Short Versus Long Term Obesity: Immune Response to Influenza Vaccination

Emily Wheeler

BSPH Nutrition

University of North Carolina at Chapel Hill

Gillings School of Global Public Health

April 25, 2016

ABSTRACT

Background: Obesity, a growing health crisis in the United States, is an independent risk factor for increased morbidity and mortality from infection with influenza. Annual vaccination is the preferred strategy to prevent infection with influenza virus, but obesity is associated with decreased protective antibody titer against influenza compared to that of healthy-weight individuals post-vaccination. No previous studies have compared the immune response to influenza vaccine with duration of obesity.

Objective: This study employed a sample of twenty short-term obese individuals and twenty long-term obese individuals to determine the IgG antibody response to the 2014-15 inactivated trivalent influenza vaccine pre-vaccination and thirty days post-vaccination.

Results: There was no significant difference in mean IgG 1, 2 or total IgG antibody titer for short term obese and long term obese individuals as detected by ELISA. However, long-term obese individuals did have a higher IgG 2 antibody titer than did short-term obese individuals. Additionally, the youngest, long-term obese individuals had the lowest pre-vaccination IgG 1 and IgG Total antibody titers, which started higher and negatively correlated with age for short term obese individuals pre- and post-vaccination as well as long-term obese individuals post-vaccination (Figures 4 and 6).

Conclusions: While we did not find differences in immune response to influenza vaccination as measured by IgG antibody titer due to duration of obesity, these data that the age of obesity onset may have an effect on immune response to influenza vaccination.

INTRODUCTION

The annual burden of influenza in the United States is estimated to be 25-30 million cases, leading to 30,000-40,000 deaths.[1] Approximately 10-20% of the population in developed countries is infected during influenza epidemics each year. Additionally, obesity is creating an additional health burden in the United States, with 34.9% of adults suffering from obesity in the US in 2015[2], as well as 42 million obese children under the age of 5 in the US in 2013.[3]

Obesity related conditions such as heart disease, type 2 diabetes, and some types of cancer have become some of the leading causes of preventable death in the US, and obesity itself is suggested to impair immunity in obese patients infected with pandemic H1N1 influenza.[4]

Annual influenza vaccination is the preferred strategy for decreasing the risk of infection with influenza virus. In 2011, Sheridan et al. examined the immune response to influenza vaccination among healthy weight and obese humans, becoming the first study to suggest that obesity may negatively affect human immune response to influenza vaccine.[5] Another study found that obese children had lower anti-tetanus IgG antibodies when compared to age-matched healthy weight controls, providing additional evidence of

obesity-related impairment of the immune response.[6] Mechanistically, over-nutrition increases anabolic hormones, including insulin and IGF-1 in the body. mTOR integrates these hormonal inputs from metabolic pathways and senses cellular nutrient content, but the mTOR pathway is dysregulated and continuously activated in obesity, which is a chronic state of over-nutrition in the most basic sense. Disrupted mTOR signaling and mitochondrial dysfunction increases cytokine production and decreases B and T cell proliferation, reducing the effectiveness of an immune response.[7]

Based on this prior research, I chose to compare individuals who had been obese for more than ten years with other individuals, who had been obese for less than ten years and were matched for age, BMI, and diabetic and smoking status to see if the duration of obesity and chronic over-nutrition further impaired the immune response to influenza vaccine compared to the simple presence of obesity. I chose ten years as the cut point for long term obesity because I hypothesized that it would be a reasonably long amount of time for the immune system to exhibit any obesity-fueled changes and because it was also a reasonable amount of time to allow me to find regular and continuous evidence of obesity over a period of ten years in the patient medical records. A longer cut-point for long term obesity would have been unfeasible for this study because many patients at the UNC Family Medicine Center did not have significantly more than ten years of data in their medical records. I, therefore, designed and carried out a small study using serum samples from 40 participants in the fifth year (2014-2015) of the same population as the Beck clinical research population.

I hypothesized that individuals who have been obese for ten years or longer would have a decreased immune response to influenza vaccine than would obese individuals who have been obese for less than ten years, as measured by influenza specific IgG antibodies detected by ELISA.

METHODS

Study design and subjects

This study was carried out at the University of North Carolina at Chapel Hill. The subjects were part of an ongoing, prospective observational study being conducted at the University of North Carolina Family Medicine Center. Forty participants who received the 2014-2015 seasonal trivalent influenza vaccine were selected post-collection for this analysis based on medical record data.

The 40 subjects were selected based on one of two criteria: The first was classified as "Short Term Obese (STO)" and consisted of subjects whose medical records showed a BMI change from healthy weight (BMI 18.5 ≤ 24.9) or overweight (BMI 25 ≤ 29.9) to obese (BMI > 30.0) within the last 10 years. The second group was classified as "Long Term Obese (LTO)" and consisted of subjects who had been medically obese (BMI > 30.0) for at least ten years, as verified by medical records. The 20 subjects in the two groups were matched based on sex, race, diabetic status, age, and BMI.

Table 1: Overall Demographic Data of Study Participants

	Short Term Obese (STO)	Long Term Obese (LTO)
Sex		
Female	12 (60%)	12 (60%)
Male	8 (40%)	8 (40%)
Race		
Caucasian	14 (70%)	14 (70%)
African American	5 (25%)	6 (30%)
Hispanic	1 (5%)	0 (0%)
Diabetic Status		
Non-diabetic	15 (75%)	16 (80%)
Type II	4 (20%)	4 (20%)
Type I	1 (5%)	0 (0%)
Age Range	26 - 75	22 - 70
BMI Category		
4 (BMI 30.0 – 34.9)	20 (100%)	17 (85%)
5 (BMI 35.0 – 39.9)	0 (0%)	3 (15%)

Table 2: Demographic Characteristics of Each Study Participant

Short Term Obese Subjects	First BMI	Date of 1st BMI	BMI approx 10 yrs later	Sex	Race	Diabetes?	Age	Long Term Obese Subjects	Sex	Race	Age	Diabetes?	BMI	Earliest BMI & 10 year BMI if available
1	24.21	12/2007	32.8	Female	Caucasion	No	48.9	1	Female	Caucasian	60.9	No	42.9	9/2009= 41.47
2	23.13	9/2005	29.5	Female	Caucasion	No	45.7	2	Female	Caucasion	54	No	39.3	6/2008=39.93 (first BMI)
3	27.27	1/2007	32.4	Female	Caucasion	No	43.2	3	Female	Caucasion	43.4	No	32.9	6/2004=30.45
4	28.1	3/2004	32.29	Female	Caucasion	No	59.7	4	Female	Caucasion	58.3	No	33.5	10/2006=32.25; 10/2002=30.6
5	28.22	3/2005	38.4	Female	Af Am	No	42	5	Female	Af Am	46.6	No	33.5	10/2003=33.59
6	28.59	10/2005	31.4	Female	Af Am	Type II	75.1	6	Female	Af Am	67.8	Type II	33.8	5/2004=36.30, 9/2004=34.01, 4/2006=36.96
7	26.02	6/2004	31.8	Female	Caucasion	No	63.5	7	Female	Caucasion	63.6	No	34.6	9/2004=40.57
8	27.45	6/2007	35	Male	Af Am	Type II	51.9	8	Male	Af Am	60	Type II	31.3	8/2004=33.1
9	25.29	1/2012	30.1	Male	Caucasion	Type II	55.4	9	Male	Af Am	62.7	Type II	34.5	5/2009=34.73
10	29.21	10/2009	34.5	Female	Caucasion	No	31.7	10	Female	Caucasion	38.4	No	41.2	5/2009=34.6
11	29.09	7/2009	33.5	Male	Caucasion	No	67.9	11	Male	Caucasion	70.8	No	32.6	12/2010=31.65
12	29.15	1/2007	34.2	Male	Af Am	No	60.7	12	Male	Af Am	61.9	No	33.9	9/2004=31.0
13	27.51	4/2011	30.7	Male	Caucasion	No	51.2	13	Male	Caucasion	59.6	No	33.9	3/1999=37.75, 3/2005=35.48
14	26.45	7/2006	37.3	Female	Af Am	No	38.6	14	Female	Af Am	34.6	No	37.9	8/2006=34.10 (first BMI)
15	26.76	8/2009	30.8	Male	Caucasion	No	49.8	15	Male	Caucasion	56.4	No	38.3	12/2008=39.89
16	24.02 25.12	6/2004 6/2005	32.6	Female	Caucasion	No	55.4	16	Female	Caucasion	56.7	No	44.8	10/2004=42.93
17	20.04 25.62	12/2004 2/2011	30	Male	Hispanic	No	26.2	17	Male	Caucasion	22.2	No	38.9	11/2004=33.13 at 12 years of age
18	27.60 26.79	5/2004 12/2006	32.9	Female	Caucasion	Type I	36.4	18	Female	Caucasion	35	No	38.6	4/2004=32.9;
19	28.86 27.45	6/2008 5/2009	30.5	Male	Caucasion	Type II	48.2	19	Male	Caucasion	63.3	Type II	30.8	9/2004 32.01
20	27.0 30.45	5/2002 7/2004	38.1	Female	Caucasion	No	59.6	20	Female	Caucasion	61.9	No	38.4	8/2004=40.34

ELISA

Immune response was quantified using enzyme linked immunosorbent assay (ELISA) and pre- and post-vaccination serum samples from each subject. IgG antibodies were quantified by ELISA, using the 2014-2015 trivalent influenza vaccine as antigen. Vaccine was diluted in PBS coating buffer, added to plates, and allowed to sit overnight in order for vaccine antigens to bind to the plate wells. Blocking buffer was then applied to plates and allowed to incubate for one hour to coat the plate wells and prevent any antibodies from binding nonspecifically to the well walls. After plates were washed, serum samples were diluted in a solution of nonfat milk and PBS and were plated and allowed to bind vaccine antigen. The quantity of vaccine specific antibodies present in the human serum correlates with how much serum derived antibody binds to vaccine-derived antigen. Antibodies from human serum bound to antigen were detected and bound by goat anti-human IgG conjugated to horseradish peroxidase, followed by a colored TMB substrate solution. The binding of the goat anti-human IgG to the human antibodies in the well, followed by the catalysis of the color-change reaction of the TMB substrate by the horseradish peroxidase bound to the goat anti-human IgG, creates a color intensity proportional to the quantity of human IgG antibody titer found in the serum sample. Color intensity was measured by absorbance at 450nm and internal control serum samples were included in each plate. Pre- and post-vaccination serum samples from each subject were tested on the same plate.

Statistics

Data were analyzed using Graphpad Prism and 2-way ANOVA analysis.

RESULTS

For each subject, ELISA antibody titer tests were used to measure IgG1, IgG2, and IgG Total as indicators of immune response. Antibody titer increased significantly from pre-vaccination to post-vaccination for IgG1, 2, and Total ($p < 0.0001$), indicating a significant immune response to vaccination for both short and long term obese individuals, as expected. However, Two-Way ANOVA analysis revealed that there was no significant difference in antibody titer increase between the short-term obese and long term obese groups for IgG1 ($p = .2615$) or IgG Total ($p = 0.0721$), contrary to my hypothesis. There was a significant difference in increase in IgG 2 antibody titer ($p = 0.0147$) between the short term and long-term obese subjects, with the long-term obese subjects showing greater increase in IgG 2 antibody titer after vaccination compared to the short-term obese group.

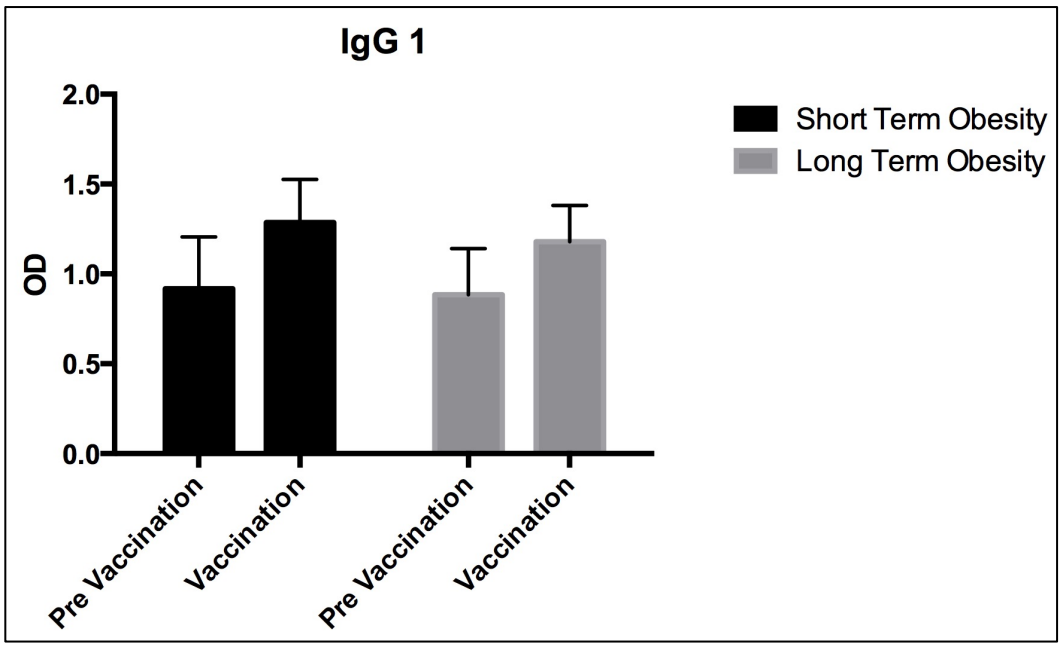

Figure 1: Mean IgG1 antibody titer pre- and post-vaccination for short term obese (STO) and long term obese (LTO) individuals.

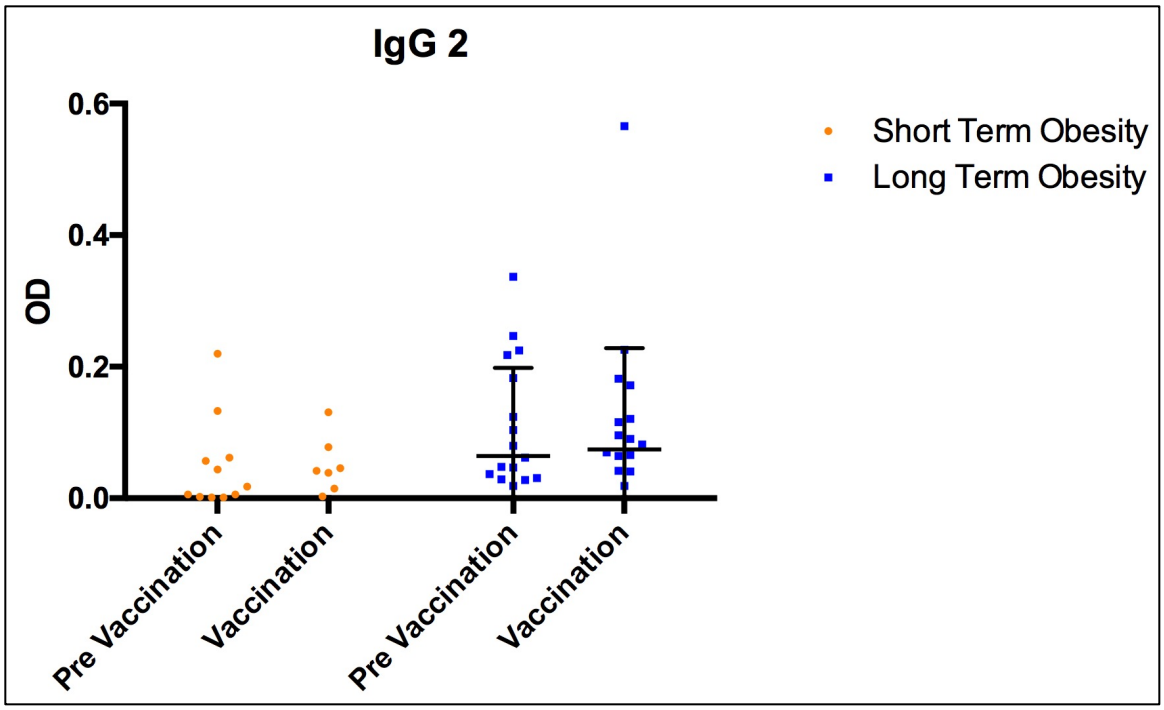

Figure 2: Mean IgG 2 antibody titer pre- and post-vaccination for STO and LTO individuals.

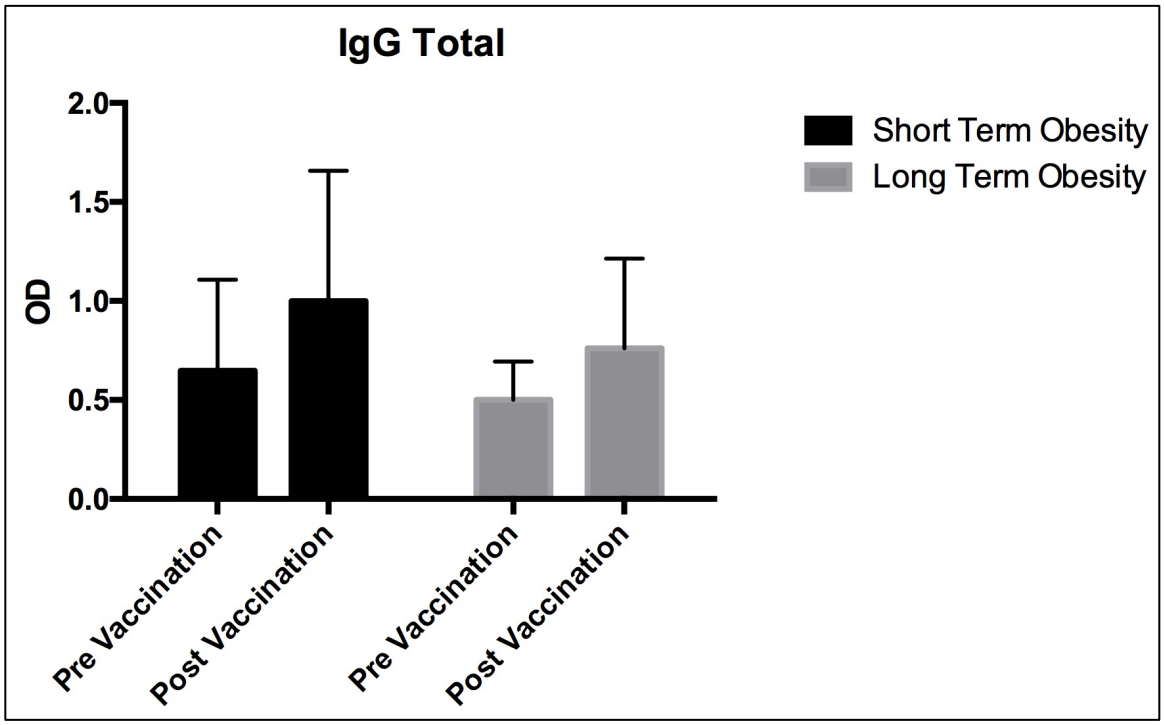

Figure 3: Mean total IgG antibody titers pre- and post-vaccination for STO and LTO individuals.

Interesting observations were also seen from the graphs of IgG versus age for the following four categories: short term obesity pre-vaccination, short term obesity post-vaccination, long term obesity pre-vaccination, and long term obesity post-vaccination. Short-term obese individuals had lower IgG 2 antibody titers at nearly all ages pre and post-vaccination compared to long-term obese individuals. Antibody titers for IgG 1 and IgG T exhibited more typical patterns of decline with age for short term obesity (pre and post-vaccination) and long term obesity post-vaccination. However, both IgG 1 and IgG Total exhibited the lowest antibody titer for young, long-term obese individuals pre-vaccination, which then increased with age.

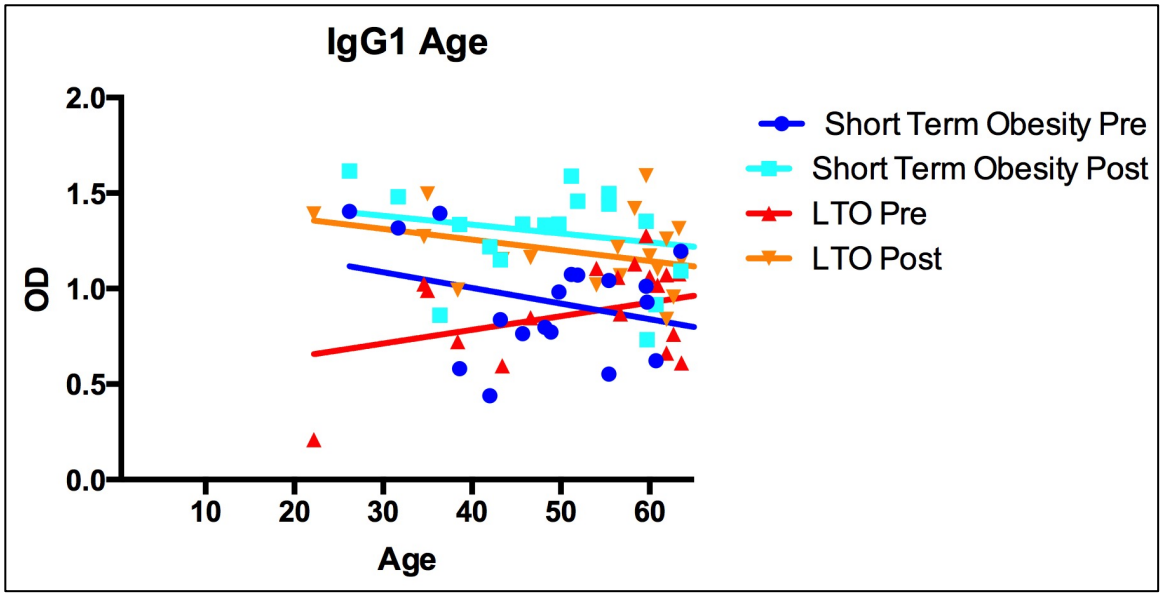

Figure 4: IgG 1 antibody titer versus age pre- and post-vaccination for STO and LTO individuals.

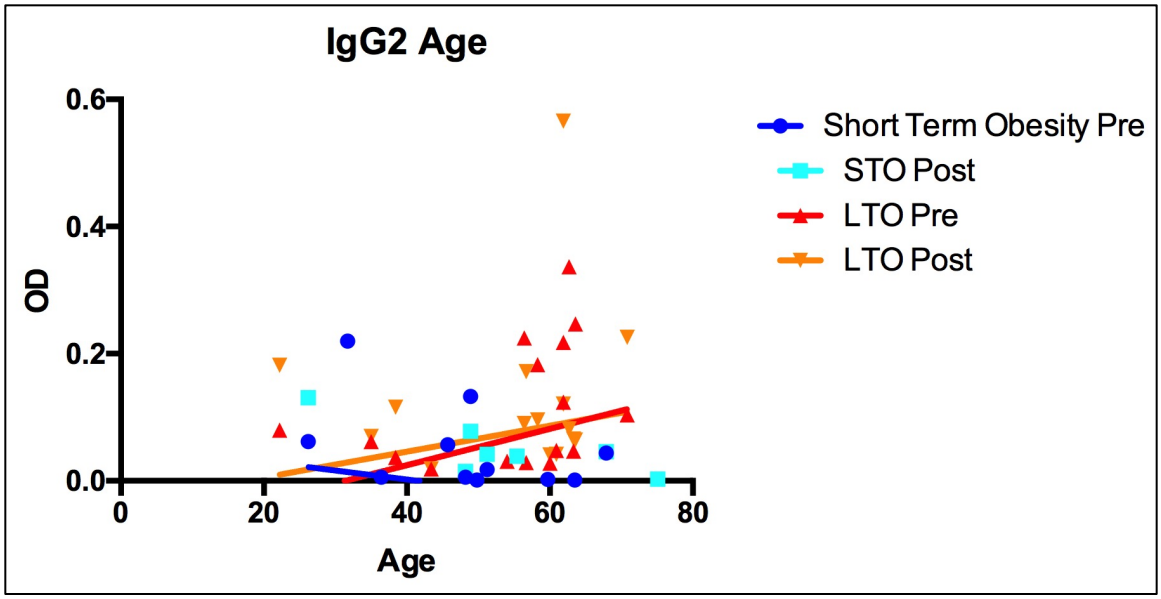

Figure 5: IgG 2 antibody titer versus age pre- and post-vaccination for STO and LTO individuals.

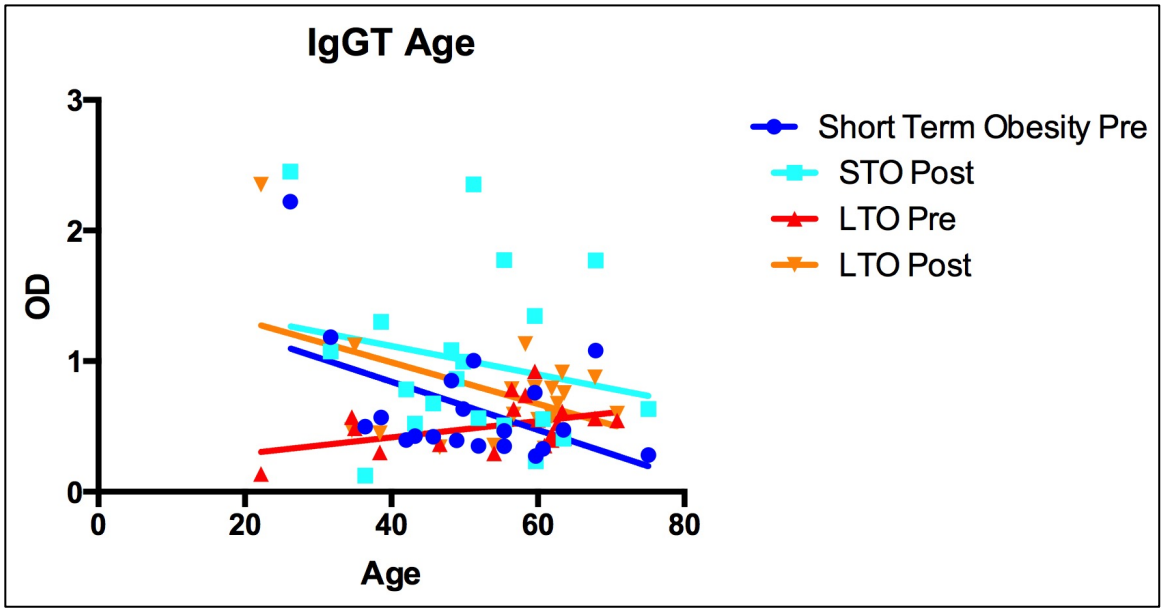

Figure 6: Total IgG antibody titer versus age pre- and post-vaccination for STO and LTO individuals.

