Immunogenicity of Split Inactivated Quadrivalent Influenza Vaccine in Adults with Obesity in the 2017/2018 Season

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Source of support: Research Foundation, Medical University of Warsaw

Background: Obesity is associated with susceptibility to severe influenza infection and several disturbances of the immune response to the influenza vaccine. However, the effect of obesity on the immunogenicity of the influenza vaccine is not fully understood.
               Our objective here was to assess the immunogenicity of the split, inactivated quadrivalent influenza vaccine (QIV) in Polish adults with obesity.

Material/Methods: Fifty-three subjects with obesity aged 21-69 years were vaccinated with the QIV in 2017/2018 season. Antibody titers against the 4 vaccine strains were measured using the hemagglutination inhibition (HI) assay. The mean fold antibody increase (MFI), seroprotection rate (protection rate, PR), and seroconversion rate (response rate, RR) were calculated to assess vaccine immunogenicity.

Results: The vaccine elicited a significant increase in the anti-HI titers against the QIV antigens. The MFI, PR, and RR for the QIV antigens also reached the required age-specific values, indicating the QIV meets current immunogenicity criteria. Individuals with class I and class II/III obesity had similar anti-HI titers, MFI, PR, and RR to each of the vaccine strains. Adults aged <60 years had similar anti-HI titers, MFI, PR, and RR to the QIV strains to those aged ≥60 years.

Conclusions: Our results indicate that the split virion, inactivated QIV is immunogenic in adults with obesity regardless of their degree of obesity and age (ie, <60 and ≥60 years).

Keywords: **Influenza Vaccines • Influenza, Human • Obesity • Vaccination**

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/929572

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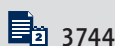
Background

Influenza is a serious viral respiratory disease that impacts all countries worldwide. Each year there are an estimated 1 billion cases globally, including 3-5 million severe cases and 290 000-650 000 influenza-related deaths [1]. The impact of body weight on the immune response to influenza infection and to the influenza vaccine was recognized after the 2009 pandemic, when an excessive incidence of hospitalization and mortality rates were noted among patients with obesity [2,3]. Based on these observations, morbid obesity was recognized by the World Health Organization (WHO) as one of the priority health conditions requiring routine influenza vaccination [4,5]. However, a limited number of studies have examined the effects of obesity on the immune response to influenza vaccines, and the data remain inconclusive [6-9]. Humoral and cellular immune disturbances may be associated with the immuno-modulatory properties of adipose tissue, although the precise mechanisms are not fully understood [10,11]. Unfortunately, despite the increasing prevalence of obesity, data on the efficacy and immunogenicity of the influenza vaccine in individuals with varying degrees of obesity are scarce and mainly concern the trivalent influenza vaccine (TIV). Sheridan et al showed that while the humoral response to the TIV may not initially be impaired in subjects with obesity, there is a greater decline in titers to influenza antigens after 12 months [6,12]. In addition, African Americans had slightly higher antibody responses to the TIV than did White people in Sheridan's study, indicating ethnic-based differences may exist [6]. Meanwhile, Talbot et al studied people aged ≥50 years and found no consistent relationship between obesity and either seroprotection or seroconversion to all 3 antigens included in the TIV [9]. Finally, Callahan et al showed that a single dose of monovalent vaccine to the pandemic A/H1N1 strain induced higher hemagglutination inhibition (anti-HI) titers at day 21 in adults with obesity compared to non-obese adults, but no significant differences in responses to the A/H1N1 strain were observed in adults of various BMI following 2 vaccine doses [13].

The seasonal influenza vaccine requires annual reformulation due to rapid changes in the hemagglutinin (HA) viral surface protein, which result in new antigenically distinct virus strains. TIVs contain 2 strains of influenza A (subtypes A/H1N1 and A/H3N2) and a single type B influenza strain. However, in the 1980s, 2 antigenically different influenza B lineages capable of infecting humans appeared globally (Victoria and Yamagata). Consequently, there was a mismatch between circulating B strains and those included in the vaccine, which reduced the efficacy of TIVs [14]. Therefore, quadrivalent influenza vaccines (QIV) were formulated for better influenza prevention and control by adding a second B virus lineage (Yamagata lineage) to the already-licensed TIVs. WHO first recommended a QIV for use in the Northern Hemisphere in the 2012/2013 season [15], and a QIV became available for the first time in Poland in the 2017/2018 season.

This study aimed to assess the immunogenicity of a split virion, inactivated QIV (available for the first time on the Polish pharmaceutical market in Autumn 2017), in a population of Polish adults with obesity in the 2017/2018 flu season.

Material and Methods

Study population

Fifty-three adult patients of the Polyclinic for Metabolic Diseases of the Food and Nutrition Institute in Warsaw, Poland, were recruited for the administration of the split virion, inactivated QIV (Vaxigrip Tetra®, Sanofi Pasteur, Lyon, France) from October 2017 to January 2018. Eligible subjects were aged 18-70 years, had a body mass index (BMI) of ≥30.0 kg/m² or 25.0-29.9 kg/m², a waist circumference of >80 cm in women and >94 cm in men, and were willing to provide informed consent. Exclusion criteria were exacerbation of chronic disease, acute infectious disease, known allergy to the vaccine component, any history of anaphylaxis after vaccination against influenza or any serious reaction after vaccination, history of Guillain-Barré syndrome up to 6 weeks after previous vaccination, or received immunosuppressive therapy.

