good protection from flu infection occurs. If the prediction is less accurate, then the flu vaccine is less protective\textsuperscript{18}.

Upon vaccine inoculation, the immune system responds to the viral antigens in the vaccine, inducing both T and B memory cells that are poised to quickly respond to a future flu infection. Exposure to the viral antigen by a vaccine sustains memory of the antigen by long-lived antigen-specific B lymphocytes that were induced by the original exposure and that persist until a second encounter with the pathogen. The number of memory cells for a given antigen is highly regulated, remaining practically the same throughout the memory phase. When restimulated by the same antigen, the cells are able to respond quickly and remove the infection\textsuperscript{18}.

1.5 Immunoglobulin G Subclasses

The glycoprotein immunoglobulin G (IgG) is a major effector molecule of the human humoral immune response and accounts for about 75% of the total immunoglobulins in plasma of healthy individuals. The other immunoglobulins, IgA, IgM, and IgD make up the remaining 25% of the total immunoglobulins\textsuperscript{29}.

The four IgG immunoglobulin subclasses (IgG1, IgG2, IgG3 and IgG4) are more than 95% homologous in their amino acid sequences of the constant domains of the \(\gamma\)-heavy chains but show the most differences amongst each other in the amino acid composition and structure of the 'hinge region.' The length and flexibility of this hinge region varies among the IgG subclasses\textsuperscript{27}.

One of the most important attributes of IgG is its role in the complement activation initiation. Activation is started when C1 binds to sites on the Fc portion of human IgG\textsuperscript{27}. IgG3 is the most potent activator of the complement system, followed by IgG1 and then IgG2. IgG4 is generally inactive in complement activation. Another important role of IgG is its role in clearance of antigens via phagocytosis\textsuperscript{28}.

Generally, antibody responses to protein antigens, including viral proteins, mainly involve IgG1 and IgG3\textsuperscript{28}. The precise roles of each IgG subclass are still
unclear, but certain antigens (bacterial and viral) can cause increases in selective subclasses of IgG\textsuperscript{28}.

1.6 Differences in the Immune Response by Racial Background

There is a large amount of information documenting the significant differences in the prevalence of obesity and chronic conditions within different ethnic groups, but the reasoning behind this is still largely unexplained. Differences in the levels of cytokines, immunoglobulins, and response after infection may contribute to these chronic conditions. Preliminary studies have been conducted to determine if genetics plays a role in these differences. One study by Ness, Haggerty, and Ferrell showed that African-American women were significantly more likely to carry allelic variants in cytokine genes known to upregulate proinflammatory cytokines. Many chronic conditions are tied to an inflamed state. The upregulation of the proinflammatory immune response may suggest that there is a race-specific genetic component that predisposes African American to chronic inflammatory conditions\textsuperscript{21}. In addition, this upregulation of a proinflammatory state may also affect the immune response to vaccines. There is more information needed in order to see if there is a difference in immune response to influenza vaccination between the African-American and the White-American populations. This study was done to determine if there is a difference in immune responses within the African-American and White-American populations.

I hypothesized that the African-American population will have a stronger immune response to the vaccine compared to the White-American population due to the increase in allelic variants in cytokine genes overrepresented in the African-American population.
CHAPTER 2
Specific Aim and Hypothesis

Specific Aim: To determine if the antibody response to the Influenza Vaccine differs in African Americans compared to that in Caucasians by looking at IgG1, IgG2, IgG3, and IgG4 subclasses of immunoglobulin G.

Hypothesis: The antibody response to the Influenza Vaccine will be higher in African Americans compared to that in Caucasians.
CHAPTER 3
Methods:

3.1 Study Design:

Subjects for this study were selected from participants of an ongoing, prospective, observational study taking place at the University of North Carolina Family Medicine Center. The original study gave participation eligibility to adult patients (≥ 18 years of age) that were scheduled to receive the 2013-2014 trivalent, inactive seasonal vaccine. Participants were excluded upon the following criteria: under the age of 18, acute febrile illness, under immunosuppressive medicines in the last 4 weeks, pregnant or breastfeeding women, and diseases such as cancer (except skin cancer that is not melanoma), Human Immunodeficiency Virus, and Hepatitis C.