DISCUSSION

Overall, long term obese individuals did not have a significantly poorer immune response to influenza vaccination when compared to short term obese individuals as measured by ELISA IgG antibody titer tests. However, an interesting trend shown in the graphs of IgG 1 and IgG T versus age revealed that the lowest antibody titers existed among the youngest long term obese individuals. Contrary to the typical pattern of antibody titer decreasing as age increases, the opposite held true for the long term obese individuals: the IgG 1 and IgG T antibody titers both increased with age among this group. Subjects who were 60-70 years old exhibited higher IgG 1 and IgGT antibody titers than subjects 20-40 years old. This surprising trend may reveal that the *age* of onset of obesity may be more impactful for immune response to influenza vaccine than the duration of obesity

Data for IgG 3 showed no significant differences between the antibody titers for the LTO and STO groups. Several negative values indicated that the background for these readings may have been too high considering that IgG 3 constitutes only 5-10% of total IgG present in humans and is therefore expected to be present in low concentrations among both groups.

The only statistically significant difference between the STO and LTO groups was exhibited in the IgG 2 antibody titers. The LTO individuals had higher IgG 2 antibody titers than the STO individuals. This outcome was unexpected due to the limited role of IgG 2 in the typical human response to influenza vaccine and because this finding was

contrary to the original hypothesis that LTO individuals would have lower antibody titers than STO individuals.

Because Immunoglobulin G is the major immunoglobulin found in serum and detection is typically indicative of prior infection or vaccination, I chose to use IgG as the major indicator of immune response to influenza vaccine. Of the four subclasses of IgG, IgG 1, 3, and 4 are involved in the immune response to protein and polypeptide antigens and IgG 2 is involved in the immune response to carbohydrate or polysaccharide antigens.[8] ELISA results for IgG 1 and IgG Total showed the least error, likely due the quantity present as IgG 1 comprises 60 – 65% of total IgG. Results for IgG 3 showed a large amount of variation, and error had a greater impact because IgG 3 comprises only 5-10% of total IgG. Based on the results of ELISAs for IgG 3, I chose not to run ELISAs for IgG 4 because it comprises less than 4% of total IgG and would have likely produced unreliable results due to error.[9]

IgG 2, the second most abundant IgG subtype, was the only IgG subtype for which there was a statistically significant difference between the long term and short term obese groups. The long term obese subjects had a higher IgG 2 antibody titer than the short term obese subjects, representing an unexpected immune response both because I hypothesized that long term obesity would further repress the immune response, as well as because increased IgG 2 antibody titer is not a typical response to influenza virus, which has no carbohydrate components. This response is therefore indicative of some

possible impairment in the immune response of long term obese individuals to influenza vaccine.

Although there were few statistically significant differences shown between the antibody titers of the long term individuals and the short term individuals, the findings displayed in Figures 1 and 3 indicate that the age of onset of obesity may have greater implications on immune response to influenza vaccine than does that actual duration of obesity. Future research has the potential to explore these mechanisms, possibly by comparing obese individuals who became obese before the age of twenty with obese individuals who became obese after the age of forty rather than comparing two groups of individuals on the criteria of the duration of their obesity.

Limitations

This study contained several limitations related to the subject pool. The sample size was small and the number of individuals with ten years of available medical record data from which to extract BMI history was limited. Additionally, many of the short term obese individuals had been obese for five to nine years and many of the long term obese individuals had been obese for eleven to fifteen years, decreasing the obesity duration gap between the short term and long term obese groups. Many of the individuals classified as short term obese had been overweight (BMI between 25 and 29.9) for several years prior to reaching a BMI greater than 30.0 and therefore being classified as obese. Due to the relatively arbitrary nature of using BMI to characterize obesity, the long-term overweight

individuals may have an immune response relatively similar to the long-term obese individuals, minimizing the differences in response between the two groups.

ACKNOWLEDGEMENTS

Dr. Melinda Beck
Principal Investigator and Mentor
Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Scott Neidich
PhD Student, Second Reader
Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Kim Bartholomew
Clinical Research Coordinator
Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

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