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Full-length Article

Positive mood on the day of influenza vaccination predicts vaccine effectiveness: A prospective observational cohort study

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ABSTRACT

Influenza vaccination is estimated to only be effective in 17–53% of older adults. Multiple patient behaviors and psychological factors have been shown to act as 'immune modulators' sufficient to influence vaccination outcomes. However, the relative importance of such factors is unknown as they have typically been examined in isolation. The objective of the present study was to explore the effects of multiple behavioral (physical activity, nutrition, sleep) and psychological influences (stress, positive mood, negative mood) on the effectiveness of the immune response to influenza vaccination in the elderly. A prospective, diary-based longitudinal observational cohort study was conducted. One hundred and thirty-eight community-dwelling older adults (65–85 years) who received the 2014/15 influenza vaccination completed repeated psycho-behavioral measures over the two weeks prior, and four weeks following influenza vaccination. IgG responses to vaccination were measured via antigen microarray and seroprotection via hemagglutination inhibition assays at 4 and 16 weeks post-vaccination. High pre-vaccination seroprotection levels were observed for H3N2 and B viral strains. Positive mood on the day of vaccination was a significant predictor of H1N1 seroprotection at 16 weeks post-vaccination and IgG responses to vaccination at 4 and 16 weeks post-vaccination, controlling for age and gender. Positive mood across the 6-week observation period was also significantly associated with post-vaccination H1N1 seroprotection and IgG responses to vaccination at 16 weeks post-vaccination, but in regression models the proportion of variance explained was lower than for positive mood on the day of vaccination alone. No other factors were found to significantly predict antibody responses to vaccination. Greater positive mood in older adults, particularly on the day of vaccination, is associated with enhanced responses to vaccination.
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1. Introduction

Between 250,000 and 500,000 deaths are estimated to occur worldwide annually as a result of seasonal influenza epidemics (World Health Organization, 2016). The vast majority of these deaths (in excess of 90% in industrialised countries), as well as non-fatal influenza-associated hospitalizations, occur in those aged 65 years or older (Thompson et al., 2003). Influenza vaccination is comparatively poor at inducing clinical protection in those 65 years of age and older. Clinical efficacy is estimated to be only 17–53% in older adults compared to 70–90% in younger adults (Goodwin et al., 2006). This means influenza vaccination is least effective amongst those in most need of protection. While pharmacological solutions to this issue have shown some promise, the effectiveness of influenza vaccination in older adults remains relatively poor and novel methods of improving vaccine outcomes in this population are needed.

Patient behaviors and psychological well-being can influence immune responses to vaccination. Physical activity (Pascoe et al., 2014), nutrition (Calder, 2013), sleep (Prather et al., 2012), stress (Pedersen et al., 2009), and mood (Marsland et al., 2006) have all been identified as 'immune modulators' sufficient to impact on

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vaccination responses. This raises the possibility that interventions targeting these factors could act as non-pharmacological adjuvants to improve vaccination outcomes. To date, no study has explicitly examined all of the above behavioral and psychological 'immune modulators' and their influence on vaccination responses simultaneously. This is needed to determine their relative importance, and thus identify the factors that should form the focus of intervention. Furthermore, previous research in this area has relied on limited assessments of these factors: typically using one-off retrospective self-report measures that are subject to recall biases. Here we report findings from a prospective, diary-based longitudinal study with the primary objective of exploring the effects of physical activity, nutrition, sleep, stress, and mood on the vaccine-induced protective antibody response in older adults.

2. Methods

2.1. Study design & participants

A prospective, diary-based longitudinal observational cohort study of psychological and behavioral influences on influenza vaccination responses in older adults was conducted between August 2014 and March 2015. Ethical (REC: 14/EM/0201) and all research governance approvals were obtained prior to study commencement. One hundred and thirty-eight community-dwelling older adults aged 65–85 were recruited through 4 primary care practices in Nottingham, UK. *A priori* sample size calculations based on observed effects of stress on vaccine response in elderly caregivers (Vedhara et al., 1999) indicated a sample of 121 would give 80% power at 5% significance to detect a similar small-to-medium sized effect ($r = 0.25$) in individual regression models. As responses to influenza vaccination are influenced by previous influenza vaccine history and exposure (Sasaki et al., 2008), we controlled for prior vaccine history by limiting recruitment to those who had been vaccinated the previous year (2013/14). This meant that all participants had similar recent influenza exposure histories, thus minimising between-person differences in prior exposure. Alternative approaches including statistically controlling for vaccine history or recruiting only participants who had not previously received an influenza vaccination were rejected due to concerns with poor record keeping and reduced generalisability respectively. By chance, the antigen strains included in the previous year were identical to those included in the 2014/15 vaccine, however this was not known prior to the study design being finalised. Exclusion criteria were kept minimal to maximise generalizability. They included: deemed by health care provider to be too physically frail to participate; diagnosed with a cognitive condition (e.g., dementia) that would make participation difficult; to have insufficient command of the English language; or having a contraindication for influenza vaccination.

The flow of participants through the study is shown in Fig. 1. Attrition was low with 136 older adults returning at four weeks post-vaccination (1.4% attrition) and 122 at 16 weeks post-vaccination (11.6% attrition). There was no evidence of systematic drop-out bias, with no significant differences in age, gender, body mass index and pre-vaccination Immunoglobulin G (IgG) levels between completers and non-completers (all p's > 0.05). Table 1 presents demographic and clinical characteristics of participants, as well as pre-vaccination IgG and hemagglutination seroprotection levels (traditionally defined as a hemagglutination inhibiting antibody (HAI) titre $\geq$40). In keeping with the vaccine strains for the 2014/15 year being identical to those in the previous year, a substantial proportion of participants reached seroprotection thresholds for the H3N2 (63.1%) and B (47.2%) strains of the vaccine at baseline.

