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Increased risk of influenza among vaccinated adults who are obese

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ABSTRACT

Background: Influenza infects 5-15% of the global population each year, and obesity has been shown to be an independent risk factor for increased influenza-related complications including hospitalization and death. However, the risk of developing influenza or ILI in a vaccinated obese adult population has not been addressed.

Objective: This study evaluated whether obesity was associated with increased risk of influenza and influenza-like illness among vaccinated adults.

Subjects and Methods: During the 2013-2014 and 2014-2015 influenza seasons, we recruited 1042 subjects to a prospective observational study of trivalent inactivated influenza vaccine (IIV3) in adults.1022 subjects completed the study. Assessments of relative risk for laboratory confirmed influenza and influenza-like illness were determined based on BMI. Seroconversion and seroprotection rates were determined using pre-vaccination and 26-35 days post-vaccination serum samples. Recruitment criteria for this study were adults 18 years of age and older receiving the seasonal trivalent inactivated influenza vaccine (IIV3) for the years 2013-2014 and 2014-2015. Exclusion criteria were immunosuppressive diseases, use of immunomodulatory or immunosuppressive drugs, acute febrile illness, history of Guillain-Barre syndrome, use of theophylline preparations, or use of warfarin.

Results: Among obese, 9.8% had either confirmed influenza or influenza-like-illness compared with 5.1% of healthy weight participants. Compared with vaccinated healthy weight, obese participants had double the risk of developing influenza or influenza-like illness (relative risk= 2.01, 95% CI 1.12, 3.60, p=0.020). Seroconversion or seroprotection rates were not different between healthy weight and obese adults with influenza or ILI.

Conclusions: Despite robust serological responses, vaccinated obese adults are twice as likely to develop influenza and influenza-like illness compared to healthy weight adults. This finding challenges the current standard for correlates of protection, suggesting use of antibody titers to determine vaccine effectiveness in an obese population may provide misleading information.

INTRODUCTION

Influenza is a serious worldwide public health problem. Seasonally, 5-10% of adults and 20-30% of children contract influenza virus, resulting in up to 500,000 deaths[1] and influenza pandemics greatly increase the number of infections and deaths. Indeed, the 1918 influenza pandemic was estimated to have infected 20-40% of the world's population, causing approximately 50 million deaths[2]. Historically, the highest risk groups for increased morbidity and mortality from influenza infection include the elderly[3], the very young[4], individuals with chronic diseases such as diabetes[5] or congestive heart failure[6], and pregnant women[7]. During the 2009 H1N1 pandemic, obesity was recognized as an independent risk factor for complications from influenza[8] and continues to be a risk factor for seasonal influenza strains[9] as well as for emerging influenza virus strains such as A(H7N9)(ref. 10). Obesity is not only a concern in the US, with 37% of adults obese[11], but also affects 14% of the worldwide adult population[12]. Therefore, with a growing obesity epidemic, complications from influenza infection would be expected to increase.

Influenza vaccine remains the primary method currently available for prevention of influenza infection. Each year, vaccines are formulated based on evaluations of previously circulating influenza strains. Typically, the vaccine contains two influenza A strains and one, or more recently two, influenza B strains. Vaccine-generated antibodies against the viral surface protein hemagglutinin (HA) are considered to be protective, therefore vaccines are standardized to the quantity of HA, generally 15 µg of HA per strain[13]. A serum hemagglutination inhibition (HAI) titer of 40 or greater has historically been considered an immunological correlate of protection from influenza infection, corresponding to 50% protection[14]. Protection against influenza infection increases up to an HAI titer of 160, beyond which further protective capacity is minimal[15]. High risk groups for influenza infection, including the elderly and children under 6 years of age, may need to reach titers greater than 40 to achieve protection[16]. To determine if obesity altered the risk of developing influenza or ILI in a vaccinated adult population, we

report the incidence of influenza infection and influenza-like illness (ILI) in vaccinated obese and healthy weight adults as well as the extent to which participants with influenza infection and ILI produced influenza specific antibodies.