Data on age, sex, race, and comorbidities were collected at study entry. At inclusion, patients were also subjected to anthropometric and body composition measurements.

Anthropometric and Body Composition Measurements

The following data were collected from the patients: height, weight (measured using a medical scale Radwag WPT 200 OW, Radom, Poland), waist circumferences (measured halfway between the last rib and the anterior superior iliac spine using a centimeter tape), non-dominant arm circumference (measured halfway between the shoulder joint and the elbow joint using a centimeter tape), skinfold thickness above the tricep muscle of the non-dominant arm (ie, at the vaccine injection site; measured halfway between the shoulder joint and the elbow joint using Harpenden Calipers, Burgess Hill, United Kingdom) [16], and body composition (measured using Bioelectrical Impedance Analyzer BioScan 920-II-S, Maltron, Rayleigh, United Kingdom, according to the manufacturer's instructions). BMI was calculated as body weight in kilograms divided by the height in meters squared (kg/m²). Following the recommendations of the WHO and the American Association of Clinical Endocrinologist/American College of Endocrinology (AACE/ACE), BMI, waist circumferences, and obesity-related comorbidities were used to classify obesity [17,18]. The

measurements of body fat percentage, abdominal obesity or total body water were carried out in accordance with the recommendations of the WHO, the International Diabetes Federation (IDF), and other published guidelines, provided with a software of Bioelectrical Impedance Analyzer BioScan 920-II-S, Maltron, Rayleigh, United Kingdom [18-20].

Vaccine and Vaccination

Subunit, inactivated QIV (Vaxigrip Tetra®, Sanofi Pasteur, Lyon, France) contained the antigen composition recommended by the WHO for the Northern Hemisphere for the 2017/2018 season [21] as follows: 15 μg hemagglutinin (HA) of A/Michigan/45/2015 (A/H1N1/pdm09)-like strain (A/Michigan/45/2015, NYMC X-275), 15 μg HA of A/Hong Kong/4801/2014 (A/H3N2/)-like strain (A/Hong Kong/4801/2014, NYMC X-2638), 15 μg HA of B/Brisbane/60/2008-like strain (Victoria lineage) (B/Brisbane/60/2008 wild strain), and 15 μg HA of B/Phuket/3073/2013-like strain (Yamagata lineage) (B/Phuket/3073/2013 wild strain). The vaccine was provided in prefilled syringes with a 15-mm-long needle and given as a 0.5-ml dose into the deltoid muscle or deeply subcutaneously into the non-dominant arm. The cold chain was preserved, and the vaccines were stored at 2-8°C.

Serological Testing

Blood samples of 5 mL of venous peripheral blood were drawn for analyses of serum anti-hemagglutinins antibodies just before vaccination and 4-8 weeks after vaccination. Samples (3 mL) were transported at 2-4°C immediately after collection from the laboratory of the Polyclinic for Metabolic Diseases of the Food and Nutrition Institute in Warsaw, Poland, to the laboratory at the Department of Influenza Research (National Influenza Center) at the National Institute of Public Health, Warsaw, Poland. Hemagglutination inhibition (HI) assay is traditionally used to measure influenza-specific titers as a correlate of protection and it quantifies the ability of HA to block the N-acetyl-neuraminic acid-mediated viral agglutination of red blood cells [22].

Assessment of Influenza Vaccine Immunogenicity

Immunogenicity of the vaccine was assessed using criteria established by the Committee for Proprietary Medicinal Products (CPMP) and the European Agency for the Evaluation of Medicinal Products (EMEA, now the European Medicine Agency, EMA) [23]. The HI assay was carried out in accordance with WHO recommendations [24] using turkey erythrocytes at the Department of Influenza Research (National Influenza Center) at the National Institute of Public Health, Warsaw, Poland. The anti-HI titers were expressed as the reciprocal of the highest serum dilution at which hemagglutination of erythrocytes was completely inhibited. Antibody response to virus HAs was presented as geometric mean titers (GMT) of HI antibodies, calculated at baseline (day 0) and 28-36 days after vaccination. The evaluated parameters were: 1) Mean Fold Increase (MFI) as the post- to pre-vaccination GMT ratio; a ratio of >2.5 is required for subjects aged 18-60 years and >2.0 for those aged ≥60 years); 2) seroprotection rate (protection rate, PR) as the percentage of subjects with anti-HI titers of >1: 40; PR of ≥70% is required for subjects aged 18-60 years and ≥60% for people aged ≥60 years; 3) seroconversion rate (response rate, RR) as the percentage of subjects with a pre-vaccination anti-HI titer <10 and a post-vaccination anti-HI titer ≥40 or a ≥4-fold increase in the post-vaccination anti-HI titer if the pre-vaccination anti-HI titer was ≥10 for people aged 18-60 years and a ≥2-fold increase in post-vaccination anti-HI titer for people aged ≥60 years. The required RR is ≥40% for people aged 18-60 years or ≥30% for people aged ≥60 years [23,25]. The effect of the degree of obesity and age on vaccine immunogenicity was also investigated. Regarding obesity, 2 groups of individuals were studied: those with class I obesity and those with class II or III obesity. People with class II and III obesity were analyzed together due to the small number of patients with a high degree of obesity. Furthermore, the effect of age on vaccine immunogenicity was studied in subjects aged <60 years and those aged ≥60 years.