2.2. Procedure

A diagram of the study timeline is shown in Fig. 2. At baseline, participants provided written informed consent, demographic data (e.g., gender, level of education), had height and weight measurements taken and had a pre-vaccination blood sample taken. Participants then began a 6-week intensive data collection period with psychological (stress, positive mood, negative mood) and behavioral factors (physical activity, nutrition, sleep) measured via daily diaries and pedometers on three consecutive, but randomly selected days each week (18 response days in total, further details below). At two weeks, participants attended their local GP surgery, received a standard dose of the 2014/15 northern hemisphere influenza vaccine and completed questionnaire measures of positive and negative mood. Post-vaccination serum samples were scheduled to assess both short- and long-term antibody responses to vaccination. Short-term antibody responses were measured at 4 weeks post-vaccination, which represents the peak of IgG responses to vaccination (Gross et al., 1996). For a more clinically relevant end-point, long-term antibody responses were measured at 16 weeks post-vaccination, as this represents the minimum time period for most participants between vaccination (September/October) and when influenza viruses circulate most frequently in the UK population (January–March).

2.3. Measures

2.3.1. Behavioral measures

Dietary intake was assessed on each response day using the 24-h EPIC-Norfolk food diary (McKeown et al., 2001) and processed using DietPlan 6 Software. Specifically, we extracted data for energy, protein, iron, zinc, selenium, vitamin A, vitamin C, vitamin D, and vitamin E. For each dietary component, participants were classified based on meeting UK recommended nutrient intake levels (Committee on Medical Aspects of Food Policy, 1991; Scientific Advisory Committee on Nutrition, 2011).

Physical activity levels were estimated using Yamax SW-200 pedometers, which have previously been shown to correlate highly with gold-standard accelerometer data (Motl et al., 2006).

Sleep duration, latency and efficiency was measured using adapted items from the Pittsburgh Sleep Quality Index (Buysse et al., 1989). On each response day, participants were asked to record at what time the previous night they had gone to bed, how long it took them to fall asleep, the time they got up, and the total amount of sleep they had (accounting for any disturbances in the night).

2.3.2. Psychological measures

Stress was measured using the four-item perceived stress scale (average $\alpha = 0.74$) (Cohen and Williamson, 1988). On each response day, participants were required to indicate how often they experienced negative thoughts and feelings because of overloading, unpredictable or uncontrollable situations (e.g., "In the last few days, how often have you felt that you were unable to control the important things in your life?") on a 5-point scale (never – very often).

Positive and negative mood was measured using the International Positive and Negative Affect Schedule Short Form (I-PANAS-SF) (Thompson, 2007). It was considered that completion of the full I-PANAS-SF on each response day during the diary period would be too burdensome, therefore we assessed positive and negative mood during the diary period using 4 items each response day (2 positive and 2 negative). These items were randomly selected with randomisation determined by a computer algorithm in cycles without replacement, so that all items appeared at equal