SUBJECTS AND METHODS

Study Design

Participants were recruited as a part of a prospective observational study carried out at the University of North Carolina at Chapel Hill Family Medicine Center, an academic outpatient primary care facility in Chapel Hill, North Carolina. All procedures were approved by the Biomedical Institutional Review Board at the University of North Carolina. At enrollment, informed written consent was received.

Participants

Recruitment criteria for this study were adults 18 years of age and older receiving the seasonal trivalent inactivated influenza vaccine (IIV3) for the years 2013-2014 and 2014-2015. Exclusion criteria were immunosuppressive diseases including HIV, use of immunomodulatory or immunosuppressive drugs, acute febrile illness, history of hypersensitivity to any influenza vaccine components, history of Guillain-Barre syndrome, use of theophylline preparations, or use of warfarin. Height and weight were measured and a baseline serum sample drawn. BMI for each participant was calculated as weight (kg)/height(m)^2. Healthy weight was defined as a BMI of 18.5-24.9, overweight as a BMI of 25-29.9 and obese as a BMI of ≥30.

Vaccines and Sample Collection

One dose of 2013-2014 trivalent inactivated influenza vaccine (0.5 mL Fluzone; Sanofi Pasteur, Swiftwater PA, USA) containing A/California/07/2009 H1N1, A/Texas/50/2012 H3N2, and B/Massachusetts/02/2012 or 2014-2015 trivalent inactivated influenza vaccine (0.5 mL Fluvirin; Novartis Vaccines and Diagnostics Limited, Basel, Switzerland) containing A/California/07/2009 H1N1, A/Texas/50/2012 H3N2, and B/Massachusetts/02/2012 was administered in the deltoid muscle, using an inch and half needle, at baseline. Participants returned 26-35 days later for a post-vaccination blood

draw. In the 2013-2014 vaccine year, vaccination of participants started on September 16, 2013 and the last vaccination was given on November 4, 2013. Influenza was first detected in NC on November 30, 2013, and cases peaked on January 11, 2014, with influenza levels back to baseline on May 17, 2014. In the 2014-2015 vaccine year, vaccination of participants started on September 15, 2014 and were completed on October 28, 2014. Influenza was first detected in NC on November 29, 2014 and cases peaked on December 27, 2014. Influenza levels were back to baseline on April 25, 2015. Serum samples were stored at -80^{o} C until analyzed. During the 2013-2014 season in North Carolina, influenza 2009 A/H1/N1 was the predominant circulating strain, and during the 2014-2015 season, influenza A/H3N2/Switzerland was the predominant circulating strain.

Surveillance and diagnosis of influenza and/or influenza-like illness (ILI)

Participants were contacted weekly beginning with the first report of influenza activity in the community and contact was discontinued when influenza was no longer active. Participants were contacted by phone or email and asked to report any symptoms of fever, cough, runny nose, sore throat, muscle aches, headaches and fatigue to assess for influenza symptoms. Medical records of all study participants, whether they reported ILI or not, were reviewed at the end of each season for medically reported influenza-like illness or laboratory confirmed influenza. Participants were also instructed to contact the study nurse if they developed ILI. Laboratory confirmed influenza infection was determined from the medical records which reported a positive influenza specimen using the FDA cleared Cepheid Xpert Flu assay (Cepheid, Sunnyvale, CA). This assay distinguishes between influenza A and influenza B strains, but does not subtype the strains. All participants who tested positive for influenza were diagnosed with influenza A. ILI was defined using the CDC guidelines[17] as fever greater than 100^{o} F with a cough and in the absence of any other medical diagnosis. All subjects with laboratory confirmed influenza also met our criteria for ILI.

Immunogenicity

Hemaglutination Inhibition (HAI) Assay: The HAI titer was blindly determined in accordance with World Health Organization guidelines[18] for all patients reporting laboratory confirmed influenza or ILI, as well as matched non-illness reporting participants.