Ethics Approval

The study was approved by the Ethics Committee of the Medical University in Warsaw, Poland (approval number: KB/165/2017). Written informed consent was obtained from all study participants.

Statistical Analyses

All calculations were done using the SPSS Statistics V25 software (IBM, Armonk, NY, USA). Data distribution was tested using skewness and kurtosis. For comparisons of anti-HI titers before and after vaccination, the paired Wilcoxon rank test was applied. Associations between immunogenicity indicators and body composition variables, skinfold thickness, and the number of non-communicable diseases were assessed using the Spearman's rank correlation test. The Mann-Whitney U test was used to assess differences in the change in anti-HI titer depending on the route of vaccine administration. The effect of the route of vaccine administration on PR and RR was evaluated using the Pearson's chi-squared test. An analysis of variance with Sidak correction was performed to investigate the effect of obesity degree, age, or both on vaccine immunogenicity parameters. A P value of <0.05 was considered significant.

Table 1. Data on height, weight, BMI, body composition of subjects and the number of comorbidities (N=53).

Parameter	M	±SD	Me	Min	Max
Height (cm)	169.89	9.30	170.00	148.00	187.00
Weight (kg)	103.41	14.90	103.80	73.30	146.30
BMI (kg/m²)	35.74	4.02	35.60	28.20	46.10
Abdominal fat (cm²)	227.15	61.49	223.00	130.00	350.00
Waist circumference (cm)	115.39	10.50	114.00	94.00	155.00
Resting energy expenditure (kcal)	1747.10	313.18	1771.00	1251.00	2405.00
Fat free mass (kg)	61.26	12.74	62.84	38.88	80.92
Fat free mas (%)	59.21	8.88	58.47	44.09	74.97
Fat mass (kg)	42.13	11.06	43.99	21.40	81.79
Fat mass (%)	40.79	8.88	41.54	25.03	55.91
Total body water (l)	46.36	9.68	46.47	29.53	62.15
Total body water (%)	44.83	6.96	43.77	33.56	59.11
Fat free mass hydration (%)	75.71	2.17	75.91	71.60	80.92
Excess Fluid (l)	2.36	2.10	2.26	-0.44	9.56
Malnutrition index	0.66	0.05	0..65	0.56	0.79
No of comorbidities	2.57	1.38	2.00	0.00	7.00

M – mean; SD – standard deviation; Me – median; Min – minimum; Max – maximum.

Results

Characteristics of study participants

The study involved 28 (52.8%) women and 25 (47.2%) men of White race. The mean age of the subjects was 54±11.19 years (range 21-69). Thirty-two (60.4%) subjects were aged <60 years, and 21 (39.6%) were aged ≥60 years. Data on the anthropometric dimensions of the subjects, their body composition, and the number of comorbidities are presented in **Table 1**.

According to BMI, 4 subjects were overweight (BMI <30 kg/m²), 17 had class I obesity (BMI 30-34.9 kg/m²), 24 had class II obesity (BMI 35-39.9 kg/m²), and 8 had class III obesity (BMI ≥40 kg/m²). Body composition analysis showed that 51 (96.2%) subjects had an elevated percentage of fat mass; the remaining 2 (3.8%) had an increased waist circumference and excessive abdominal fat. According to abdominal fat content, 12 (22.6%) subjects were overweight and 41 (77.4%) had abdominal obesity. The percentage of total body water was within the normal range in 45 (86.5%) subjects. Based on the malnutrition index, 39 (75%) subjects were overweight or obese. In terms of comorbidities, 49 (92.5%) subjects had at least 1 non-communicable disease. Subjects had 2 comorbidities on average (range 0-7) (**Table 1**), with hypertension, dyslipidemia, type 2 diabetes mellitus, and hypothyroidism most commonly reported.

According to the AACE/ACE guidelines, obesity is diagnosed when the BMI exceeds 30 kg/m² or when the BMI exceeds 25 kg/m² and there is at least 1 mild-to-moderate obesity-related complication (class I obesity); or at least 1 severe obesity-related complication (class II obesity). As all overweight individuals in our study had comorbidities and they were classified as having class I or class II obesity.

QIV Immunogenicity

The QIV elicited a significant increase in the level of antibodies against each of the 4 influenza HA contained in the vaccine (**Table 2**). An inadequate antibody response to the vaccine (post-vaccination HI titer equal to zero) was noted in 2 subjects for the A/Michigan/45/2015 (H1N1)pdm09-like strain, 3 subjects for the A/Hong Kong/4801/2014 (A/H3N2/)-like strain, 1 subject for the B/Brisbane/60/2008 strain, and 1 subject for the B/Phuket/3073/2013 strain. Parameters of vaccine immunogenicity are shown in **Table 3**.