At enrollment, informed consent, height, weight, and baseline blood samples were obtained from each participant. They were then administered one dose of the inactive 2013-2014 trivalent inactive vaccine one to one and a half inches into their deltoid muscle. Participants returned to the center 25-28 days after the first administration to give a post-vaccination blood sample. PBMCs and serum were isolated from each blood sample collected pre and post vaccination. For this study, serum from women with BMIs between 30 and 35 kg/m² with little, inconsistent, or no exercise were used. These participants were separated into one of two groups: African American or White American.

3.2 Serum Collection:

Serum was collected before receiving the vaccine and again 25-28 days after receiving the vaccine. Blood was collected from patients at this time into a vacutainer tube and allowed to clot for 30-60 minutes at room temperature, then moved to a refrigerator. During time of collection, the tubes were centrifuged at 800 x g for 10 minutes at 4°C using for IEC Centra MP4R. Serum was aliquoted into 500 microliters amounts using serum tubes and placed into an -80°C freezer.

3.3 Influenza-specific ELISA for IgG
The enzyme-linked immunosorbent assay (ELISA) was used to identify the level of influenza-specific antibodies in serum. This method enabled analysis of antibody protein levels by immobilizing them in microplate wells using antigen-antibody binding. The method employed by this study was the indirect ELISA method. The vaccine antigen, diluted in coating buffer, was immobilized to the surface of the polystyrene microplate wells. The plate was then blocked with a block buffer of milk in order to cover all unsaturated surface-binding sites of the microplate wells. Next, the subject serum was added. Following an incubation and a washing, a secondary, mouse anti-human IgG (IgG1-IgG4) antibody conjugated with horse radish peroxidase (HRP) was added to bind to the specific primary antibody from the participant’s serum. Following a wash, the enzyme substrate was added to catalyze a reaction that induces a color change in the substrate due to HRP activity. This color change signal was measured with an ELISA plate reader by measuring the absorbance at 450 nm. Increased enzyme activity (detected by a darker color reaction) was directly correlated with antibody concentration.

The ELISA procedure spanned over two days. Prior to the start of the experiment, an optimization assay was run for each ELISA component at different concentrations to determine the optimal dilutions for vaccine antigen, serum, and secondary antibody with HRP. For this project, each subclass of IgG (IgG1, IgG2, IgG3 and IgG4) was measured, and therefore each one was optimized individually. The optimal vaccine antigen dilution for each antibody was chosen to be 1:160.

Following optimization, the serum from the participants and the secondary antibody with HRP was diluted according to the following table:

<table>
<thead>
<tr>
<th>Antibody Plate</th>
<th>Secondary antibody with HRP</th>
<th>Serum Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>1:1000</td>
<td>1:6400</td>
</tr>
<tr>
<td>IgG2</td>
<td>1:1000</td>
<td>1:100</td>
</tr>
<tr>
<td>IgG3</td>
<td>1:1000</td>
<td>1:400</td>
</tr>
<tr>
<td>IgG4</td>
<td>1:500</td>
<td>1:200</td>
</tr>
</tbody>
</table>
Table 1. Dilutions of secondary antibodies and serum dilutions used during ELISA runs for IgG1, IgG2, IgG3, and IgG4.

### 3.4 Specific Details for Analysis of Antibody Levels in Serum using ELISA:

**Materials:**
- Clear 96 well plates
- Plate covers
- Multi-channel precision pipettors with disposable plastic tips
- Plate Reader

**Reagents**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coating Buffer</td>
<td>0.2 M sodium carbonate/bicarbonate solution in PBS wash</td>
</tr>
<tr>
<td>Wash buffer</td>
<td>330 mL phosphate buffer solution, 3000 mL of distilled H₂O, and 1.6 mL of Tween-20</td>
</tr>
<tr>
<td>Block Buffer</td>
<td>2.4 grams of nonfat dry milk in 80 mL of Coating Buffer</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td></td>
</tr>
<tr>
<td>Detection/Secondary</td>
<td>Mouse anti-human antibody for IgG1, IgG2, IgG3, and IgG4 with HRP conjugate</td>
</tr>
<tr>
<td>Antibody</td>
<td></td>
</tr>
<tr>
<td>Vaccine Antigen</td>
<td>Diluted in Coating Buffer – 1:160 dilution</td>
</tr>
<tr>
<td>Horseradish Peroxidase</td>
<td>Attached to detection antibody; acts as enzyme conjugate</td>
</tr>
<tr>
<td>Enzyme Substrate</td>
<td>TMB Substrate in Peroxide solution</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>2M sulfuric Acid</td>
</tr>
<tr>
<td>Primary Antibody</td>
<td>Antibodies in the serum of study participants</td>
</tr>
</tbody>
</table>

Table 2. Reagents used during ELISA.