Microneutralization (MN) Assay: Standard microneutralizations (MN) were blindly performed against cell-grown A/Texas/50/2012 (H3N2) according to WHO guidelines[18]. Luminescent MN assays were blindly performed as previously described using a reverse genetics A/California/04/2009 (pdmH1N1) virus containing an NLuc on its polymerase segment[19]. MN were conducted for participants with laboratory confirmed influenza and matched controls who did not report any ILI during influenza season.

Matched non-illness reporting participants: For every participant who either had laboratory confirmed influenza or reported ILI, we matched them with a non-illness reporting participant. Matching of the non-illness reporting participants was done on a one-to-one basis with the 74 participants with either confirmed influenza or reporting ILI based on the following criteria in the order provided: 1) same vaccine year; 2) sex; 3) Race; 4) weight category; 5) diabetes status; 6) statin use; 7) smoking status; 8) age (within 10 years). All samples were uniquely matched.

Statistics

Individuals were categorized as underweight (BMI < 18.5), healthy weight (BMI 18.5 - 24.9), overweight (BMI 25.0 - 29.9), or obese (BMI ≥ 30). The Jonckheere–Terpstra test was employed to assess associations between baseline covariates and the ordinal weight category. Risk ratios for laboratory confirmed influenza and influenza-like Illness (ILI) were estimated by fitting a log-binomial model using generalized estimating equations (GEE) with an exchangeable working correlation structure to account for repeated observations per individual. Logistic regression models fit using GEE were utilized to examine associations of diabetes and statin use with obesity and risk of influenza/ILI. Microneutralization and HAI results were analyzed via 2-way repeated measures analysis of variance

(ANOVA). The Wilcoxon signed-rank test was used for comparisons between matched pairs. Seroconversion and seroprotection were analyzed by the chi-square test of independence. P-values less than 0.05 were considered statistically significant. Data presented in tables were analyzed using R[20] . Data presented in Figure 1 were analyzed using Graphpad Prism 6.0h for Mac OSX.

RESULTS

Demographics of Participants

During the 2013-2014 vaccine year, we enrolled 587 participants and 575 (98.0%) completed the study. During the 2014-2015 vaccine year, we enrolled 455 participants and 447 (98.2%) completed the study. As shown in Table 1, overall, our participants were 27% healthy weight, 28% overweight and 44% obese. In both years of the study, approximately 60% of the participants were Caucasian and 30% African-American. Female participants represented approximately 63% of the total participants. As has been reported for other studies, African-Americans[21,] and diabetics[22] were more likely to be obese, and statin use was associated with higher BMIs. However, statin use and diabetes was not independently associated with influenza or influenza-like illness. Most of the participants were either non-smokers, or had never smoked, with approximately 17% current smokers. There were 184 study subjects who participated in both years of the study, fifteen of which reported ILI in one or both years of the study.

Relative Risk for Influenza and ILI

In the total vaccinated adult participants for both vaccine years, there were 10 laboratory confirmed cases of influenza A and 64 cases of ILI (Table 2). Of the 74 participants with either confirmed influenza or ILI, 19% were healthy weight, 22% were overweight and 59% were obese. Relative to influenza incidence in vaccinated healthy weight adults, vaccinated obese adults had double the risk for laboratory confirmed influenza considered together with ILI (estimated risk ratio 2.06 with 95% confidence interval (CI) 1.14, 3.71). The risk ratio estimate was similar when adjusting for vaccine

year, age, sex, and smoking status using log-binomial regression (2.01, 95% CI 1.12, 3.60). Diabetes and statin use were not associated with influenza or influenza-like illness, however, as expected, BMI category was a significant predictor of diabetes and statin use.