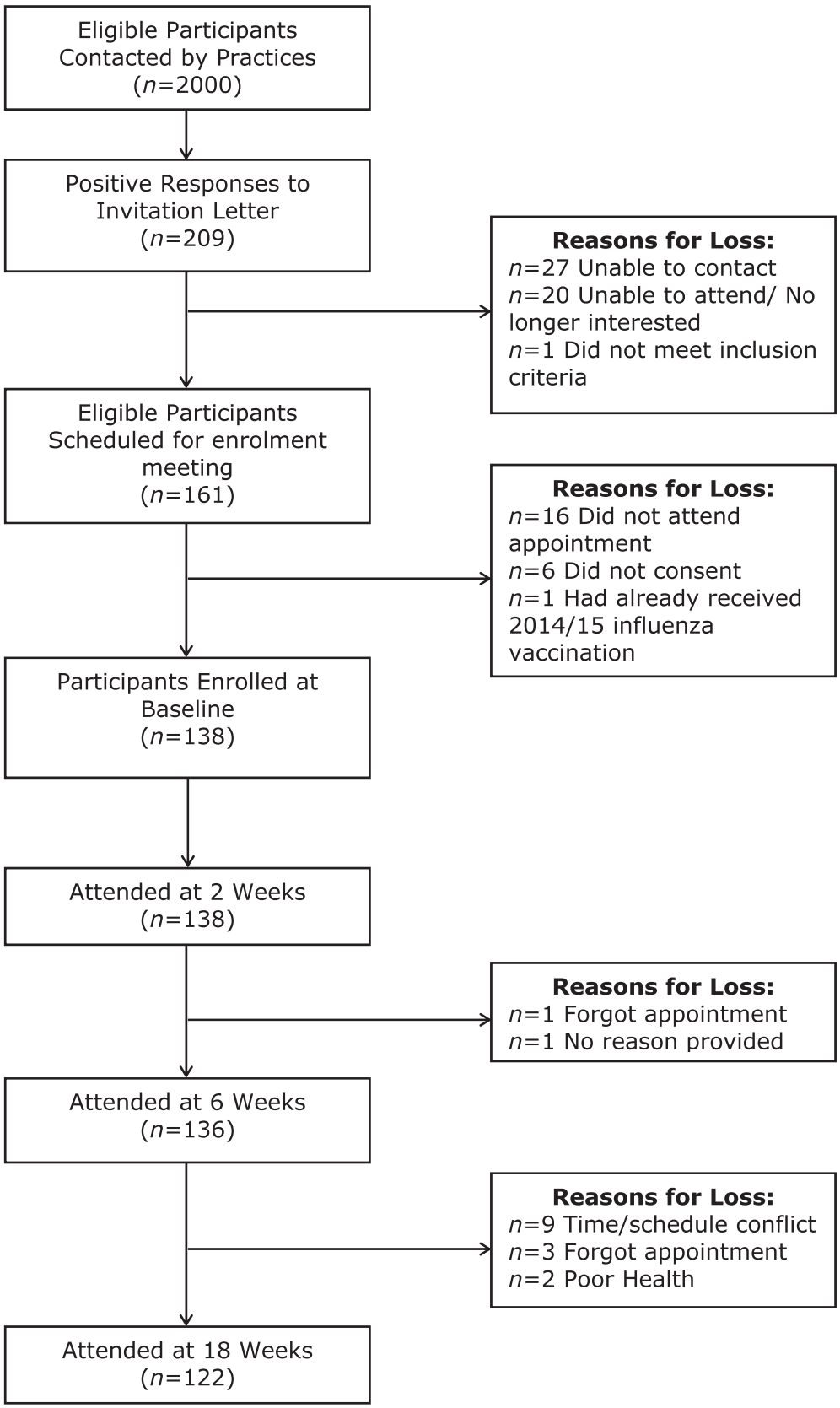

Fig. 1. Flow of participants.

frequencies throughout the diary period. Participants all completed the same items at the same time points. On each response day, participants were presented with emotion adjectives that were positive (e.g., active) or negative (e.g., nervous) in nature and asked to rate on a five-point scale (very slightly or not at all - extremely) the extent to which they felt that way "at that moment". On the day of vaccination, participants also completed the full 10-item version of this scale ($\alpha = 0.89$ for PA, $\alpha = 0.64$ for NA).

Table 1
Participant demographics and medical information at baseline[+].

Variable	
Age – yr	72.87 ± 5.41
Female sex – n (%)	61 (44.2)
White Ethnicity – n (%)	135 (97.8)
Marital Status – n (%)	
Married	89 (64.5)
Single, never married	7 (5.1)
Separated/Divorced	14 (10.1)
Widowed	26 (18.8)
Co-habiting	1 (0.7)
Did Not Respond	1 (0.7)
Highest Level of Education – n (%)	
School	88 (63.8)
University (Undergraduate)	33 (23.9)
Other	13 (9.4)
Did Not Respond	4 (2.9)
Highest Ever Total Household Income	
≤ £14,999	46 (33.3)
£15,000–£24,999	29 (21.0)
£25,000–£34,999	21 (15.2)
£35,000–£49,000	14 (10.1)
£50,000–£74,999	11 (8.0)
£75,000–£99,000	8 (5.8)
≥ £100,000	4 (2.9)
Did Not Respond	5 (3.6)
Current Smoker – n (%)	10 (7.2)
No. Medical Conditions	2.07 ± 1.45
No. Prescribed Medications	3.57 ± 2.79
Body Mass Index	27.9 ± 5.79
Pre-Vaccination IgG[‡]	
H1N1	1.93 ± 1.88
H3N2	6.96 ± 6.81
B	5.18 ± 4.66
Pre-Vaccination Seroprotection rate – n (%)	
H1N1	19 (15.6)
H3N2	77 (63.1)
B	59 (47.2)

[+] Mean ± standard deviation unless otherwise specified.
[‡] Interpolated μg/ml values against a human IgG standard calibration curve in 1:8000 diluted sera.

2.3.3. IgG and HAI antibodies

Venous blood samples (8 ml) were collected via venipuncture in tubes containing clot activator and gel for separating serum. After clotting at room temperature, samples were centrifuged at 2000*g* for 10 min after which sera were separated and stored at -80 °C until analysis. Influenza IgG antibodies were measured in thawed sera via antigen microarray as previously reported (Ayling et al., 2017). As pre-vaccination IgG levels can influence responses to vaccination, we corrected for pre-vaccination levels using a procedure developed by Beyer and colleagues (2004). While IgG levels outcomes provide highly specific quantitative measures of immune response to vaccination, established thresholds for adequate IgG levels to indicate clinical protection are lacking. Therefore, we also measured HAI titres in accordance with WHO guidelines (World Health Organization, 2011), which does not have the specificity of IgG levels measured by antigen microarray but does have well-established clinical protection thresholds, with seroprotection traditionally defined as a titre ≥40 (Hobson et al., 1972). Following processing, a substantial proportion (>50%) of H3N2 samples obtained at 16 weeks post-vaccination could not be reliably interpreted for HAI titres, likely due to a technical error. Therefore, the H3N2 viral strain was excluded from HAI analyses at 16 weeks.

2.4. Treatment of data & statistical analyses

The proportion of missing data for each variable measured over the diary period (averaged across all time-points) was as follows: positive affect – 14.3%; negative affect – 14.4%; perceived stress – 14.3%; physical activity – 12.9%; sleep duration – 11.2%; sleep latency – 14.6%; sleep efficiency – 23.6%; and nutritional factors – 11.7%. The nature of missing data was assessed using Little's MCAR test, which showed no significant deviation from randomness ($\chi 2(29420) = 13616.39$, $p = 1.00$). No imputation of missing data was performed.

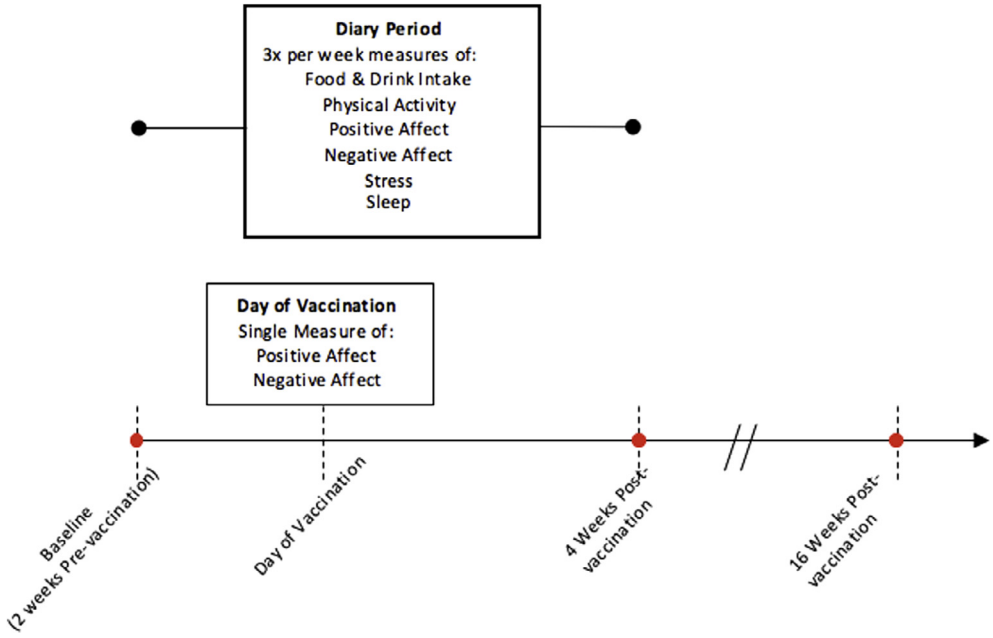

Fig. 2. Overview of Study Timeline *Note:* Red dot indicates times of blood sampling. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

To aggregate repeated measures of psycho-behavioral factors, random intercept latent growth modelling was performed, fitting linear regression lines for each participant. As patterns of change over time were not of interest for these analyses, slopes of the regression lines were fixed to 0, so that the intercepts represented the average level for that variable over diary period. Autocorrelation was assessed for each psycho-behavioral factor by examining the correlation between adjacent and subsequent residuals and was retained in the model if significant ($p < 0.05$). Intercepts were then used as the aggregated measure for each psycho-behavioral predictor in subsequent analyses. An advantage of this approach is that it can accommodate reasonable levels of missing data, meaning that for all variables measured during the diary period nearly all participants provided sufficient responses for an aggregated measure to be derived. The maximum number of participants for whom no intercept could be calculated for a variable measured over the diary period was 10 out of 138 (7.2%). Sensitivity analysis performed using unadjusted mean levels produced similar findings to those reported below.

IgG levels were $\log_2$ transformed to improve distribution normality. Negative mood scores were found to be significantly positively skewed, however attempts at transformation (log, square root, and inverse) were unsuccessful at sufficiently improving the distribution. Therefore, in subsequent analyses the untransformed scores were used, with non-parametric tests employed where possible.

To assess relationships between antibody outcomes and psychological/behavioral factors, exploratory correlational and chi-squared analyses were first performed to identify significant bivariate relationships. Due to the exploratory nature of these analyses and a desire not to increase type-2 errors, no corrections for multiple comparisons were applied. Following this, hierarchical linear and logistic regression models were built to predict antibody outcomes. Based on previous findings that age and gender can influence immune function and, in particular, vaccination responses (Giefing-Kroll et al., 2015), we decided *a priori* to control for age and gender by entering them into step 1 of each regression model with significant psychological and behavioral factors added sequentially in steps thereafter, starting with the predictor with the highest correlation coefficient. All analyses were performed using SPSS (version 23) or Mplus (version 7.4) software.

3. Results

3.1. Responses to vaccination

The profile of IgG levels and HAI seroprotection levels at each time point are presented in Figs. 3 and 4 respectively. For all strains, repeated measures ANOVAs showed a significant increase in $\log_2$-transformed mean IgG antibody levels following vaccination [H1N1: $F(2,238) = 3.76$, $p = 0.03$; H3N2: $F(2,238) = 9.30$, $p < 0.001$; B: $F(2,238) = 6.48$, $p = 0.002$]. Post-hoc pairwise comparisons revealed IgG antibody levels significantly increased in all strains from pre-vaccination to 16 weeks post-vaccination (H1N1: $p = 0.004$; H3N2 $p < 0.001$; B: $p = 0.001$), from pre-vaccination to 4 weeks for H3N2 ($p = 0.020$) and B ($p = 0.007$) strains, but not the H1N1 strain ($p = 0.095$). IgG levels did not increase for any strains between 4 and 16 weeks post-vaccination (H1N1: $p = 0.34$, H3N2: $p = 0.086$; B: $p = 0.775$).

For HAI antibody responses, Cochran's Q tests showed the proportion of participants achieving seroprotection for all strains significantly increased following vaccination [H1N1: $Q(2) = 43.28$, $p < 0.001$; H3N2: $Q(2) = 11.57$, $p = 0.0013$; B: $Q(2) = 27.45$, $p < 0.001$]. Post-hoc pairwise McNemar tests indicated the proportion of seroprotected participants increased from pre-vaccination to both four and 16 weeks post-vaccination in all strains (p's < 0.001–0.007), but there was no significant change between four and 16 weeks post-vaccination, except for in the B strain ($p = 0.011$).

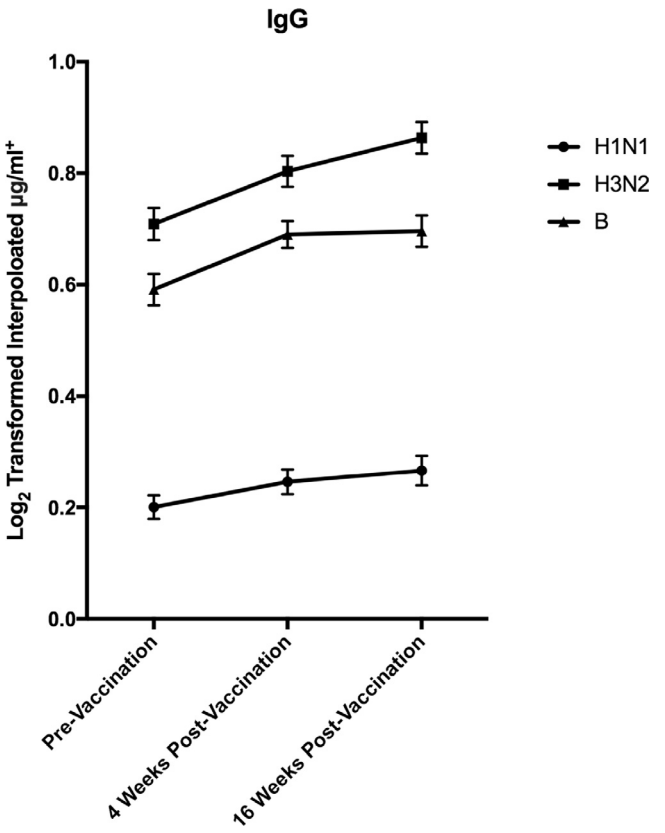

Fig. 3. Serum IgG levels pre- and post-vaccination. Bars represent standard error of the mean. +Florescence values from a 1:8000 diluted sample interpolated against a human IgG calibration curve.

3.2. Psychological and behavioral factors

Table 2 presents a summary of behavioral and psychological factors measured over the diary period. For the dietary components, nearly all participants (range 80–98%) met recommended intakes for protein, iron and vitamin C, but did not meet recommended intakes for selenium, and vitamin D. Therefore, only those components in which some variability was evident were examined as potential predictors of the antibody response to vaccination i.e., energy, zinc, vitamin A, and vitamin E.

3.3. Relationships between psycho-behavioral factors and IgG responses to vaccination

Exploratory bivariate correlations between psychological and behavioral factors measured and IgG levels are presented in Table 3. For the H1N1 strain, this revealed a significant relationship between positive mood over the diary period and IgG levels at 4 weeks ($r = 0.20$, $p = 0.03$) and 16 weeks post-vaccination ($r = 0.30$, $p = 0.002$), such that those with greater levels of positive mood had higher IgG levels. Positive mood on the day of vaccination was significantly positively correlated with IgG levels at 4 ($r = 0.26$, $p = 0.004$) and 16 weeks post-vaccination ($r = 0.35$, $p < 0.001$), with marginally higher correlation coefficients. For the H3N2 strain, significant relationships between IgG levels and negative mood over the diary period (rho = −0.27, $p = 0.004$) and on

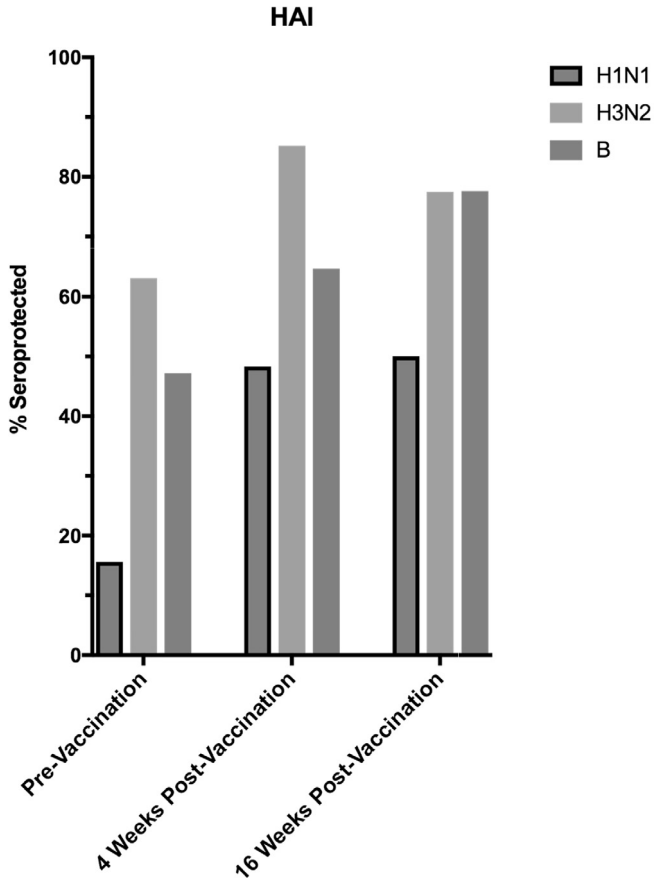

Fig. 4. Percentage of patients achieving seroprotection based on HAI responses.

Table 2
Participant behavioral and psychological characteristics over the diary period[+].

Variable	
Positive Mood	6.41 ± 1.55
Negative Mood	2.35 ± 0.53
Perceived Stress	3.26 ± 2.34
Steps – Per Day	5984 ± 2913
Sleep Duration – hours	7:05 ± 0:53
Sleep Latency – minutes	22.38 ± 17.30
Sleep Efficiency –%	82.15 ± 10.16
Met recommended nutrient intake levels for – n (%)	
Energy	41 (34.2)
Protein	118 (97.5)
Iron	98 (80.3)
Zinc	77 (63.6)
Selenium	5 (4.1)
Vitamin A	32 (26.4)
Vitamin C	109 (89.3)
Vitamin D	2 (1.6)
Vitamin E	71 (58.7)

[+] Mean ± Standard Deviation unless otherwise specified.

the day of vaccination (rho = −0.21, $p = 0.031$) were found at 16 weeks post-vaccination, such that those with greater levels of negative mood had lower IgG levels. For the B Strain, positive mood on the day of vaccination was significantly associated with IgG levels at 4 weeks post-vaccination (r = 0.19, $p = 0.038$), such that those with greater levels of positive mood had higher IgG levels. No other psychological or behavioral factors measured during the diary period were significantly correlated with IgG responses to influenza vaccination.

Linear regression models showed that positive mood over the diary period was an independent predictor above age and gender for H1N1 IgG levels at both 4 [$\beta = 0.19$, $p = 0.04$; model $F(3,120) = 2.48$, $p = 0.07$, $R^2 = 0.06$] and 16 weeks post-vaccination [$\beta = 0.30$, $p = 0.002$; model $F(3,106) = 4.08$, $p = 0.009$, $R^2 = 0.10$], although at 4 weeks the overall model was non-significant. Separate linear regression models focusing on positive mood on the day of vaccination showed this to be a significant independent predictor of H1N1 IgG levels above age and gender at both 4 [$\beta = 0.24$, $p = 0.01$; model $F(3,116) = 3.15$, $p = 0.028$, $R^2 = 0.08$] and 16 weeks post-vaccination [$\beta = 0.38$, $p < 0.001$; model $F(3,103) = 5.65$, $p = 0.001$, $R^2 = 0.14$]. In each case, the proportion of variance explained (R^2) was larger than for equivalent models containing aggregated measures of positive mood over the complete diary period.

For the H3N2 strain, linear regression models revealed negative mood during the diary period [$\beta = -0.06$, $p = 0.512$; model $F(3,106) = 1.13$, $p = 0.339$, $R^2 = 0.03$], and on the day of vaccination [$\beta = -0.11$, $p = 0.253$; model $F(3,103) = 1.15$, $p = 0.245$, $R^2 = 0.04$] were not significant independent predictors of IgG levels at 16 weeks post-vaccination above age and gender. For the B Strain, positive affect on the day of vaccination did not significantly independently predict IgG levels at 4 weeks post-vaccination [$\beta = 0.16$, $p = 0.078$; model $F(3,116) = 2.20$, $p = 0.092$, $R^2 = 0.05$].

3.4. Relationships between psycho-behavioral factors and HAI responses to vaccination

A similar pattern of findings was observed for HAI responses to vaccination. Specifically, exploratory analyses showed the aggregated measure of positive mood over the complete diary period ($r_{biserial} = 0.28$, $p = 0.01$), and positive mood on the day of vaccination ($r_{biserial} = 0.38$, $p = 0.002$), were significantly positively associated with having H1N1 seroprotection at 16 weeks post-vaccination, although not at 4 weeks post-vaccination (Table 4). For the H3N2 strain, associations were observed between dietary zinc intake ($\chi^2(1) = 4.13$, $p = 0.042$, $\varphi = -0.195$) at 4 weeks post-vaccination, with those who met recommended nutrient intake levels being less likely to be seroprotected. No other significant associations between psycho-behavioral factors and seroprotection were observed.

Logistic regression models indicated the aggregated measure of positive mood significantly improved the model predicting H1N1 seroprotection at 16 weeks containing age and gender alone [$\chi^2(1) = 4.796$, $p = 0.03$], but the model as a whole remained non-significant [$\chi^2(3) = 6.640$, $p = 0.08$]. In contrast, positive mood on the day of vaccination independently predicted H1N1 seroprotection at 16 weeks post-vaccination above age and gender [B = 0.15 (0.56), Wald = 7.60, $p = 0.006$, Exp(B) = 1.166, 95% CI: 1.05–1.30], with the overall model being significant [$\chi^2(3) = 9.873$, $p = 0.02$, $R^2 = 0.12$ (Nagelkerke), 0.09 (Cox & Snell)]. For the H3N2 strain, dietary zinc status significantly improved the model predicting seroprotection at 4 weeks containing age and gender alone [$\chi^2(1) = 5.212$, $p = 0.022$], but the model as a whole remained non-significant [$\chi^2(3) = 5.418$, $p = 0.144$].

3.5. Post-hoc exploratory analyses

As relationships were observed for positive affect over the whole diary period and on the day of vaccination, we examined whether these factors potentially influenced vaccination outcomes through independent pathways by conducting additional exploratory analyses entering both measures into hierarchical regression models simultaneously for outcomes where both had been identified as significant predictors when entered alone. For the H1N1 strain, only positive affect on the day of vaccination remained an

Table 3
Exploratory analyses between psycho-behavioral factors and IgG responses (Pearson's correlation coefficients unless otherwise indicated).

Variable	H1N1		H3N2		B	
	4 weeks	16 weeks	4 weeks	16 weeks	4 weeks	16 weeks
Over diary period						
Positive Mood	0.199*	0.296**	0.085	0.083	0.077	0.136
Negative Mood+	−0.018	−0.168	−0.081	−0.269**	−0.022	−0.177
Perceived Stress	−0.054	−0.134	−0.025	−0.096	0.014	−0.050
Steps – Per Day	0.078	0.153	0.093	0.032	0.004	0.027
Sleep Duration – hours	−0.117	−0.070	−0.018	−0.050	−0.086	−0.087
Sleep Latency – minutes	0.064	−0.099	0.050	0.058	0.069	−0.034
Sleep Efficiency – %	−0.051	−0.073	−0.044	−0.100	−0.069	−0.050
Met Energy EAR†	0.170	0.162	0.205	0.098	0.207	0.207
Met Zinc RNI†	0.042	0.109	−0.021	−0.030	0.056	0.064
Met Vitamin A RNI†	−0.034	0.026	−0.037	0.101	0.081	−0.036
Met Vitamin E RNI†	0.198	0.025	0.019	−0.064	0.146	0.087
On day of vaccination						
Positive Mood	0.256**	0.350***	0.162	0.101	0.188*	0.187
Negative Mood+	−0.102	−0.101	−0.091	−0.208*	−0.117	−0.096

+ Non-parametric correlation (rho).
† Biserial correlation.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 4
Exploratory analyses between psycho-behavioral factors and seroprotection (Biserial correlation coefficients unless otherwise indicated).

Variable	H1N1		H3N2		B	
	4 weeks	16 weeks	4 weeks	16 weeks	4 weeks	16 weeks
Over diary period						
Positive Mood	0.109	0.280*	0.104	–	−0.158	−0.008
Negative Mood	−0.075	−0.019	0.024	–	−0.128	−0.029
Perceived Stress	−0.099	−0.172	−0.052	–	0.011	0.063
Steps – Per Day	−0.039	0.117	0.066	–	−0.022	−0.008
Sleep Duration – hours	0.034	0.162	−0.011	–	−0.101	−0.034
Sleep Latency – minutes	−0.054	−0.029	−0.097	–	0.086	−0.025
Sleep Efficiency – %	0.061	0.046	−0.049	–	−0.142	−0.086
Met Energy EAR†	$\chi^2 = 0.373$, p = 0.541	$\chi^2 = 0.111$, p = 0.739	$\chi^2 = 0.529$, p = 0.467	–	$\chi^2 = 0.975$, p = 0.323	$\chi^2 = 2.59$, p = 0.107
Met Zinc RNI†	$\chi^2 = 0.019$, p = 0.890	$\chi^2 = 0.686$, p = 0.407	$\chi^2 = 4.13$, p = 0.042*	–	$\chi^2 = 0.580$, p = 0.446	$\chi^2 = 0.098$, p = 0.754
Met Vitamin A RNI†	$\chi^2 = 0.998$, p = 0.318	$\chi^2 = 0.083$, p = 0.773	$\chi^2 = 0.001$, p = 0.982	–	$\chi^2 = 3.25$, p = 0.071	$\chi^2 = 0.011$, p = 0.915
Met Vitamin E RNI†	$\chi^2 = 0.028$, p = 0.867	$\chi^2 = 0.281$, p = 0.596	$\chi^2 = 0.001$, p = 0.980	–	$\chi^2 = 0.3.78$, p = 0.052	$\chi^2 = 0.806$, p = 0.369
On day of vaccination						
Positive Mood	0.148	0.376**	0.026	–	−0.129	−0.03
Negative Mood	−0.110	−0.127	0.070	–	−0.066	0.001

A substantial proportion of H3N2 16 week HAI assays could not be reliably interpreted and were therefore excluded from the analysis.
† Chi-squared tests.

independent predictor above other variables entered in the model (age, gender, positive affect across diary period) for IgG levels [β = 0.352, $p = 0.034$; model $F(4,101) = 4.182$, $p = 0.004$, $R^2 = 0.14$] and seroprotection [B = 0.20 (0.09), Wald = 7.57, $p = 0.033$, Exp(B) = 1.218, 95% CI: 1.02–1.46; Model: $\chi^2(4) = 10.718$, $p = 0.03$, $R^2 = 0.13$ (Nagelkerke), 0.10 (Cox & Snell)] at 16 weeks post-vaccination. For IgG levels at 4 weeks post-vaccination, neither positive affect measure was an independent predictor when entered together.

To examine whether relationships between psychological and behavioral factors and vaccination outcomes differed according to the time relative to vaccination, post hoc correlational and chi-squared analyses were conducted between weekly mean levels for each psychological and behavioral variable measured across the diary period and antibody outcomes. These are presented in the supplementary appendix (Table S1). These analyses show there was no consistent pattern across variables. In addition, further exploratory correlational analyses were performed exploring the relationships between specific positive and negative affect items completed on the day of vaccination and antibody outcomes, to explore which aspects of affect may be driving observed effects (Table S2). However, no specific items appeared to be more strongly associated with antibody outcomes than others.

3.6. Discussion

The present study sought to examine the effects of known behavioral and psychological immune modulators on influenza vaccination responses in older adults. We found that greater positive mood, whether measured repeatedly over a 6-week period around vaccination, or on the day of vaccination, significantly predicted greater antibody responses to influenza vaccination in the least immunogenic viral strain (H1N1). This effect was observed when measuring both absolute levels of IgG and seroprotection as determined by HAI. However, comparable effects were not found for the H3N2 and B strains of the vaccination. No other behavioral or psychological factor measured significantly predicted vaccination outcomes.

Our findings in relation to positive mood are consistent with, and extend, previous findings that positive mood can act as an 'im-

mune modulator' (Marsland et al., 2007) and that trait positive affect is associated with greater antibody responses to Hepatitis B vaccination (Marsland et al., 2006). Interestingly, we found the effects of positive mood on the day of vaccination appeared to be more pronounced than positive mood over the 6-week period surrounding vaccination as a whole. This was evidenced by the relative size of the effects (see Tables 3 and 4) and post hoc exploratory analyses for 16 week post-vaccination H1N1 outcomes - in which only positive mood on the day of vaccination remained a significant independent predictor when both measures were entered into regression models. This suggests the day of vaccination may be a particularly salient time-point at which positive mood influences immune responses to vaccination.

While positive mood on the day of vaccination may be particularly salient for vaccine outcomes, this does not appear to be completely independent of positive affect over the longer period surrounding vaccination (i.e., these may not necessarily act through different pathways). This raises an interesting question regarding whether it might be sufficient to change mood on the day of vaccine to achieve an improved antibody response to vaccination or if it is necessary to change mood over longer periods? There is of course extensive experimental research which suggests that relatively brief mood-enhancing interventions can impact on immune parameters. For example, watching a comedy film has been found to increase S-IgA concentration and amend cytokine secretions (Dillon et al., 1985; Mittwoch-Jaffe et al., 1995), a session of yoga has been associated with increases in soluble IL-6 receptors (Kiecolt-Glaser et al., 2010), and briefly writing about one's self-congruencies has been found to increase leukocyte, lymphocyte and NK cell counts in sera (Strauman et al., 2004). Although, whether these immunological changes are sufficient to impact meaningfully on vaccination responses, or whether such mood-enhancing interventions could be implemented within the confines of existing vaccination practices (e.g., short-time windows, acceptability to older adults and healthcare professionals), remains unclear.

When considering potential mechanisms, it is likely that these may be both direct and indirect. Indirectly, positive mood is associated with healthier lifestyles (Grant et al., 2009) and better health status is associated with improved responses to vaccination (Gross et al., 1989). More directly, biological pathways between sub-cortical regions of the brain responsible for affective processing and the immune system have been described previously, including endocrine-mediated mechanisms (Marques-Deak et al., 2005) and the direct innervation of lymphatic organs extending from the central nervous system (Felten and Felten, 1991). Mood induction and ambulatory measurement studies show links between positive affective states and CNS parasympathetic activation; cortisol secretions; greater NK cytotoxicity, c-reactive protein levels, and flow rates of secretory IgA (Pressman and Cohen, 2005). Together, these biological correlates of positive mood provide some evidence of mechanisms by which immune function, and therefore antibody responses to vaccination, might be influenced.

Notwithstanding the issue of potential mechanisms, it is noteworthy that the relationship between positive mood and antibody responses was only observed for the weakest immunogenic strain (H1N1) and not for H3N2 and B strains. One explanation is that any influence of psychological or behavioral factors on antibody responses may only be observable when the host immune response to the antigen is relatively weak. This explanation has been put forward previously by other researchers in this field and is supported by evidence from comparative trials between full and half-dose vaccines. These have repeatedly shown that psychobehavioral influences are often only seen in the weakest immunogenic strains (Edwards et al., 2012, Edwards et al. (2010), Edwards et al. (2007)). For example, Edwards et al. (2012) compared young adults' antibody responses to either a full or 50% dose of pneumococcal vaccination, administered immediately after completing a 15-min resistance exercise program or resting. Those performing exercise in the half-dose group showed greater average post-vaccination antibody levels over resting participants. For those receiving the full dose, there was no significant difference in antibody responses between exercisers and resting controls, indicating that where vaccination induces high levels of antibody, additional benefits of psychological or behavioral factors may not be evident.

An alternative explanation relates to ceiling effects obscuring psycho-behavioral influences on antibody responses. This is particularly relevant in this study as participants received a vaccination which was identical to that administered in the previous year. Consequently, we observed that 63% and 47% of participants had seroprotective levels of H3N2 and B strains respectively prior to being vaccinated. This was unexpected. While the recommended composition of influenza vaccines often includes some strains two years running, all three strains remaining the same has only previously happened twice since the turn of the century (2002/3–2003/4 & 2010/1–2011/2) for the northern hemisphere vaccination. This may partly explain why other factors did not significantly predict post-vaccination antibody responses despite previous findings to the contrary (Calder, 2013; Pascoe et al., 2014; Pedersen et al., 2009; Prather et al., 2012). Accordingly, we speculate that the observed effects may differ for more novel antigens and could potentially be larger. As such, we do not argue that the other psychological and behavioral factors measured do not influence vaccination response, rather we suggest that this study provides preliminary evidence that positive affect may be particularly influential given that the effect was observed despite the potential for ceiling effects. It is noteworthy that other researchers have also failed to find relationships between many of the behavioral and psychological factors measured with vaccine response (e.g., Bunout et al., 2004 [nutrition]; Dopp et al., 2007 [sleep]; Hayney et al., 2014 [physical activity]; Long et al., 2013 [physical activity]; Marsland et al., 2001 [stress]; Wong et al., 2013 [stress]) and as such replication of the present findings with more novel antigens or in a subsequent influenza vaccination season would give greater confidence in their validity.

This study has several limitations. The presented relationships are observational in nature and therefore it is possible that other, unmeasured factors are acting as confounders. Further, the self-selecting sample may not be representative of the older adult population. This is particularly true with respects to ethnicity, which was primarily limited to Caucasians in this study. This is noteworthy because of previous reports of race-based differences in immune responses to vaccination (Haralambieva et al., 2013). Extrapolation of these results should therefore be made with due caution. Further, null findings in relation to stress and negative affect may in part be related to their relatively low prevalence in the present cohort. Previous studies that have demonstrated detrimental effects of negative affect or stress on vaccination responses (e.g., Vedhara et al., 1999) have often been conducted in high-stress contexts such as caregiving. However, comparatively few of our participants experienced high levels of negative affect, as evidenced by the non-normal distribution of negative affect scores in our sample (skewed towards having low levels of negative affect).

A further limitation is that we only recruited individuals who had been vaccinated in the previous year, potentially limiting generalisability. However, as influenza vaccination uptake rates for older adults in the US and UK annually are between 60–65% and 70–75% respectively (Centers for Disease Control and Prevention, 2015; Public Health England, 2015) the proportion of older adults not having previously been vaccinated is comparatively small. Additionally, as we examined many variables it is possible that

some relationships between psychological and behavioral factors and vaccine responses with effect sizes lower than that observed for positive affect were not found to be significant due to insufficient power (type-II errors). We attempted to mitigate this possibility to a degree by not further reducing power by correcting for multiple comparisons, however this may in turn have inflated the possibility of type-I errors. Replication of these findings is needed to be confident in their veracity.

Finally, while antigen-specific IgG and HAI levels are widely accepted correlates of vaccine-induced protection, they do not perfectly predict protection against real-world clinical infections. In part, this is because exposure to influenza differs across individuals. Additional research focusing on the relationship between positive mood and non-surrogate influenza outcomes would help clarify the clinical importance of these findings. Despite these limitations, this is the first study to comprehensively examine patient behaviors and psychological factors on the vaccine-induced protective antibody response in older adults using a robust methodology (prospective, diary-based longitudinal study).

Conflicts of interest

All authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2017.09.008.

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