Depending on the vaccine strain, pre- and post-vaccination GMT ranged from 1.98 to 34.82 and from 59.44 to 166.53, respectively. The pre- and post-vaccination GMT were lower for the A/H1N1/pdm09-like strain and A/H3N2 strain than for both B (Victoria and Yamagata lineages) strains. The MFI against the vaccine strains ranged from 3.7 (the B Victoria lineage) to

Table 2. Pre-vaccination and post-vaccination anti-HI titers against influenza virus strains (N=53).

Antibodies	Pre-vaccination					Post-vaccination					P value*
	M	±SD	Me	Min	Max	M	±SD	Me	Min	Max	
A/Michigan/45/2015 (A/H1N1/pdm09)-like strain	11.5	34.2	0	0	160	184.2	211.1	80	0	640	<0.01
A/Hong Kong/4801/2014 (A/H3N2/)-like strain	5.7	24.4	0	0	160	175.5	201.1	80	0	640	<0.01
B/Brisbane/60/2008-like strain (Victoria lineage)	24.3	27.7	20	0	160	230.6	196.2	160	0	640	<0.01
B/Phuket/3073/2013-like strain (Yamagata lineage)	49.8	26.5	40	0	80	181.5	153.8	160	0	640	<0.01

M – mean; SD – standard deviation; Me – median; Min – minimum; Max – maximum; * (pre- versus post-vaccination).

Table 3. Parameters of vaccine immunogenicity (N=53).

Antibodies	GMT		MFI	PR (%)		RR (%)
	Pre	Post		Pre	Post	
A/Michigan/45/2015 (A/H1N1/pdm09)-like strain	1.98	59.44*	42.2	11.3	77.4*	84.9
A/Hong Kong/4801/2014 (A/H3N2/)-like strain	1.55	73.38*	47.3	3.8	83.0*	86.8
B/Brisbane/60/2008-like strain (Victoria lineage)	11.87	166.53*	14.0	30.2	96.2*	88.7
B/Phuket/3073/2013-like strain (Yamagata lineage)	34.82	129.25*	3.7	83.0	96.2*	66.0

Pre – pre-vaccination; Post – post-vaccination; GMT – Geometric Mean antibody Titers; MFI – mean fold increase; PR – protection rate; RR – response rate. * p<0.05 (pre- versus post-vaccination).

47.3 (the A/H3N2 strain) and comparable MFI values for the A/H1N1/pdm09-like and A/H3N2 strains were observed. Pre- and post-vaccination PR to the vaccine strains ranged from 3.8% to 83.0% and from 77.4% to 96.2%, respectively. Higher post-vaccination PRs were obtained for both B lineage strains compared to the A/H1N1/pdm09-like and A/H3N2 strains. The RRs to the vaccine strains ranged from 66% to 88.7%, with the lowest value recorded for the B Yamagata strain. All parameters (MFI, PR, RR) for all 4 vaccine strains reached the levels required for the respective age range of subjects. Thus, the QIV met the immunogenicity criteria established by CPMP/EMA for influenza vaccines in the study cohort.

Effect of Degree of Obesity, Age, and Body Composition on QIV Immunogenicity

The magnitude of change in HI titers following vaccination, GMT (pre- and post-vaccination), MFI, PR, and RR to each influenza virus strain contained in the vaccine did not differ significantly between individuals with class I obesity and those with class II/III obesity, or between individuals aged <60 years and those ≥60 years. However, if both factors were considered (ie, degree of obesity and age), a greater increase in anti-HI titers against the A/H3N2 strain was observed in younger subjects (aged <60 years) with class I obesity than in those with class II/III obesity ($P<0.001$) (**Figure 1**). The opposite trend was observed in subjects aged ≥60 years. Moreover, the RR to A/H3N2 strain at the analyzed level was achieved by all individuals with class I obesity aged <60 years compared to only 67% of individuals aged ≥60 years ($P<0.05$) (**Figure 2**). However, in both age groups, the RR met the criteria recommended by the CPMP/EMA.