Seroprotection and Seroconversion

Among the 74 cases of confirmed influenza or ILI during 2013-2014 and 2014-2015, 30 (41%) seroconverted (four-fold increase from pre- to post-vaccination HAI titer) to vaccine strain A/H1N1/California/pdm2009 and 34 (46%) seroconverted to vaccine strain A/H3N2/Texas/50/2012. For these same 74 participants, 70% reached a seroprotective titer (26-35 day post vaccination HAI titer $\geq$ 40) for A/H1N1/California/pdm2009 and 80% reached a seroprotective titer for A/H3N2/Texas/50/2012. However, no differences were observed in seroprotective or seroconversion rates based on BMI (Table 3 and Figures 1a-b).

During the 2014-2015 influenza season, the H3N2 vaccine strain was a poor match for the circulating strain[23]. Therefore, for the 43 cases of laboratory confirmed influenza or ILI during 2014-2015, we measured HAI titers pre and post vaccination against the circulating influenza A/H3N2/Switzerland/9715293/2013 strain. Seroconversion for this strain occurred in 19 (44%) participants, and seroprotection was present in 30 (70%) participants. Again, no differences were observed based on BMI (Table 3 and Figures 1c). Higher, alternative cutoffs for seroprotection levels of HAIs at 80, 160 and 320 were also determined, and no differences were observed based on BMI (Table 3).

Laboratory confirmed Influenza and ILI compared to non-illness reporting matched controls

All 74 participants with either laboratory confirmed or ILI were matched with non-illness reporting participants and their demographics are shown in Supplemental Table 1. There were no differences in pre or post HAI titers for the vaccine strains A/California/H1N1/pdm2009 (Figure 1d) and A/Texas/H3N2/50/2012 (Figure 1e) or for the circulating 2015 influenza strain A/H3N2/Switzerland/9715293/2013 (Figure 1f) between participants reporting ILI and their non-reporting matched controls. Similarly, there was no difference in HAI (Figures 1g-h) or MN influenza titers (Figures 1 j-k) between participants with laboratory confirmed influenza and their matched, uninfected controls.

DISCUSSION

The first influenza pandemic of the 21st century resulted in identifying obesity as an independent risk factor for increased severity from infection with Influenza A/pH1H1/2009[8]. Since that time, obesity has also been identified as a risk factor for seasonal and emerging influenza strains. This is highly significant, in that obesity levels in the US population are at epidemic proportions, with 37% of adults overall obese[11] and even higher rates in non-Hispanic blacks (48%)[21]. Obesity rates worldwide have doubled since 1980 and currently 13% of the world's adult population is obese[12], leaving a large number of obese adults in the US and worldwide at significant risk for infection with influenza virus.

Influenza vaccination represents the best method of protection from infection with influenza virus. Several studies have suggested that overweight and obesity impairs vaccine response to several pathogens. For example, non-responders to hepatitis B vaccination are overrepresented in obese adults[24], while tetanus toxoid response in overweight children is similarly impaired[25]. A recent review on the association of obesity with vaccine responses points to a number of studies that demonstrate

diminished vaccine-induced immune responses in both obese adults and children[26]. We have also documented impaired vaccine-specific T cell responses in influenza vaccinated obese adults[27], and a waning serological response one year post vaccination[28]. Despite the growing number of studies implicating obesity in poor responses to vaccination, and specifically influenza vaccination, a key question remains unanswered: in obesity and healthy weight, does vaccination offer the same protection from influenza and ILI?

Here, for the first time, we demonstrate that obese adult recipients of IIV3 have two times greater incidence of influenza and/or ILI despite being vaccinated. One obvious hypothesis for the increase in influenza and ILI in obese adult participants is a failure to seroconvert or reach seroprotective levels of antibody. Serological responses to influenza vaccination are typically assessed as seroprotection, defined as an HAI titer of 40 or greater post vaccination, or seroconversion, defined as a 4 fold or greater increase in HAI from prevaccination titer to post vaccination titer. However, we found that the increased susceptibility to influenza and ILI in the obese adults was not associated with a failure to reach a seroprotective titer or to seroconvert. Indeed, we found no statistical differences in serological responses to vaccine between healthy weight and obese vaccinated adults. For the H1N1 strain, 36% of healthy weight adults seroconverted compared with 43% of obese adults. Similarly, seroconversion rates to the H3N2 vaccine strain were 43% of healthy weight adults and 50% of obese adults. When using the commonly defined seroprotective HAI titer of ≥40, more than 70% of the healthy weight and obese participants reached this HAI level.

