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The antibody response to influenza vaccination is not impaired in type 2 diabetics

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ABSTRACT

Background: Diabetics are considered to be at high risk for complications from influenza infection and type 2 diabetes is a significant comorbidity of obesity. Obesity is an independent risk factor for complications from infection with influenza. Annual vaccination is considered the best strategy for protecting against influenza infection and it's complications. Our previous study reported intact antibody responses 30 days post vaccination in an obese population. This study was designed to determine the antibody response to influenza vaccination in type 2 diabetics.
Methods: Subjects enrolled were 18 or older without immunosuppressive diseases or taking immunosuppressive medications. A pre-vaccination blood draw was taken at time of enrollment, the subjects received the influenza vaccine and returned 28–32 days later for a post-vaccination blood draw. Height and weight were also obtained at the first visit and BMI was calculated. Antibody levels to the vaccine were determined by both ELISA and hemagglutination inhibition (HAI) assays.
Results: As reported in our previous work, obesity positively correlates with the influenza antibody response ($p = 0.02$), while age was negatively correlated with antibody response ($p < 0.001$). In both year 1 and year 2 of our study there was no significant difference in the percentage of the type 2 diabetic subjects classified as seroprotected or a responder to the influenza vaccine compared to the non-diabetic subjects.
Conclusions: These data are important because they demonstrate that diabetics, considered a high risk group during influenza season, are able to mount an antibody response to influenza vaccination that may protect them from influenza infection.

1. Introduction

Type 2 diabetes (T2D) is a significant comorbidity associated with obesity. The comorbidities associated with obesity and infection with influenza virus are significant public health concerns. Currently, greater than two-thirds of the US population is classified as overweight or obese, with 34% of the population being classified as obese [1]. Twenty nine million Americans (9.3% of the population) have diabetes with an additional 35% classified as having pre-diabetes [2]. Infection with influenza results in 3000–49,000 deaths in non-pandemic years [3,4] and during the pH1N1 pandemic of 2009, studies suggested that diabetics were at a greater risk for hospitalization and increased complications from influenza [5–7].

Influenza vaccination remains the single most effective way to prevent serious influenza infection. The Centers for Disease Control considers diabetics to be at a higher risk for morbidity and mortality from influenza [9]. Diabetics are at greater risk for "complicated" influenza and longer hospital stays when infected, therefore, the CDC recommends that all diabetics over 6 months of age receive the trivalent inactivated form of the influenza vaccine [9,10]. Despite this recommendation, there are very few

Abbreviations: HAI, hemagglutination inhibition assay; HbA1c, glycosylated hemoglobin; T2D, type 2 diabetes; TIV, trivalent influenza vaccine.

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Table 1
Demographic characteristics of 2010–2011 participants[a].

		Underweight[b]	Healthy weight	Overweight	Obese	Total
Year 2						
Enrolled[a]		5 (1.1)	113 (24.4)	145 (31.3)	200 (43.2)	463
Age[c]		62.6 ± 21.4 (27–83)	54.2 ± 15.8 (19–88)	56.9 ± 16.4 (22–86)	52.0 ± 13.7 (18–83)	
Gender[a]	Male	1 (0.2)	43 (9.3)	65 (14.0)	73 (15.8)	182 (39.3)
	Female	4 (0.9)	70 (15.1)	80 (17.3)	127 (27.4)	281 (60.7)
Race[a]	White	4 (0.9)	87 (18.8)	108 (23.3)	113 (24.4)	312 (67.4)
	AA	1 (0.2)	19 (4.1)	35 (7.6)	84 (18.1)	139 (30.0)
	Other	0 (0)	7 (1.5)	2 (0.4)	3 (0.6)	12 (2.6)
Diabetes[a]	Yes	0 (0)	9 (1.9)	21 (4.5)	72 (15.6)	102 (22.0)
	No	5 (1.1)	104 (22.5)	121 (26.1)	123 (26.6)	353 (76.2)
	Unknown	0 (0)	0 (0)	3 (0.6)	5 (1.1)	8 (1.7)

[a] Number (percentage, out of $n = 463$).
[b] BMI: underweight (<18.5), healthy weight (18.5–24.9), overweight (25–29.9), obese (≥30).
[c] Mean ± SD (Range).

studies that have examined the response to vaccination in T2D. A systematic review of hepatitis B vaccine studies in diabetic populations suggests that older diabetics have an impaired response to vaccine compared to older non-diabetics [11]. A small study of an adult, mixed diabetic population (both Type 1 and Type 2, $n = 49$) showed that the antibody response to the monovalent pH1N1 vaccine suggest there was a negative correlation between HbA1c levels and seroprotection. To determine if the antibody response to the trivalent influenza vaccine is impaired in T2D subjects, we measured serum antibody titers in influenza vaccinated T2D and healthy controls. Here, we report that T2D did not affect influenza specific antibody titers 30 days post influenza vaccination.