Although some vaccine immunogenicity parameters were correlated with body composition variables, these associations were only moderate (**Table 4**). Larger fat mass and fat percentage were associated with a higher average PR (r_s=0.325 and 0.347, respectively, $P<0.05$) and a greater change in the anti-HI titer against the A/H3N2 strain (r_s=0.301 and 0.307, respectively, $P<0.05$). However, the percentage of total body water and the
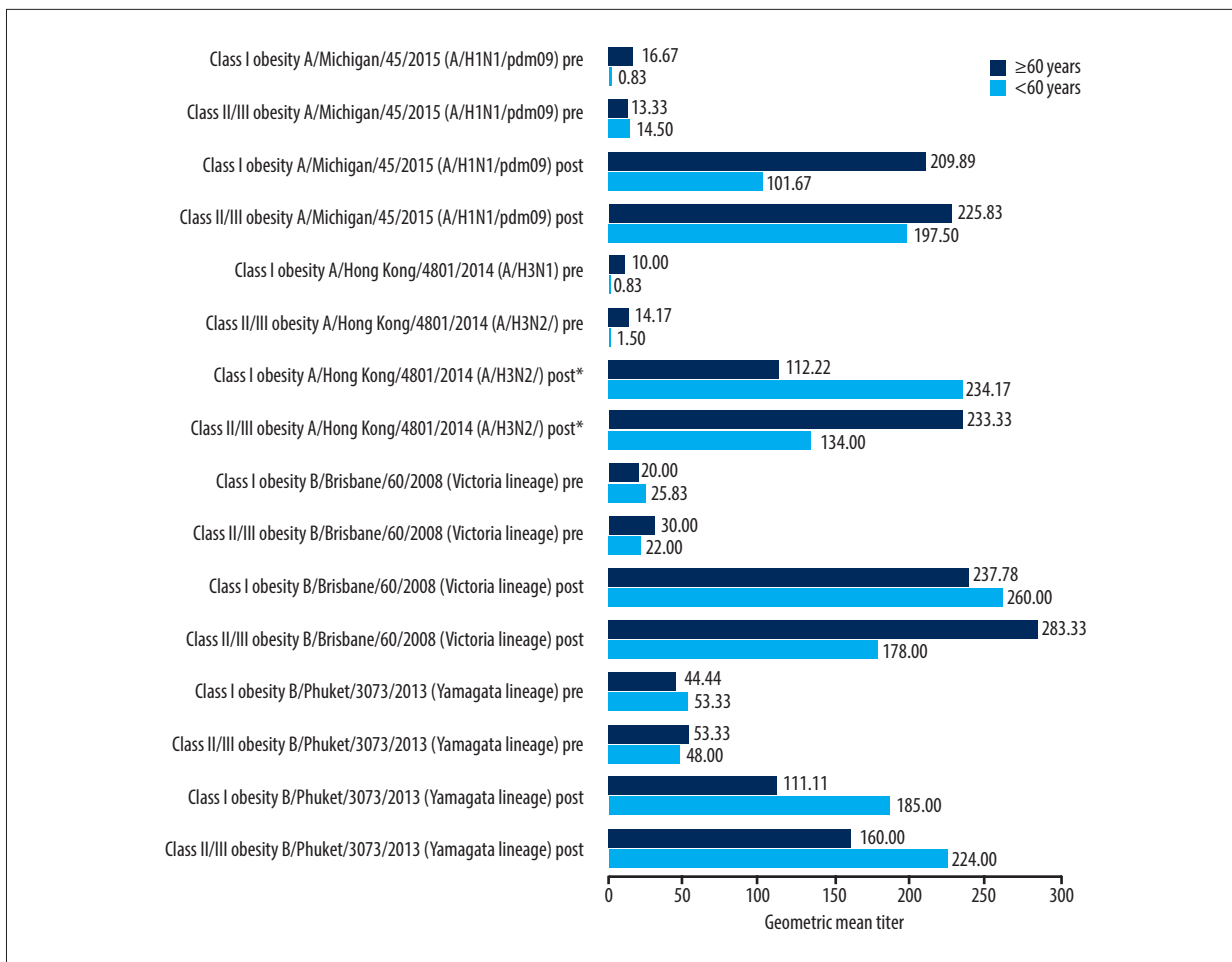
Figure 1. Pre-vaccination and post-vaccination geometric mean anti-hemagglutinins titers in subjects grouped by obesity class and age (N=53). Asterisks indicate groups that differ significantly (*P*<0.001). Pre – pre-vaccination; Post – post-vaccination; GMT – Geometric Mean antibody Titers.
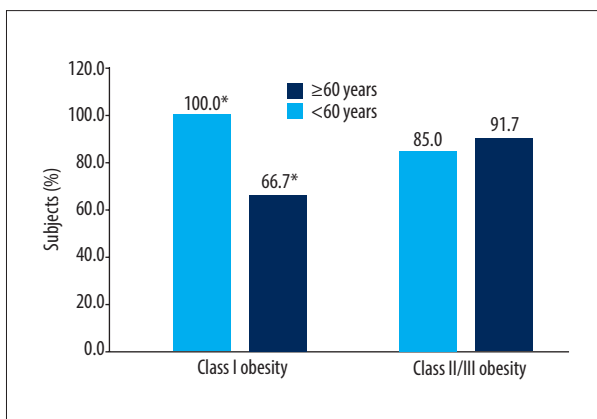

Figure 2. The effect of the degree of obesity and age on the response rate to the A/Hong Kong/4801/2014 (A/H3N2/)-like strain (N=53). Asterisks indicate groups that differ significantly (*P*<0.05).

percentage of fat-free mass were correlated negatively with the average PR (r$_s$=-0.347 and -0.335, respectively, *P*<0.05) and the magnitude of the change in the anti-HI titer against the A/H3N2 strain (r$_s$=-0.307 and -0.290, respectively, *P*<0.05). In addition, the average pre-vaccination GMT correlated positively with the percentage of fat-free mass hydration (r$_s$=0.337, *P*<0.05) and the excess fluid volume (r$_s$=0.362, *P*<0.01).