The presence of a “seroprotective” level of antibody against influenza A strains demonstrates that, despite the vaccine inducing this this correlate of protection, the obese adults were still 2X more likely to develop influenza and ILI. This lack of protection, even with a seroprotective antibody titer, has also

been observed in elderly adults[29] and children[16], where a higher HAI definition as a correlate of protection has been proposed. Our data, however, do not suggest an elevated definition is protective for obese adults. Raising the seroprotective cutoff level to 80, 160 and 320 still failed to differentiate healthy weight adults from obese adults.

The 2014-2015 influenza vaccine effectiveness overall was reduced (13% vs 61% in 2013-2014) due to the circulating Influenza A H3N2 strain having drifted from the H3N2 vaccine strain[23]. Therefore, for all participants who had influenza or ILI during the 2014-2015 vaccine season, we measured HAI antibody titer against the circulating A/Switzerland/9715293/2013 strain. Despite the mismatch with the vaccine strain, IIV3 induced seroconversion among 67% of the healthy weight and 32% of the obese participants. For a seroprotective level of ≥40 HAI, 67% of healthy weight and 60% of obese participants achieved this level. There were no statistical differences in seroprotection or seroconversion rates between healthy weight and obese adults.

We found no differences in HAI titers between non-illness reporting participants and participants reporting ILI. In addition to HAI, virus microneutralization (MN) titers are a highly sensitive and specific method for detecting antibodies that inhibit viral entry or exit out of the cell. Cheng *et al*.[30] reported that, compared to HAI titers, MN titers demonstrated a greater seroconversion rate and fold increase and suggested that neutralizing antibody titers may be a better correlate of protection for understanding influenza vaccine effectiveness. However, as was found for HAI titers, there were no differences in MN titers between uninfected controls and infected participants.

Our study has several limitations. Although we used the CDC's stringent definition for ILI and ILI is widely used for influenza surveillance reporting, we did not do specific testing for influenza in subjects

with ILI. Therefore we could be over-reporting, as some of the ILI subjects may be positive for a respiratory virus other than influenza, or under-reporting, as the more stringent CDC criteria may miss some milder ILI symptoms that are influenza positive. By only collecting ILI data during times of influenza circulating in the community, this helps to reduce over-reporting, but it doesn't eliminate this possibility. In addition, our study does not address the possibility that obese adults may be more exposed to influenza compared with healthy weight adults. Under this possibility, the influenza vaccine may equally protect healthy weight and obese adults, however an increased rate of infection exposure in obese adults could lead to an increased rate of infection in vaccinated obese adults compared with healthy weight adults. However, Murphy et al.[31] used data from the 2010 Health Survey for England, which asked in a survey question administered during the year following the 2009 H1N1 influenza pandemic whether participants had experienced "flu-like illness where [respondents] felt feverish and had a cough or sore throat," and considered cases between May and December 2009 to be flu-like illness in that study. The investigators found no relationship between ILI (including laboratory confirmed influenza) and obesity. This finding may suggest that influenza infection rates in healthy weight and obese adults are similar, and therefore our findings are related to a failure of the vaccine to protect obese adults to the same extent as healthy weight adults. Indeed, in an animal model, Karlsson *et al.*[32] reported that although lean mice were protected from influenza infection following vaccination, diet-induced obese mice were still susceptible to influenza infection despite vaccination. This contrasts with school aged children, where live attenuated influenza vaccination was shown to reduce risk for laboratory confirmed influenza similarly for healthy weight and obese[33]. These contrasting findings may be driven by differences in vaccine preparation (live versus inactivated), or by differences between obese adults and children.