2. Materials and methods

2.1. Study design and subjects

This is an ongoing, prospective observational study carried out at the University of North Carolina Family Medicine Center, an academic outpatient primary care facility in Chapel Hill, NC. Eligible participants were adult patients at the Center scheduled to receive the 2009–2010 or 2010–2011 seasonal trivalent influenza vaccine (TIV). Enrollment and data analysis were conducted independently for each year because of the annual change in vaccine composition. Exclusion criteria were 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 [12,13]. Diabetes status (Type 2) was self-reported and confirmed from medical records (physician diagnosis, glycosylated hemoglobin (HbA1c) and fasting glucose levels). HbA1c values from within 6 months of vaccination were obtained from the medical records of subjects enrolled in the study. The medications that the diabetic subjects were taking at the time of enrollment are listed in Supplemental Table 1. These medications were not used as part of the analysis. All procedures were approved by the Biomedical Institutional Review Board at the University of North Carolina.

In year 1 of the study (September–November 2009), we enrolled 499 participants. At enrollment, informed consent, height, weight and a baseline serum sample were obtained. One dose of 2009–2010 seasonal TIV ((0.5 ml Fluzone (Sanofi Pasteur, Swiftwater, PA, USA) containing A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2) and B/Brisbane/60/2008)) was administered in the deltoid muscle. Participants (461, 92% completion rate) returned 28–35 days later for a post-vaccination blood draw. Pre- and post-vaccination serum samples were stored at −80 °C until analyzed.

In year 2 of the study, (September–November 2010) we enrolled 489 and 463 completed the study (94.6% completion rate). The procedures for enrollment, consent, sample collection and storage were the same as year 1. The 2010–2011 seasonal TIV contained A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008.

The full demographic table for year 1 of the study has been previously published [8]. The data for year 2 are in Table 1.

2.2. ELISA for total anti-vaccine IgG

The method for the anti-influenza vaccine ELISA has been previously published [8]. This ELISA allows for the measurement of the total IgG made to all components of the influenza vaccine. Run in conjunction with the hemagglutination inhibition assay (described below), the combination of these two assays allows for a fuller description of antibody response to the vaccine in each subject. Briefly, vaccine was diluted and adsorbed to microtitration plates in a carbonate coating buffer. After washing, triplicate serum dilutions in PBS were allowed to react with antigen, and bound antibodies were detected by a peroxidase-conjugated goat anti-human IgG (Abcam, Cambridge, MA), followed by a chromogenic substrate. Color intensity was measured by absorbance at 450 nm. Internal control sera were included in each run. Pre- and post-vaccination sera from each subject were tested in the same run. The intra-assay coefficient of variation of the assay was 4%.

2.3. Hemagglutination inhibition assay

HAI assays were conducted to determine the level of antibodies in sera as previously described [14]. In contrast to the total vaccine component ELISA, the HAI assay measures the total antibody response to each individual strain of influenza present in the vaccine. Briefly, sera were treated with receptor destroying enzyme (RDE; Denka Seiken, Tokyo, Japan) overnight, followed by inactivation at 56 °C for 1 h, and a final dilution to 1:10 with PBS. RDE-treated sera was then incubated in duplicate with either A/Brisbane/59/2007, ABrisbane/10/2007 or the influenza B virus B/Brisbane/60/2008 for those vaccinated in year 1 of the study. For year 2, serum was incubated with A/California/4/2009 (H1N1), A/Perth/16/2009 (H3N2) influenza A viruses or the influenza B virus B/Brisbane/60/2008 for 15 min at room temperature. After a 1 h incubation at 4 °C with either 0.5% turkey red blood cells (A/California/4/2009 virus) or 0.5% chicken red blood cells for the other viruses, HAI titer was determined by the reciprocal dilution of the last well. Positive and negative controls as well as back titrations of virus were included on each individual plate.

An HAI titer of 1:40 is considered to be the threshold for seroprotection against influenza, while a 4-fold increase in HAI titers from

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