Subcutaneous fat deposition affects the route of vaccine administration, as intramuscular injection using the 15-mm-long needle supplied by the manufacturer may not be possible if the skinfold thickness exceeds 15 mm. In our study, regardless of whether the vaccine was given intramuscularly (in subjects with skinfold <15 mm) or deeply subcutaneously (in subjects with skinfold >15 mm), no differences were observed in the anti-HI titer rise, PR, or RR against all 4 HA (**Supplementary Tables 1-4**). These results indicate that the thickness of the skinfold at the vaccine injection site does not significantly influence the immunogenicity of the QIV.

Table 4. Relations between anthropometric dimensions, BMI, and body composition variables and antibody response – Spearman's correlation coefficient (N=53).

Parameter	Averaged GMT Pre	Averaged GMT Post	Averaged MFI	Averaged PR	Averaged RR
Height (cm)	0.04	0.00	-0.04	-0.16	-0.07
Weight (kg)	0.07	0.12	0.04	0.02	0.01
BMI (kg/m^2)	0.02	0.19	0.15	0.17	0.13
Abdominal fat (cm^2)	-0.02	0.12	0.12	0.09	0.11
Waist circumference (cm)	-0.04	0.15	0.14	0.10	0.10
Resting energy expenditure (kcal)	0.09	-0.02	-0.10	-0.13	-0.11
Fat free mass (kg)	0.11	-0.05	-0.11	-0.20	-0.12
Fat free mas (%)	0.07	-0.22	-0.22	-0.35*	-0.20
Fat mass (kg)	0.04	0.26	0.14	0.33*	0.13
Fat mass (%)	-0.07	0.22	0.22	0.35*	0.20
Total body water (l)	0.14	-0.04	-0.11	-0.20	-0.11
Total body water (%)	0.14	-0.22	-0.24	-0.34*	-0.22
Fat free mass hydration (%)	0.34*	-0.07	-0.19	-0.00	-0.09
Malnutrition index	0.07	0.16	0.07	0.152	0.11
Excess fluid (l)	0.36**	-0.10	-0.21	-0.02	-0.06

| Parameter | Change in titer# | | | |
	A/Michigan/45/2015 (A/H1N1/pdm09)-like strain	A/Hong Kong/4801/2014 (A/H3N2/)-like strain	B/Brisbane/60/2008-like strain (Victoria lineage)	B/Phuket/3073/2013-like strain (Yamagata lineage)
Height (cm)	-0.09	-0.21	0.08	0.18
Weight (kg)	-0.05	0.00	0.11	0.21
BMI (kg/m^2)	0.09	0.21	0.08	0.18
Abdominal fat (cm^2)	0.15	0.12	0.13	0.06
Waist circumference (cm)	0.09	0.03	0.18	0.13
Resting energy expenditure (kcal)	-0.12	-0.17	0.04	0.17
Fat free mass (kg)	-0.13	-0.20	0.04	0.11
Fat free mas (%)	-0.22	-0.31*	-0.07	-0.05
Fat mass (kg)	0.07	0.30*	0.16	0.15
Fat mass (%)	0.22	0.31*	0.07	0.05
Total body water (l)	-0.12	-0.19	0.05	0.09
Total body water (%)	-0.22	-0.29*	-0.07	-0.09
Fat free mass hydration (%)	-0.08	0.08	-0.03	-0.23
Malnutrition index	0.09	-0.06	0.21	0.12
Excess fluid (l)	-0.09	0.04	-0.01	-0.21

Pre – pre-vaccination; Post – post-vaccination; GMT – Geometric Mean antibody Titers; MFI – mean fold increase; PR – protection rate; RR – response rate; # difference in anti-HI titers pre- and post-vaccination; * p<0.05; ** p<0.01.

Discussion

Our study provides data on the immunogenicity of the split virion, inactivated QIV in an ethnically homogeneous population of Polish adults with obesity while it was first introduced in Poland in 2017/2018 season. The QIV fulfilled the CPMP/EMA criteria for acceptance of the immunogenicity of an influenza vaccine in the studied subjects with obesity. Depending on the type of influenza virus strain, protective levels of titers were achieved in 77.4-96.2% of subjects. The PR values for the A/H3N2, B Victoria lineage, and B Yamagata lineage strains were comparable with those reported by other researchers [26,27]. Although a lower PR value than that described in the literature was observed for the A/H1N1/pdm09 strain [26-30], it still met the CPMP/EMA criteria for vaccine immunogenicity. Prior to vaccination, the GMT ranged from 1.55 (for the A/H3N2 strain) to 34.82 (for the B Yamagata lineage strain) in studied subjects with obesity, which is lower than that observed in the general adult population [28,29,31-33]. This finding is in line with other researchers, who showed reduced levels of anti-HI titers before vaccination in individuals with excessive body weight compared to people with normal weight [6,8,13]. The reason for this may be a sharp decrease in anti-HI titers against vaccine strains after 12 months following influenza vaccination, which has been observed in people with obesity [6]. The post-vaccination GMT were also lower in studied subjects compared to the general adult population [26-28,32-34]. However, it cannot be excluded that differences in GMT levels described in the literature likely reflect, at least in part, the inter-laboratory discrepancies in titer measurements.

Despite these relatively lower pre- and post-vaccination GMT, we observed significant MFIs in studied adults with obesity. The MFI for the B Victoria lineage strain was above 14, and was above 40 for both the A/H1N1/pdm09 and A/H3N2 strains. A lower MFI (3.71) was only observed for the B Yamagata lineage strain compared to other vaccine HAs. This phenomenon can be associated with the higher pre-vaccination GMT observed for the B Yamagata lineage strain compared to those for the other 3 influenza strains [35], and was also confirmed in an elderly Polish population in a study by Ganczak et al carried out 1 season later (2018/2019) [36]. Interestingly, Bart et al observed that subjects who were seronegative at baseline demonstrated substantially higher RRs than those who were seropositive at baseline [35]. Furthermore, the PRs obtained in our study were comparable to those described by Ofori-Anyinam et al [37], but the RRs were higher than those previously reported [33]. Indeed, the relatively wide ranges for the MFI, PR, and RR (3.71-47.3%, 83-96.2%, and 66-88.7%, respectively) noted in this study have already been described by others and may be associated with the simultaneous exposure to 4 influenza HAs [29,31,38].

In our study, over 90% of participants had comorbidities. However, there was no correlation between the number of non-communicable diseases that accompanied obesity and the change in anti-HI titers following QIV vaccination (data not shown). These observations are consistent with the results of a meta-analysis indicating that obesity, regardless of comorbidities, is associated with influenza-related complications [39]. Moreover, we noted that the immunogenicity of the QIV in studied adults was at least as high as in other groups with different non-communicable conditions in Poland (ie, patients with acute lymphoblastic leukemia or non-Hodgkin's lymphoma, women with breast cancer, patients with Wegener's granulomatosis, splenectomized patients, patients on hemodialysis, or those who are immunosuppressed) [40-47].

Further analyses of the elicited anti-HI titers, GMT, MFI, PR, or RR did not show statistically significant differences in the vaccine immunogenicity to the A/H1N1/pdm09 and both B (Victoria and Yamagata lineages) strains among patients according to the degree of obesity, age, or both of these factors. Regarding the A/H3N2 strain, a greater increase in anti-HI titers was observed in younger subjects (aged <60 years) with class I obesity than those with class II/III obesity. The interaction effect of the degree of obesity and age was also significant in the case of the RR to the A/H3N2 strain, which at the analyzed level was observed more frequently in younger adults (<60 years) than in older adults (≥60 years) with class I obesity. Although the PR and RR for the A/H3N2 and B Yamagata lineage strains calculated in this study were similar to those previously demonstrated in individuals with obesity [9,13], our RRs for the A/H1N1/pdm09 and A/H3N2 strains were twice as high as those previously described for people with obesity by Neidich et al [8]. We observed higher values of MFI, PR, and RR for A/H1N1/pdm09, B Victoria lineage, and B Yamagata lineage strains and the similar values of MFI, PR, and RR for A/H3N2 strain for adults with obesity aged ≥60 years compared to data published by Ganczak et al from a study carried out on elderly Polish people 1 season later (2018/2019) [36]. The assessment of the QIV-induced antibody response in relation to the body composition of subjects indicated that fat mass and fat percentage were positively correlated with the magnitude of the HI antibody response to the A/H3N2 strain and the average PR, while the fat-free mass was negatively correlated. However, these correlations were moderate, and their clinical implications are uncertain.

The route of vaccine administration can also influence its immunogenicity [48]. However, our results indicate that the skinfold thickness at the injection site, which determines whether the vaccine is administered intramuscularly or subcutaneously, does not significantly affect the antibody response to all virus strains contained in the QIV. Hence, a practical aspect of our work may be the indication that it is not necessary to use longer needles for vaccinating people with obesity, as considered by others [48,49].

According to WHO, CDC, and Polish guidelines for influenza vaccination, it is recommended to use standard-dose inactivated (TIV or QIV) or live attenuated influenza vaccines (LAIVs) [5,50,51]. LAIVs are recommended for naïve populations like children, as they are able to induce a more protective and longer-lasting immune response [5,52]. TIV or QIV are recommended for the general population, especially for pregnant women, children aged 6 months to 2 years, people with chronic diseases, or adults with certain comorbidities such as obesity, diabetes, or liver disease [5,50,51]. Our results indicate the immunogenicity of the standard-dose QIV in adults with obesity fulfilled the CPMP/EMA criteria, which might result in the observed effectiveness of traditional seasonal influenza vaccines [52]. The observed lower increase in anti-HI titers or RR to the A/H3N2 strain of standard-dose QIV in older adults (≥60 years) with obesity suggests use of high-dose influenza vaccines in this age group of patients with obesity, which was described in the ECDC Technical Report as being more effective in prevention of influenza-like illness, respiratory illness, and respiratory-related or pneumonia-related hospital admissions [52].

The limitation of our study is the relatively small number of participants, but groups of similar numbers of subjects have already been investigated in studies assessing the humoral response to an influenza vaccine [7,45,46].

Conclusions

In conclusion, this is one of the first reports on the immunogenicity of the QIV in a Polish population with obesity. Our results indicate that the split virion, inactivated QIV is immunogenic in adults with obesity regardless of their degree of obesity and age (ie, <60 and ≥60 years). The effectiveness of the QIV measured as an epidemiological effect in observational studies of vaccinated and unvaccinated adults with obesity remains an interesting issue for evaluation in future research.

Conflict of Interest

None.

Supplementary Data

Supplementary Tables 1-4. The effect of route of administration on QIV immunogenicity.

Subjects were grouped according to the thickness of skinfold at the injection site: with a skinfold <15 mm enabling intramuscular injection and with a skinfold >15 mm which determines subcutaneous injection. Vaccine immunogenicity by route of administration is presented (**Supplementary Tables 1-4**).

Table 1. Antibody response to A/Michigan/45/2015 (A/H1N1/pdm09)-like strain by route of administration.

Parameter	Route of administration	N	M	SD	Me	Min	Max	P value
Pre-vaccination anti-HI titer	IN	23	6.96	18.45	0.00	0.00	80.00	0.8505*
	SUB	30	15.00	42.57	0.00	0.00	160.00	
Post-vaccination anti-HI titer	IN	23	186.52	224.41	80.00	0.00	640.00	0.9356*
	SUB	30	182.33	204.21	160.00	0.00	640.00	
Change in anti-HI titer	IN	23	179.57	225.52	80.00	0.00	640.00	0.9000*
	SUB	30	167.33	212.57	80.00	-80.00	640.00	
%								
PR	IN	23	83					0.4239**
	SUB	30	73					
RR	IN	23	83					0.9444**
	SUB	30	83					

IN – intramuscularly; SUB – subcutaneously; PR – protection rate; RR – response rate; N – number of subjects; M – mean; SD – standard deviation; Me – median; Min – minimum; Max – maximum; * Mann-Whitney U test; ** Pearson's chi-squared test.

Table 2. Antibody response to A/Hong Kong/4801/2014 (A/H3N2/)-like strain by route of administration.

Parameter	Route of administration	N	M	SD	Me	Min	Max	*P* value
Pre-vaccination anti-HI titer	IN	23	3.91	16.72	0.00	0.00	80.00	0.5067*
	SUB	30	7.00	29.14	0.00	0.00	160.00	
Post-vaccination anti-HI titer	IN	23	157.39	206.80	80.00	0.00	640.00	0.2362*
	SUB	30	189.33	198.94	160.00	0.00	640.00	
Change in anti-HI titer	IN	23	153.48	209.69	80.00	-40.00	640.00	0.2471*
	SUB	30	182.33	188.31	155.00	0.00	640.00	
					%			
PR	IN	23			74			0.1221**
	SUB	30			90			
RR	IN	23			74			0.503**
	SUB	30			93			

IN – intramuscularly; SUB – subcutaneously; PR – protection rate; RR – response rate; N – number of subjects; M – mean;
SD – standard deviation; Me – median; Min – minimum; Max – maximum; ** Mann-Whitney U test; ** Pearson's chi-squared test.

Table 3. Antibody response to B/Brisbane/60/2008-like strain (Victoria lineage) by route of administration.

Parameter	Route of administration	N	M	SD	Me	Min	Max	*P* value
Pre-vaccination anti-HI titer	IN	23	20.00	19.54	20.00	0.00	80.00	0.4896*
	SUB	30	27.67	32.56	20.00	0.00	160.00	
Post-vaccination anti-HI titer	IN	23	273.91	217.54	160.00	20.00	640.00	0.2026*
	SUB	30	197.33	176.07	160.00	0.00	640.00	
Change in anti-HI titer	IN	23	253.91	215.15	150.00	20.00	640.00	0.1102*
	SUB	30	169.67	181.08	120.00	0.00	640.00	
					%			
PR	IN	23			96			0.8477**
	SUB	30			97			
RR	IN	23			96			0.0557**
	SUB	30			77			

IN – intramuscularly; SUB – subcutaneously; PR – protection rate; RR – response rate; N – number of subjects; M – mean;
SD – standard deviation; Me – median; Min – minimum; Max – maximum; * Mann-Whitney U test; ** Pearson's chi-squared test.

Table 4. Antibody response to B/Phuket/3073/2013-like strain (Yamagata lineage) by route of administration.

Parameter	Route of administration	N	M	SD	Me	Min	Max	P value
Pre-vaccination anti-HI titer	IN	23	48.70	28.81	40.00	0.00	80.00	0.8365*
	SUB	30	50.67	25.04	40.00	0.00	80.00	
Post-vaccination anti-HI titer	IN	23	186.96	166.42	160.00	20.00	640.00	0.9642*
	SUB	30	177.33	146.19	160.00	0.00	640.00	
Change in anti-HI titer	IN	23	138.26	162.69	80.00	0.00	600.00	0.9571*
	SUB	30	126.67	145.87	120.00	0.00	620.00	
%								
PR	IN	23			96			0.8477**
	SUB	30			97			
RR	IN	23			70			0.9571**
	SUB	30			63			

IN – intramuscularly; SUB – subcutaneously; PR – protection rate; RR – response rate; N – number of subjects; M – mean; SD – standard deviation; Me – median; Min – minimum; Max – maximum; * Mann-Whitney U test; ** Pearson's chi-squared test.

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