The findings reported here demonstrate that, compared to vaccinated healthy weight adults, vaccinated obese adults were 2X more likely to develop influenza infection and ILI. Notably, HAI antibody titers,

widely viewed as correlates of protection against influenza, were unreliable as predictive of disease protection in obese adults. Previously, we[28] and others[34-36] have reported that HAI antibody titers 30 days post vaccination in obese adults or children are either slightly higher or no different from vaccinated healthy weight individuals. The present study confirmed these earlier reports on vaccine-induced antibody titers. However, here we found that an HAI antibody titer of 40 or higher was not a serological correlate for vaccine-induced protection and did not prevent laboratory confirmed influenza and ILI in obese adults. Additionally, MN titers in obese adults were also inadequate predictors of protection and these studies directly correlate with studies conducted in obese mice[32]. Although our study does not compare vaccinated obese adults with unvaccinated obese adults, it is clear that vaccinated obese adults are at a higher risk for influenza and ILI compared to vaccinated healthy weight adults.

The mechanism for increased risk of influenza and ILI in the obese population may be due to poor T cell function. As we have reported previously, compared with T cells from vaccinated healthy weight adults, T cells from influenza vaccinated obese adults are less activated when stimulated with vaccine strains of influenza[26,27]. As T cells are necessary for protection and recovery from influenza, impaired T cell function, despite a robust serological response, may render vaccinated obese adults more susceptible to influenza infection. Indeed, vaccinated elderly adults are also less protected from influenza infection despite having an adequate serological response, which has been attributed to poor T cell responses[29].

Taken together, these results suggest that the effectiveness of influenza vaccines, and perhaps other vaccines as well, should be fully assessed in obese adults. For example, use of adjuvanted influenza vaccines such as MF59 (FLUAD, Seqirus) or high-dose vaccine preparations that are designed for vaccinating adults over 65 may be warranted for use in an obese population. However, animal studies suggest that even adjuvanted vaccines or increased dosage may not overcome the increased

susceptibility of the obese host[32]. Therefore, alternative approaches may be needed to protect obese adults from both seasonal and pandemic influenza virus infection.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Figure 1. Influenza HAI and MN antibody titers for influenza confirmed and ILI participants and uninfected controls. Pre and post influenza vaccination HAI titers of participants with influenza confirmed and ILI against (**a**) A/California/pdm2009, (**b**) A/Texas/50/2012 and (**c**) A/Switzerland/9715293/2013. Pre and post vaccination titers were not statistically different among healthy weight (white with open circles), overweight (checkered with black circles) or obese (grey with grey circles) participants. Pre and post influenza vaccination HAI titers in participants with ILI (open circles and grey boxes) and demographically matched uninfected controls (black circles and black boxes) for (**d**) A/California/pdm2009, (**e**) A/Texas/50/2012 and (**f**) A/Switzerland/9715293/2013. Pre and post vaccination titers were not statistically different between ILI participants and non-illness reporting matched controls. Pre and post influenza vaccination HAI (**g, h, i**) and MN **(j,k**) titers in participants with confirmed influenza (open circles) and matched uninfected controls (black circles). Pre and post HAI or MN vaccination titers were not statistically different between confirmed influenza participants and matched controls. a,b: n=14 for healthy weight, n=16 for overweight, n=44 obese; c: n=9 healthy weight, n=9 for overweight, n=25 for obese. d, e: n=74; f: n=43, g, h, j, k: n=10; I: n=7.