On the first day, vaccine was diluted to a 1:160 using Coating buffer solution. The plates were coated with the diluted vaccine, adding 50 microliters of the vaccine solution into each well of a 96 well plate. The plates were stored in a moist plastic bag in a 4°C refrigerator overnight.

On the second day, the solution in the wells was discarded and each well was coated with 200 microliters of Block buffer. The wells were then incubated in
a 37°C incubator for 1 hour. The plates were then removed and washed with 1x Phosphate Buffer Saline Solution with Tween-20 twice. The serum from the participants was diluted according to Table 1. The serum was added to the wells in triplicates of 50 microliters each, filling three wells with the same diluted serum. Three wells were filled with a control serum and three wells were filled with dilution buffer without serum to provide a background measurement. The plates were then incubated for two hours at 37°C and washed with 1x Phosphate Buffer Saline Solution with Tween-20.

After this wash, the secondary antibody with HRP was added. Each plate received a different antibody: IgG1, IgG2, IgG3, or IgG4. These antibodies were diluted according to Table 1. Each well received 50 microliters of antibody solution in Dilution buffer. The plates were then incubated at 37°C for one hour and washed with 1x Phosphate Buffer Saline Solution with Tween-20.

After washing, 100 microliters of TMB substrate solution was added to each well, catalyzing the reaction of the horseradish peroxidase conjugate to change the substrate’s color. This reaction was allowed to continue for thirty minutes exactly under aluminum foil. The reaction was stopped using 100 microliters of 2 M sulfuric acid in each well. The optical density was measured for each well at 450 nm wavelength.

3.5 Data Analysis:

After each plate read, the blank measurements were compared to the readings from the positive control wells. If the absorbance measurements from the control wells were significantly higher than those of the blank wells, the plate measurements were kept. If not, the ELISA protocol was rerun for that plate. The blank measurements on each plate were subtracted from each well containing a sample. Then, the average, standard deviation, and the coefficient of variation were calculated for each triplicate. The coefficient of variation was kept if it was 15% or less. If the coefficient of variation was higher than 15%, the triplicates were observed for an outlier, which was deleted. If the coefficient remained high, then the sample was rerun.
The average of the blank measurements from all plates for a certain antibody was added to the average reads for each sample. Then, the percent change was calculated using the formula:

\[
\text{PERCENT INCREASE} = \left( \frac{\text{POST VACCINATION VALUE}}{\text{PRE VACCINATION VALUE}} \times 100 \right) - 100
\]

The data was analyzed using the program GraphPad Prism 6.0. A Wilcoxon Rank-Sum test was used to analyze the significance of the data.
CHAPTER 4
Results:

4.1 Demographics of the Study Population

Participants were classified into two groups: African American and Caucasian. All were obese, which was defined as a BMI greater than 30 and less than a BMI of 35. Past studies have been done that have demonstrated a decreased antibody response in obese individuals compared to healthy weight individuals, so women at similar BMIs were chosen to eliminate potential confounding variables. Furthermore, using a survey developed at the Gillings School of Global Public Health at the University of North Carolina Chapel Hill, levels of physical activity per week was assessed. The immune system can be influenced acutely by exercise, both positively by moderate exercise and negatively by heavy exertion\textsuperscript{19}. Thus, this study only included participants with inconsistent, little, or no exercise in order to remove this potential confounder.
Table 3. Demographics of all participants in this study. All were women with little, no, or inconsistent exercise with BMIs between 30 and 35 kg/m^2. The average age of Caucasian women participating in the study was 55 years with a standard deviation of 10 years. The average age of African American women was 53 years with a standard deviation of 11 years. The average BMI of Caucasian participants was 32.4 ± 1.5 kg/m^2 and the average BMI of African Americans was 32.4 ± 1.3 kg/m^2.