Table 1: Demographics of all participants by study year and of participants with influenza or influenza like illness

			Underweight: BMI<18.5	Healthy Weight: BMI18.5-24.9	Overweight: BMI 25.0-29.9	Obese: BMI≥30	Total	P-Value[b]
2013-2014	Overall	N	6 (1)	143 (25)	170 (29)	256 (45)	575	0.57
		Age[a]	53 ± 20	55 ± 18	54 ± 16	54 ± 12	54 ± 15	
	Sex	Female	3 (1)	92 (25)	98(26)	178 (48)	371	0.09
		Male	3 (2)	51 (25)	72 (35)	78 (38)	204	
	Race	Caucasian	6 (2)	104(29)	114 (31)	140 (38)	364	0.001
		African American	0 (0)	23 (13)	45 (26)	107 (61)	175	
		Other	0 (0)	16 (45)	11 (33)	9 (22)	36	
	Diabetes	Pre Diabetes	1 (2)	9 (15)	10 (21)	27 (62)	47	0.00002
		Type 1	0 (0)	3 (60)	1 (20)	1 (20)	5	
		Type 2	0 (0)	12 (9)	29 (23)	86 (68)	127	
		Non-Diabetic	5 (1)	119 (31)	130 (33)	142 (35)	396	
	Smoking	Current Smoker	1 (1)	29 (28)	33 (32)	38 (39)	101	0.4
		Previous Smoker	1 (1)	36 (24)	50 (31)	74 (44)	161	
		Non Smoker	4 (1)	78 (25)	87 (27)	144 (47)	313	
	Statin Use	Current Statin Use	0 (0)	41 (18)	63 (28)	119 (53)	223	0.0001
		No Statin	6 (2)	102 (29)	107 (30)	137 (31)	352	
2014-2015	Overall	N	6 (1)	134 (30)	113 (26)	194 (43)	447	0.64
		Age[a]	54 ± 21	54 ± 18	60 ± 17	55 ± 14	56 ± 16	
	Sex	Female	4 (1)	89 (31)	58 (20)	139 (48)	290	0.15
		Male	2 (1)	45 (29)	55 (35)	55 (35)	157	
	Race	Caucasian	3 (1)	98 (34)	74 (26)	112 (39)	287	0.06
		African American	0 (0)	20 (16)	34 (27)	73 (57)	127	
		Other	3 (1)	16 (4)	5(1)	9 (2)	33	
	Diabetes	Pre Diabetes	1 (3)	2 (7)	6 (21)	19 (69)	28	0.00002
		Type 1	0 (0)	0 (0)	1 (33)	2 (67)	3	
		Type 2	0 (0)	9 (11)	19 (22)	57 (67)	85	
		Non Diabetic	5(2)	123 (37)	87 (26)	116 (35)	331	
	Smoking	Current Smoker	2 (3)	24 (33)	15 (21)	31 (43)	72	0.4
		Previous Smoker	1 (1)	31 (23)	40 (29)	64 (47)	136	
		Non Smoker	3 (1)	79 (33)	58 (24)	99 (41)	239	

	Statin Use	Current Statin Use	0 (0)	34 (19)	50 (28)	94 (53)	178	0.00006
		No Statin	6 (2)	100 (37)	63 (23)	100 (37)	269	
2013-2015 Confirmed Influenza or ILI	Overall	N	0 (0)	14 (19)	16 (22)	44 (59)	74	0.41
		Age[a]		52 ± 17	59 ± 15	57 ± 12	56 ± 14	
	Sex	Female	0 (0)	10 (14)	10 (16)	36 (70)	56	0.2
		Male	0 (0)	4 (33)	6 (39)	8 (28)	18	
	Race	Caucasian	0 (0)	12 (23)	11 (25)	25 (52)	48	0.05
		African American	0 (0)	2 (12)	5 (12)	18 (76)	25	
		Other (Asian)	0 (0)	0 (0)	0 (1)	1 (0)	1	
	Diabetes	Pre Diabetes	0 (0)	2 (0)	0 (11)	7 (89)	9	0.04
		Type 1	0 (0)	0 (0)	0 (0)	0 (0)	0	
		Type 2	0 (0)	1 (0)	3 (24)	13 (76)	17	
		Non Diabetic	0 (0)	11 (29)	13 (23)	24 (48)	48	
	Smoking	Current Smoker	0 (0)	3 (23)	4 (31)	6 (46)	13	0.8
		Previous Smoker	0 (0)	2 (7)	7 (24)	20 (69)	29	
		Non Smoker	0 (0)	9 (28)	5 (12)	18 (60)	32	
	Statin Use	Current Statin Use	0 (0)	5 (17)	6 (20)	19 (63)	30	0.64
		No Statin Use	0 (0)	9 (21)	10 (23)	25 (57)	44	

[a]Age is reported in years +/- SD

[b] The p-values are shown for an overall test of association between the covariate and the weight category using the Jonckheere-Terpstra test. As previously reported and also found here, prediabetes, type 2 diabetes and statin use is associated with weight, and overweight/obesity is present at a higher rate in African-Americans[21] and females are frequently more at risk for influenza infection than males[37].
All other values reported as number of participants within each weight class with the indicated demographic.

Table 2: Obesity is associated with greater risk for influenza and influenza like illness						
		Underweight (BMI<18.5)	Healthy Weight (BMI 18.5-25)	Overweight (BMI 25-30)	Obese (BMI>30)	Total
2013-2014	Laboratory confirmed influenza	0	0	1	2	3
	Influenza-like Illness	0	5	6	17	28
	No flu-like Illness	6	138	163	237	544
2014-2015	Laboratory confirmed influenza	0	1	3	3	7
	Influenza-like Illness	0	8	6	22	36
	No flu-like Illness	6	125	104	169	404
Total		12	277	283	450	1022
Risk Ratio vs Healthy Weight[a]		-	-	1.27	2.01	
95% CI[b]		-	-	(0.64,2.52)	(1.12,3.60)	
Significance		-	-	NS[c]	p=0.020	

[a]Risk ratio estimates are for laboratory confirmed influenza and influenza-like Illness (ILI) combined. Estimates were obtained using a log-binomial model fit using generalized estimating equations (GEE) with an exchangeable working correlation structure to account for repeated observations per individual. The model was adjusted for vaccine year, age, sex and smoking. Underweight and healthy weight individuals were combined into a single referent category due to the small number of underweight individuals.

[b]CI: confidence interval.

[c]NS: not significant, p>0.05.

Table 3. Obesity was not associated with seroconversion and seroprotection levels in participants with confirmed influenza and ILI

			Healthy Weight (BMI 18.5-24.9)	Overweight (BMI 25-29.9)	Obese (BMI ≥ 30)	Total
A/H1N1 California /pdm2009	[a]Seroconversion	4-Fold Increase or greater	43%	38%	41%	41%
	[b]Seroprotection	HAI ≥ 40	71%	79%	68%	70%
		HAI ≥ 80	64%	31%	50%	49%
		HAI ≥ 160	36%	13%	25%	24%
		HAI ≥ 320	14%	6%	11%	11%
		N[c]	14	16	44	74
A H3N2 Texas 50/2012	Seroconversion	4-Fold Increase or greater	50%	44%	46%	46%
	Seroprotection	HAI ≥ 40	86%	69%	82%	80%
		HAI ≥ 80	57%	44%	55%	53%
		HAI ≥ 160	43%	25%	32%	32%
		HAI ≥ 320	14%	6%	11%	11%
		N[c]	14	16	44	74
A/H3N2 Switzerland 9715293/2013	Seroconversion	4-Fold Increase or greater	67%	56%	32%	44%
	Seroprotection	HAI ≥ 40	67%	100%	60%	70%
		HAI ≥ 80	33%	78%	28%	40%
		HAI ≥ 160	11%	33%	8%	14%
		HAI ≥ 320	11%	11%	4%	7%
		[c]N	9	9	25	43

[a]Seroconversion is defined as a ≥4-fold increase in HAI titer 25-35 days post vaccination from pre-vaccination titer.
[b]Seroprotection is typically defined as an HAI of ≥40, however multiple cut-off points were assessed as indicated. There were no significant associations between weight and the odds of seroconversion for the multiple cut-off points considered.
[c]N is number of subjects by column and varies by virus as A/H3N2/Switzerland/9715293 was not assessed in participants from the 2013-2014 influenza vaccine season.