4.2 Antibody Levels in Serum – Percent Change from Pre Vaccination to Post Vaccination

The null hypothesis for each IgG subclass was that there was no difference in the percent increase of antibody levels pre and post vaccination.
between the two groups, Caucasians and African Americans. A Wilcoxon rank-sum test was used to analyze the significance of each subclass because the assumption of a normalized population could not be assessed.

For Caucasians, the IgG1 antibody level in serum increased at an average of 191.3% from pre vaccination to post vaccination with a standard error of ± 46.2%. The sample size of this group was 22 people. For African-Americans, the IgG1 antibody level in serum increased at an average of 143.6% from pre vaccination to post vaccination with a standard error of ± 41.7%. The sample size of this group was 17 people. The difference between the means of these two groups for IgG1 was 24.41% ± 83.66%. For IgG1, the p-value was 0.4876, greater than the significance level of 0.05. Thus, the two groups did not have significantly different levels of increase in IgG1. The two groups are compared below in Graph 1.

**Graph 1.** The IgG1 antibody levels for Caucasian and African American participants increased from pre vaccination to post vaccination at 191.3% ± 46.2% and 143.6% ± 41.7% respectively.

The IgG2 antibody was in negligible levels in the population. For this year’s vaccine antigen, IgG2 was not highly expressed and was unable to be detected using the indirect ELISA.
For Caucasians, the IgG3 antibody level in serum increased at an average of 129.5% from pre vaccination to post vaccination with a standard error of ± 31.1%. The sample size of this group was 21 people. For African-Americans, the IgG3 antibody level in serum increased at an average of 153.9% from pre vaccination to post vaccination with a standard error of ± 87.1%. The sample size of this group was 16 people. The difference between these two means was 24.4% with a standard error of 83.7%. For IgG3, the p-value was 0.3818, greater than the significance level of 0.05. Thus, the two groups did not have significantly different levels of increase in IgG3. The two groups are compared below in Graph 2.

**Graph 2.** The IgG3 antibody levels for Caucasian and African American participants increased from pre vaccination to post vaccination at 129.5% ± 31.1% and 153.9% ± 87.1% respectively.

For Caucasians, the IgG4 antibody level in serum increased at an average of 75.9% from pre vaccination to post vaccination with a standard error of ± 40.9%. The sample size of this group was 22 people. For African-Americans, the IgG4 antibody level in serum increased at an average of -2.1% from pre vaccination to post vaccination with a standard error of ± 9.5%. The sample size of this group was 17 people. The difference between these two means was -78.1% with a standard error of 47.4%. For IgG4, the p-value was 0.0264, less
than the significance level of 0.05. Thus, the two groups did have significantly different levels of increase in IgG4. The two groups are compared below in Graph 3.

Graph 3. The IgG4 antibody levels for Caucasian and African American participants increased from pre vaccination to post vaccination at 75.9% ± 40.9% and -2.1% ± 9.5% respectively.

The data was also stratified to quantify how much each group’s antibody levels increased. The graphs below compare stratified percentage increases in the immunoglobulin levels pre and post vaccination within Caucasians compared to that in African Americans.

Graph 4. The percent increases in IgG1 from pre to post vaccination were stratified by groups of percentage increases.
Graph 5. The percent increases in IgG3 from pre to post vaccination were stratified by groups of percentage increases.

Graph 6. The percent increases in IgG4 from pre to post vaccination were stratified by groups of percentage increases.
CHAPTER 5
Discussion

5.1 Conclusions

In this study, the immune responses of African American and Caucasian women were compared by looking at the levels of antibody in serum pre-vaccination and post-vaccination. We had hypothesized that the African American population would have a stronger immune response due to previous studies that have shown higher levels of gene expression of proinflammatory cytokines in the African American population\(^{21}\). As with viral infection, variability in the immune response to vaccination is likely to be influenced by genotype\(^{22}\). These proinflammatory cytokines include interleukin-1 alpha and interleukin-6.

Cytokines play a major role in the innate immune system. Interleukin-1 is a cytokine produced by activated macrophages\(^{23}\). IL-1 plays a necessary role in the generation of a differentiation signal directly related to the B-cell, without which the subsequent B-cell maturation to Ig-secreting cells does not occur\(^{24}\). Interleukin-2 promotes the clonal selection of B-cells in humoral immunity. Interleukin-6, interleukin-4, interleukin-5, interleukin-10, and TGF-\(\beta\) all promote the differentiation of B-cells to plasma cells that release antibodies\(^{23}\).

The increased gene expression of these cytokines prompted a hypothesis of higher levels of antibody release with the African American population when compared to that of Caucasians. However, the study depicted no difference in the increase of IgG1 and IgG3 pre- and post- vaccination with the flu vaccine. In IgG4, there was a significantly higher average percentage increase in Caucasians compared to African Americans, which is opposite of what was expected. For IgG2, the ELISA method used to detect the antibodies was not able to detect the antibody at the titer levels currently in the population. Other types of ELISA may be more sensitive for future use including fluorescence detection.
Comparing the stratified percent increases shown in Graph 4, Caucasians had a greater percentage of participants that had a percentage increase in IgG1 of over 140% from pre to post vaccination as compared to African Americans. This trend can also be seen in graph 5, where the percentage of Caucasian participants that had a percent increase of IgG3 greater than 140% is higher than the percentage of African American participants that had a percent increase of IgG3 greater than 140%.

The significantly higher average percent increase in IgG4 within the Caucasian women compared to African American women from pre to post vaccination was supported by data displayed in Graph 6. The stratified percentage increases show that Caucasians had a less percentage of participants that had antibody increases at 0% or less compared to African Americans.

An explanation for the decreased immune response in African Americans is the high levels of chronic conditions such as diabetes and hypertension that exist within this group. The presence of secondary characteristics associated with these chronic conditions could decrease the effectiveness of their immune response and contribute to the lower average percentage increase in IgG4 antibody compared to Caucasians seen in this study. Furthermore, this could explain the lesser percentage of African Americans who experienced greater than a 140% increase pre to post vaccination in IgG3 and IgG1 as compared to Caucasians. Studies have shown a lower antibody response comparing pre and post vaccination in diabetic patients compared to non-diabetic patients.²⁵

This could have interesting implications in public health. A past study has shown that IgG4 can block cytotoxicity mediated by antibody-activated effector cells in Malaria. Thus, it is unclear now if lower levels of IgG4 can be associated with a weakened immune response to the influenza virus and may serve as a potential benefit for the African American population. Low IgG4 levels in the African American population may also be due to a natural, ancestral low levels of IgG4 that were inherent in this population for greater immunity to
Malaria. Furthermore, the stronger increases in the Caucasian population, indicated by the greater percentage of these participants who had percentage increases pre and post vaccination of IgG1 and IgG3 that were greater than 140%, could indicate that the Caucasian population may be in a chronic inflamed state. Thus, it may not be beneficial to the Caucasian population to have more participants have large increases in antibody response.

5.2 Limitations and Recommendation for Further Studies

This study had several limitations. The first was its small sample size. The study had 22 participants in the Caucasian group and 17 participants in the African American group. However, in the IgG3 group, only data from 21 Caucasians and 16 African Americans were used due to inconsistent ELISA readings. The low sample size decreases the power of this study and decreases its ability to draw significant conclusions. Further studies could be done that pulled a larger sample size by recruiting participants specifically for this study, instead of pulling from an already created subset.

Another limitation to this study was that height was self-reported. This could have skewed BMI readings because of inconsistent height reports. To avoid this in future studies, height should be recorded by data collectors. The marker of obesity used was BMI, which does not directly measure body fat and could be a limitation of the study. Furthermore, racial status was self-reported and did not distinguish between those who were racially mixed, despite the known 7-20% White admixture within African Americans, which would tend to reduce observed associations.21

Further studies could be done by stratifying each population by chronic conditions to compare the immune response between races with similar chronic conditions. In this way, the confounder of chronic disease can be removed. Another potential addition that could be added to this study would be to follow participants to see if they develop flu-like symptoms and compare this to their
IgG levels. This could provide insight into which patients have lower antibody responses as well as weakened immune systems. Another recommendation for future studies would be to look at CD8$^+$ and CD4$^+$ T-cell responses and cytokine levels to have a closer understanding of any differences in the immune response that may exist between the two races.

The age range of African Americans was 37 years to 71 years with an average age of 55 years. The age range of Caucasians was between 29 years to 73 years with an average age of 53 years. At an older age, the immune response to the flu vaccine is diminished and a similar age group should be used for that reason$^{26}$. In future studies, a set age range should be made in order to account for confounding factors of age.
References:


