

Effect of fruit and vegetable consumption on immune function in older people: a randomized controlled trial^{1–4}

Andrew Gibson, J David Edgar, Charlotte E Neville, Sarah ECM Gilchrist, Michelle C McKinley, Chris C Patterson, Ian S Young, and Jayne V Woodside

ABSTRACT

Background: Fruit and vegetable (FV) intake, which is often low in older people, is associated with reduced chronic disease risk.

Objective: We determined whether increased FV intake improves measures of immune function.

Design: We conducted a randomized controlled trial (The Ageing and Dietary Intervention Trial) in 83 healthy volunteers aged 65–85 y with low FV intakes (≤ 2 portions/d); 82 subjects completed the intervention. Participants were assigned to continue their normal diets or to consume ≥ 5 FV portions/d for 16 wk. At 12 wk, tetanus toxoid (0.5 mL intramuscular) and Pneumovax II vaccine (0.5 mL intramuscular; both vaccines from Sanofi Pasteur) were administered. FV intake was monitored by using diet histories, and biomarkers of nutritional status were assessed. The primary endpoint was the antibody response to vaccination. Specific antibodies binding to tetanus toxoid (total IgG) and pneumococcal capsular polysaccharide (total IgG and IgG2) were assessed at baseline and 16 wk. Participants were recruited between October 2006 and June 2008.

Results: The change in FV consumption differed significantly between groups [mean change in number of portions (95% CI): in the 2-portion/d group, 0.4 portions/d (0.2, 0.7 portions/d); in the 5-portion/d group, 4.6 portions/d (4.1, 5.0 portions/d); $P < 0.001$] and also in micronutrient status. Antibody binding to pneumococcal capsular polysaccharide (total IgG) increased more in the 5-portion/d group than in the 2-portion/d group [geometric mean (95% CI) of the week 16:baseline ratio: 3.1 (2.1, 4.4) and 1.7 (1.3, 2.1), respectively; $P = 0.005$]. There was no significant difference in the increases in antibody binding to tetanus toxoid.

Conclusion: Increased FV intake improves the Pneumovax II vaccination antibody response in older people, which links an achievable dietary goal with improved immune function. This trial was registered at clinicaltrials.gov as NCT00858728. *Am J Clin Nutr* 2012;96:1429–36.

INTRODUCTION

A diet that is rich in fruit and vegetables (FVs)⁵ may provide protection against cardiovascular disease (1–3), several cancers (4), and other chronic diseases. Several of the micronutrients associated with diets high in FVs, such as carotenoids, flavonoids, and vitamin C, have also been shown to improve immune function (5, 6). Randomized controlled trials have tested the effect of a particular type of FV supplementation on markers of immune function, with some trials that reported an enhancement

of natural killer cell (NKC) cytotoxicity and lymphocyte proliferation with consumption of different FV juices (7–9), whereas other intervention studies have shown no effect of prolonged tomato juice consumption (10) or 2 wk of supplementation with a tomato extract (11). Only one study has examined an increase in a mixture of FVs and showed no effect on a range of immune function markers including lymphocyte proliferation and NKC cytotoxicity in healthy men over 4 wk (12).

Aging has been associated with physiologic, social, and economic changes that can lead to a compromised nutritional status (13, 14), and older populations have low FV intakes (15, 16). Aging is also associated with dysregulation of the immune system (17), and older people are at elevated risk of infection (18), which will also have an adverse effect on nutritional status. An increase in FV intake may potentially benefit older populations because of their initially low intake of FVs and their altered immune status. A comprehensive assessment of the effects of diet on immune system status and function is methodologically complex, and both the antibody response to vaccination, which represents a functional test of the immune system, by indirectly testing innate and adaptive immunity, and NKC cytotoxicity have been recommended (19). This study was a randomized, controlled, parallel-group trial that compared the effect of increasing FV intake to the recommended 5 portions/d with a control low-FV diet (≤ 2 portions/d) on clinically relevant measures of immune function in an older population.

¹ From the Centre for Public Health, Queen's University Belfast, Institute of Clinical Science B, Belfast, United Kingdom (AG, CEN, SECMG, MCM, CCP, ISY, and JYW), and the Regional Immunology Service, Royal Victoria Hospital, Belfast, United Kingdom (JDE).

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³ Supported by the United Kingdom Food Standards Agency.

⁴ Address reprint request and correspondence to JV Woodside, Centre for Public Health, Queen's University Belfast, Institute of Clinical Science B, Grosvenor Road, Belfast, BT12 6BJ, United Kingdom. E-mail: j.woodside@qub.ac.uk.

⁵ Abbreviations used: FV, fruit and vegetable; NKC, natural killer cell; PCP, pneumococcal capsular polysaccharide.

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SUBJECTS AND METHODS

Setting and participants

This study was a randomized controlled trial that was designed to examine the effect of the recommended 5 portions FVs/d compared with ≤ 2 portions FVs/d on clinically relevant markers of immune function. The study design is summarized in **Figure 1**. Participants were recruited between October 2006 and June 2008 through press releases to local media, older people's networks, newsletters, and bulletins, and presentations to older people's community groups and from hospital outpatient clinics. All subsequent study visits were home visits. The study was approved by the Office for Research Ethics Committees Northern Ireland (ORECNI).

Healthy older free-living participants (aged 65–85 y) with low FV intakes (≤ 2 portions/d) were recruited. Exclusion criteria were as follows: the consumption of special diets, the taking of nutritional supplements or medications known to affect immune function or the absorption of nutrients, excessive alcohol consumption (>28 U/wk for men and >21 U/wk for women), BMI (in kg/m^2) >35 , history of diabetes or dementia, Pneumovax II vaccination (Sanofi Pasteur) within the previous 2 y, inability to provide informed consent, any other problem that would prevent adherence to a high FV diet, or recent infection (<3 wk since completion of any antibiotic course or symptoms of viral illness).

Eligible individuals who were willing to take part in the study gave informed written consent. Baseline demographic information (assessment of dietary intake by using a 7-d diet history, physical activity by using a validated questionnaire, alcohol consumption, and medication use) was recorded, and anthropometric data were collected (weight and height measured

by using calibrated scales and a stadiometer, respectively). A fasting baseline blood sample was also taken, separated appropriately for the proposed assays, and, where necessary, stored at -70°C until analysis.

Random assignment and interventions

Participants were randomly assigned by using a block-randomization approach (block size $n = 8$) with computer-generated random numbers to one of the 2 arms, either to increase FV consumption to ≥ 5 portions/d or to follow their normal diets (therefore, consuming ≤ 2 portions/d) for 16 wk. A portion was considered an 80-g serving (eg, one apple, orange, or banana; 3 heaped tablespoons of vegetables; or 150 mL fruit juice) as defined by the Food Standards Agency. All participants were supplied with a tablespoon and glass as an aid to estimate portion sizes.

All participants had additional assessments (a diet history and a fasting blood sample were taken) at 6 and 12 wk to monitor compliance with the intervention. At the 12-wk visit, tetanus toxoid [0.5 mL intramuscular; given as part of Revaxis vaccine (tetanus, diphtheria and poliomyelitis; Sanofi Pasteur)] and Pneumovax II vaccine (0.5 mL intramuscular; Sanofi Pasteur) were administered.

At the final 16-wk visit, all participants underwent a diet-history interview, had a fasting blood sample taken for nutrient status and immune function analyses, had weight assessed, and completed a brief questionnaire to document changes in physical activity and other lifestyle behaviors. Over the course of the intervention, participants were asked to make a note of any recent infections and illness and any newly prescribed medications and to minimize other changes to their health and lifestyle behaviors.

Maximizing and monitoring compliance

Participants received extensive personal dietetic advice and nutritional counseling to encourage the incorporation of FVs into their diets without, for example, compromising energy intake and in line with their physical capabilities. Compliance was also encouraged by the provision of menu suggestions and recipes. Participants were allowed a free choice of FVs for consumption over the study. No prescriptive list was offered to participants at any point. However, participants were encouraged to consume as wide a variety of FVs as possible. At the time of FV selection, the researchers recorded the FV choices of participants while discussing feasibility issues such as storage, cooking methods, and preparation of FVs and composite dishes. To support dietary compliance while minimizing personal expense and maximizing food freshness, each participant (in both intervention and control arms) received weekly home deliveries of FVs from a local supermarket. All participants were contacted on a weekly basis by telephone to check and encourage compliance and to monitor any difficulties they experienced. Compliance was monitored in both intervention and control groups by using a diet-history interview and laboratory assessment of micronutrient status at baseline and 6, 12, and 16 wk.

Outcomes and follow-up

All endpoint assessments were conducted by researchers who were blinded to the group allocation of participants. Daily FV intakes were assessed from diet histories by 2 independent researchers.

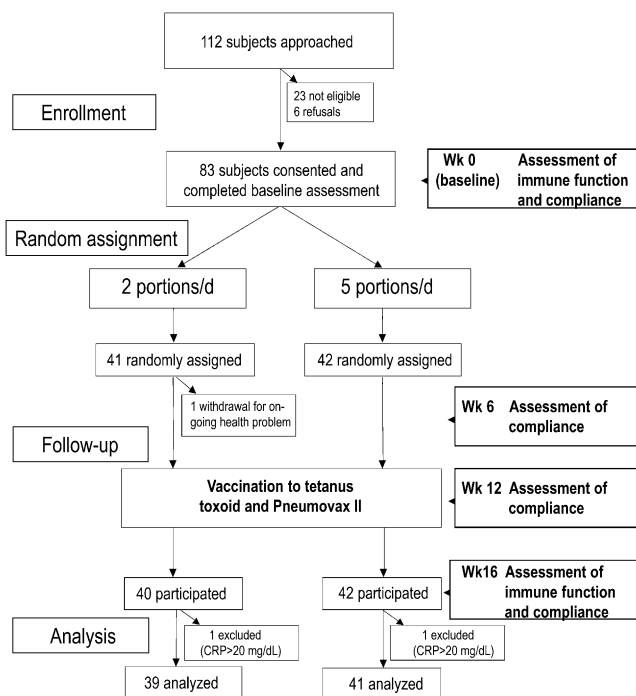


FIGURE 1. Flowchart of the overview of the study design and progression of participants through the study. Pneumovax II and tetanus toxoid vaccines are manufactured by Sanofi Pasteur. CRP, C-reactive protein.

All laboratory methods were performed with careful attention to quality control. There was participation in relevant external quality-control schemes where available, and standardization against available international standards (eg, National Institute of Standards and Technology materials for lipid-soluble vitamins and ascorbate) when possible.

Biochemical markers of nutritional status were assessed at baseline and 6, 12, and 16 wk. Serum lutein, zeaxanthin, β -cryptoxanthin, lycopene, and α - and β -carotene were assessed by using reverse phase HPLC with diode-array detection (20). Plasma ascorbate was measured with an automated fluorimetric assay by using a Cobas FARA analyzer (Roche Diagnostics) (21).

Antibody assessment was undertaken at baseline and 16 wk. Specific antibody binding to tetanus toxoid (total IgG) and pneumococcal capsular polysaccharide (PCP) (total IgG and IgG2) were assessed with an ELISA by using commercially available kits (Vacczyme Anti-tetanus toxoid IgG Enzyme Immunoassay Kit; Vacczyme Anti-PCP IgG Enzyme Immunoassay Kit; Anti-PCP IgG2 Enzyme Immunoassay Kit MK013; all from The Binding Site). NKC cytotoxicity was assessed by using flow cytometry according to an in-house method at baseline and week 16. Participant mononuclear cells were isolated over a Ficoll Histopaque (GE Healthcare) density-diffusion gradient incubated with K562 target cells at a range of effector cell-to-target cell ratios (0, 12.5, 25, and 50) both with and without IL-2 stimulation for 2 h, stained with propidium iodide, and analyzed by flow-cytometry. NKC cytotoxicity data were adjusted for the proportion of NK cells (22). Total serum Igs and IgG subclasses were assessed by nephelometry at baseline and week 12. Lymphocyte subset analysis including CD3, CD4, CD8, CD19, and CD16/56 (both relative and absolute counts) were measured by flow cytometry at baseline and week 16. Analysis was undertaken in a clinical pathology-accredited immunology laboratory. High-sensitivity C-reactive protein concentrations were determined in serum by using an immunoturbidimetric assay (Randox) on an ILab-600 biochemical analyzer (Instrumentation Laboratories). Primary endpoints were the ratio at 16 wk relative to the baseline value of the antibody response to tetanus toxoid and Pneumovax II vaccination.

Statistical analysis

This study aimed to examine whether an increase in consumption of FVs by older participants had an effect on immune

function. There were no relevant data available for any older population or a population in Northern Ireland. Therefore, power and sample-size calculations were not possible.

Analyses were performed with SPSS version 17.0 (SPSS Inc). Normally distributed continuous variables were summarized by using means \pm SDs. Skewed variables were log transformed for parametric analysis and were summarized by using the geometric mean and IQR.

Between-group comparisons of baseline values were made by using independent samples *t* tests and chi-square analysis for continuous and categorical variables, respectively. Between-group comparisons of the change in each outcome variable were made by using independent samples *t* tests, and data are presented as differences (95% CIs) for normally distributed variables or the geometric means (95% CIs) of the ratio of the week 6, 12, or 16 to baseline values for skewed variables. In an exploratory analysis, a subgroup analysis of primary endpoints by Pneumovax II vaccination history was performed by including an interaction between the intervention group and vaccination history in a linear regression analysis with a log-transformed ratio of the week 16 to baseline value as the dependent variable. The association between self-reported FV intake and changes in primary endpoints was also assessed by using linear regression analysis. All tests were 2-tailed tests, and $P < 0.05$ was considered statistically significant.

RESULTS

The number of participants at each stage of the study is summarized in Figure 1. Of the 83 participants who were randomly assigned, 82 subjects completed the study. Two participants had serum C-reactive protein concentrations >20 mg/L at at least one time point during the study and were excluded from all analyses because acute inflammation would have affected immune function endpoints. Of the 80 participant blood samples included in the analysis, there was one missing blood sample at baseline, 3 missing blood samples at week 6, and no missing blood samples at weeks 12 and 16. The usual (\pm SD) FV intake of participants was 1.4 ± 0.6 portions/d.

Preintervention characteristics are summarized in Table 1. Any significant imbalances between groups at baseline are indicated in Table 1, and additional statistical analysis was carried

TABLE 1
Baseline characteristics of participants according to intervention-group allocation

	2 portions/d ($n = 39$)	5 portions/d ($n = 41$)
Age (y) ¹	71.1 \pm 5.0	70.9 \pm 5.0
Men [n (%)]	7 (18)	21 (51) ²
BMI (kg/m ²)	28.1 (4.5)	28.5 (4.0)
Systolic blood pressure (mm Hg)	150.5 (24.4)	152.9 (20.9)
Diastolic blood pressure (mm Hg)	84.1 (10.9)	87.0 (10.9)
Current smokers [n (%)]	3 (8)	1 (2)
Former smokers [n (%)]	14 (35)	18 (44)
Alcohol consumers [n (%)]	16 (40)	18 (44)
Use of medication [n (%)]	37 (93)	38 (93)
CRP ³ (mg/L) ⁴	2.44 (1.25, 4.38)	1.74 (0.96, 2.70)

¹ All values are means \pm SDs.

² Significantly different from the 2-portion/d group, $P < 0.01$ (chi-square test).

³ CRP, C-reactive protein.

⁴ All values are geometric means (IQRs) because distributions were skewed.

out by using the change in each variable of interest to minimize the effect of any imbalances on the comparison between intervention groups. BMI did not significantly change in either group over the course of the study [the mean change (95% CI) at 16 wk was -0.1 ($-0.3, 0.1$) in the 2-portion/d group and 0.1 ($-0.1, 0.3$) in the 5-portion/d group; P -difference in change = 0.16], and, although not formally measured, participants were advised to not change physical activity habits during the study, which they were asked about at each study visit.

The change in portions of FVs consumed per day is reported in **Table 2**. The change in self-reported daily FV consumption was significantly different between the 2 groups at all time points.

The baseline and 6-, 12-, and 16-wk micronutrient concentrations for the 2 intervention groups is also shown in Table 2. Changes in vitamin C, zeaxanthin, β -cryptoxanthin, and lycopene at all time points differed significantly between the 2 intervention groups and were higher in the 5-portion/d group than in the 2-portion/d group.

The change in the primary endpoint and NKC cytotoxicity by FV intervention group is shown in **Table 3**. There was no difference in the change in antibody binding to tetanus toxoid between the 2 intervention groups, but antibody binding (total IgG and IgG2) to PCP increased more in the 5-portion/d group than in the 2-portion/d group. For the PCP antibody binding, we

TABLE 2

Self-reported FV intake and micronutrient status at baseline and during the intervention in subjects who consumed 2 or 5 portions FVs/d¹

	No. of subjects	Baseline	Change at 6 wk	Change at 12 wk	Change at 16 wk
Fruit (portions/d) ²					
2 portions/d	39	0.6 \pm 0.5	0.3 (0.1, 0.5)	0.2 (0.1, 0.4)	0.4 (0.2, 0.6)
5 portions/d	41	0.7 \pm 0.5	3.2 (2.9, 3.6)	3.0 (2.7, 3.4)	3.3 (3.0, 3.7)
<i>P</i>	—	0.41	<0.001	<0.001	<0.001
Vegetables (portions/d) ²					
2 portions/d	39	0.8 \pm 0.4	0.0 ($-0.1, 0.1$)	0.0 ($-0.1, 0.2$)	0.0 ($-0.1, 0.1$)
5 portions/d	41	0.8 \pm 0.3	1.4 (1.2, 1.6)	1.2 (1.0, 1.4)	1.2 (0.9, 1.5)
<i>P</i>	—	0.77	<0.001	<0.001	<0.001
FVs (portions/d) ²					
2 portions/d	39	1.4 \pm 0.7	0.3 (0.1, 0.6)	0.3 (0.1, 0.5)	0.4 (0.1, 0.7)
5 portions/d	41	1.4 \pm 0.5	4.6 (4.2, 5.0)	4.2 (3.8, 4.6)	4.5 (4.1, 5.0)
<i>P</i>	—	0.68	<0.001	<0.001	<0.001
Vitamin C ($\mu\text{mol/L}$) ³					
2 portions/d	35	43.0 (39.8, 60.8)	1.03 (0.93, 1.13)	1.00 (0.91, 1.10)	0.98 (0.87, 1.12)
5 portions/d	38	42.7 (35.1, 58.1)	1.42 (1.27, 1.59)	1.27 (1.13, 1.42)	1.39 (1.23, 1.57)
<i>P</i>	—	0.95	<0.001	0.002	<0.001
Lutein ($\mu\text{mol/L}$) ³					
2 portions/d	36	0.15 (0.12, 0.21)	1.07 (0.98, 1.18)	1.07 (0.96, 1.18)	1.03 (0.92, 1.16)
5 portions/d	38	0.14 (0.10, 0.20)	1.18 (1.07, 1.31)	1.16 (1.05, 1.29)	1.18 (1.04, 1.34)
<i>P</i>	—	0.52	0.17	0.24	0.12
Zeaxanthin ($\mu\text{mol/L}$) ³					
2 portions/d	36	0.03 (0.03, 0.04)	1.00 (0.88, 1.12)	1.01 (0.90, 1.14)	1.02 (0.89, 1.17)
5 portions/d	38	0.02 (0.01, 0.04)	1.41 (1.21, 1.65)	1.39 (1.19, 1.63)	1.42 (1.22, 1.66)
<i>P</i>	—	0.02	<0.001	0.002	0.002
β -Cryptoxanthin ($\mu\text{mol/L}$) ³					
2 portions/d	36	0.06 (0.04, 0.10)	1.03 (0.90, 1.18)	0.99 (0.78, 1.25)	1.12 (0.95, 1.32)
5 portions/d	38	0.06 (0.04, 0.09)	1.50 (1.29, 1.75)	1.37 (1.15, 1.63)	1.53 (1.26, 1.86)
<i>P</i>	—	0.93	<0.001	0.03	0.02
α -Carotene ($\mu\text{mol/L}$) ³					
2 portions/d	36	0.10 (0.07, 0.15)	1.19 (0.98, 1.45)	1.18 (0.99, 1.40)	1.17 (0.96, 1.44)
5 portions/d	38	0.09 (0.05, 0.18)	1.37 (1.09, 1.73)	1.33 (1.05, 1.69)	1.43 (1.04, 1.96)
<i>P</i>	—	0.52	0.35	0.40	0.30
β -Carotene ($\mu\text{mol/L}$) ³					
2 portions/d	36	0.25 (0.18, 0.38)	1.13 (0.98, 1.32)	1.13 (1.01, 1.27)	1.26 (1.08, 1.47)
5 portions/d	38	0.25 (0.14, 0.45)	1.18 (0.96, 1.45)	1.20 (0.94, 1.53)	1.23 (0.92, 1.64)
<i>P</i>	—	0.94	0.75	0.67	0.87
Lycopene ($\mu\text{mol/L}$) ³					
2 portions/d	36	0.51 (0.31, 0.99)	0.91 (0.72, 1.17)	0.94 (0.77, 1.14)	0.85 (0.65, 1.12)
5 portions/d	38	0.31 (0.16, 0.74)	1.39 (0.99, 1.96)	1.44 (1.04, 1.99)	1.34 (0.96, 1.86)
<i>P</i>	—	0.03	0.05	0.03	0.04

¹ Baseline variables and changes were compared between 2- and 5-portion/d groups by using independent samples *t* tests. FV, fruit and vegetable.

² All baseline values are means \pm SDs, and all change values are means (95% CIs). Changes were calculated as week 6 – baseline, week 12 – baseline, and week 16 – baseline values.

³ Variables were logarithmically transformed. All baseline values are geometric means (IQRs), and all change values are geometric means (95% CIs) of the ratio of week 6, 12, or 16 to baseline values.

TABLE 3

Antibody response to vaccination and NKC cytotoxicity at baseline and after the intervention in subjects who consumed 2 or 5 portions FVs/d¹

	No. of subjects	Baseline ²	Change at 16 wk ³	Subjects who achieved a 4-fold response <i>n</i> (%)
SAB tetanus toxoid (mg/L)				
2 portions/d	38	2.1 (0.6, 8.0)	8.9 (5.6, 14.2)	NM
5 portions/d	40	3.9 (0.7, 18.7)	6.9 (4.1, 11.6)	NM
<i>P</i>	—	—	0.46	NM
SAB pneumococcal capsular polysaccharide (total IgG) (mg/L) ⁴				
2 portions/d	38	143 (60, 389)	1.7 (1.3, 2.1)	2 (5)
5 portions/d	40	139 (77, 281)	3.1 (2.1, 4.4)	12 (30)
<i>P</i>	—	—	0.005	0.004
SAB pneumococcal capsular polysaccharide (IgG2) (mg/L) ⁴				
2 portions/d	38	49 (20, 130)	1.7 (1.4, 2.1)	3 (8)
5 portions/d	40	48 (22, 106)	3.1 (2.2, 4.3)	11 (28)
<i>P</i>	—	—	0.006	0.02
NKC cytotoxicity (unstimulated)				
2 portions/d	33	65.4 (28.9, 134.2)	0.70 (0.52, 0.92)	NM
5 portions/d	29	85.2 (37.0, 171.2)	0.82 (0.62, 1.10)	NM
<i>P</i>	—	0.32	0.40	NM
NKC cytotoxicity (IL-2 stimulated)				
2 portions/d	32	111.3 (58.9, 222.8)	0.61 (0.46, 0.79)	NM
5 portions/d	29	129.9 (67.4, 264.9)	0.85 (0.65, 1.10)	NM
<i>P</i>	—	0.55	0.07	NM

¹ NKC data are presented as the AUC. All variables were logarithmically transformed. FV, fruit and vegetable; NKC, natural killer cell; NM, not measured; SAB, specific antibody binding.

² All values are geometric means; IQRs in parentheses.

³ All values are geometric means (95% CIs) of the ratio of 16 wk to baseline values and were compared between the 2- and 5-portion/d groups by using independent samples *t* tests.

⁴ For Pneumovax II vaccination, the proportion of subjects who achieved a 4-fold antibody response was calculated from untransformed data and compared between groups by using the chi-square test.

also calculated the proportion that achieved a 4-fold antibody response to vaccination (23, 24), and this also differed significantly between intervention groups (Table 3). None of these findings was altered by adjustment for the sex imbalance between groups. NKC cytotoxicity, which was adjusted for the proportion of NK cells, in response to IL-2 stimulation tended to be greater in the 5-portion/d group than in the 2-portion/d group at 16 wk ($P = 0.07$). There was no effect of increasing FV intake on unstimulated NKC cytotoxicity. There was also no effect of increasing FV intake on total serum Igs, Ig subclasses, or lymphocyte subsets (both relative and absolute counts), except for IgG2, which appeared to decrease over time in the 5-portion/d group compared with in the 2-portion/d group, and the percentage of CD8 cells, which seemed to decrease in the 2-portion/d group compared with in the 5-portion/d group (see Tables 1 and 2 under "Supplemental data" in the online issue).

Statistical analysis was initially carried out between the intervention groups to which participants were randomly assigned but was also repeated according to self-reported changes in FV consumption. There was a significant positive relation between the change in FV consumption and specific antibody response to PCP (total IgG and IgG2). An extra daily portion of FVs was predicted to increase the total IgG response by 18% (95% CI: 8%, 29%), and the IgG2 response by 17% (95% CI: 7%, 27%).

The effect of increased FV consumption on PCP primary endpoints was then examined by history of Pneumovax II vaccination in an exploratory analysis. The inclusion of an interaction between intervention group and Pneumovax II vaccination history in the regression model for week 16–to–

baseline ratio provided evidence that the response to vaccination of increased FV consumption depended on the vaccination history for IgG ($F = 6.30$, $df = 2,60$; $P = 0.003$) and IgG2 ($F = 6.63$, $df = 2,60$; $P = 0.003$). The effect of increased FV consumption was only seen in subjects who had never received the Pneumovax II vaccination in the past, and this result is shown in **Figure 2**. In the vaccination-naïve group, in a similar analysis to that displayed in Table 3, PCP antibody binding (total IgG) increased more in the 5-portion/d group than in the 2-portion/d group [geometric mean (95% CI) of the week 16:baseline ratio: 9.8 (5.6, 17.0) and 3.2 (1.7, 5.9), respectively; $P = 0.008$], and corresponding estimates for the IgG2 response were 9.1 (5.0, 16.3) and 2.9 (1.7, 5.0). These estimates were not significant for subjects who had received Pneumovax II vaccination either 3–5 or >5 y before the study (data not shown).

An additional analysis of the data showed that, at weeks 12 and 16, a smaller proportion of participants reported recent infections or illnesses (defined as an infection or illness that occurred since the previous study visit) in the 5-portion/d group than in the 2-portion/d group (week 12: 20% of participants in the 5-portion/d group and 33% in the 2-portion/d group; week 16: 24% in the 5-portion/d group and 33% in the 2-portion/d group) but this was not significant ($P = 0.16$ and $P = 0.38$ at weeks 12 and 16, respectively).

DISCUSSION

We have shown, for the first time to our knowledge, an improved response to Pneumovax II vaccination, but not to tetanus

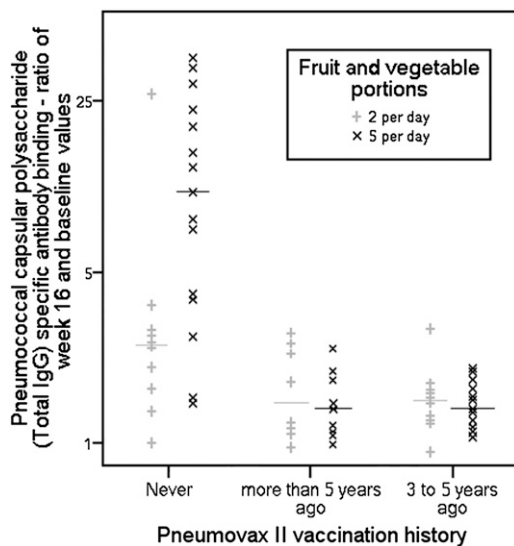


FIGURE 2. Relation between vaccination history and effect of increased fruit and vegetable consumption on specific antibody binding to pneumococcal capsular polysaccharide (total IgG) expressed as the ratio of week 16 to baseline values and plotted on a logarithmic scale. *P* values for comparisons within vaccination history category were 0.008, 0.71, and 0.55, respectively. Numbers of participants who consumed 2 and 5 portions/d were as follows—never: 10 and 15 subjects, respectively; >5 y before the study: 8 and 9 subjects, respectively; and 3–5 y before the study: 10 and 14 subjects, respectively.

vaccination, in individuals who consumed 5 portions FVs/d compared with individuals who consumed 2 portions FVs/d for 16 wk.

The mechanism behind the increased response to PCP vaccination is unclear. Significantly higher antibody responses were observed against the PCP polysaccharide (a T cell–independent B cell response) than against tetanus toxoid (a T cell–dependent B cell response). This result suggested that, in some way, the FV increase in this study augmented the T cell–independent pathway of antigen presentation with resultant enhanced immune responsiveness. The increased response to PCP vaccination was confined to subjects who had never received the vaccine before, and this vaccination-naïve group showed the largest antibody response as a whole and was, therefore, the group in which an effect of intervention was most likely to be shown. Whatever mechanism was involved, the effect was maximal on primary and not secondary immune responses, which indicated that the influence was greater on naïve B cells rather than committed memory B cells. Therefore, this effect may have implications for other components of the immunization schedule.

Such observations are potentially of great importance in the consideration of vaccination programs. In older populations, because their immune systems are known to be less effective than those of younger individuals as characterized by increased incidences of chronic diseases and susceptibility to infection, an effect on antibody response may be of particular importance (25–28). In terms of markers of immune function decline in the elderly, decreases in T and B cell numbers and the proliferation of T cells in response to mitogen stimulation, decreased NKC cytotoxicity, and decreased phagocytosis have been associated with increasing age (29, 30). However, these changes have been subject to debate (17), whereas not all changes in immune markers are inversely associated with age. Reported increases in IL-6 and other soluble mediators with increased age coupled

with increases in the number of NKCs present in blood (31), but not in cytotoxicity (32), have led to the proposal that aging is associated with a dysregulation of the immune response rather than a global decline (26). Therefore, the effect of changes in these immune markers induced by diet needs to be further investigated to establish the public health significance for healthy older individuals (33).

We have also shown no significant effect of increased FV consumption on NKC cytotoxicity, although there was a trend toward improved IL-2–stimulated NKC cytotoxicity. Watzl et al (12) also observed no effect of increased FV consumption on unstimulated NKC lytic activity. We assessed NKC cytotoxicity with and without IL-2 stimulation because resting cytotoxicity may not reflect the actual functional potential. With IL-2 enhancement, the maximal effect is assessed, and therefore, the result may be more clinically informative.

Endpoint selection in nutrition intervention studies that examine immunomodulation is important. Albers et al (19) suggested targeting a spectrum of immune system variables because there is no single marker available to predict the outcome of a dietary intervention on resistance to infection or other immune system–related diseases. Albers et al (19) classified potential immune markers as being of high, medium, or low suitability, and immune markers of high suitability included vaccine-specific serum antibody production, whereas NKC cytotoxicity was of medium suitability.

Interpretation, even of the markers of high suitability (19), is difficult. The response to vaccination was chosen as our primary endpoint because it is a functional test of an immune response that indirectly tests many elements of the innate and adaptive system. It is also a meaningful marker because pneumococcal immunization of older people is a part of the vaccination program in the United Kingdom. Definitions of normal levels of antibody response to vaccination in older people or protective levels after vaccination have not been formally established (24). Furthermore, the demographics and determinants of vaccine response require additional consideration, alongside how these vaccine responses relate to infection rates (23, 24). Therefore, the clinical and public health relevance of changes in these markers has yet to be fully established. Calder (33) has questioned the biological significance of differences in immune function and suggested that relatively small differences in indicators of immune function within normal ranges may not be relevant to the host defense. In terms of clinical and public health relevance, the inclusion of the incidence and severity of infection data within suitable study designs will be most readily interpretable. In the current study, we observed a trend toward reduced infection rates in the 5-portion/d group, but this was not significant, and data were not collected by using a validated questionnaire.

Although we failed to detect any effect of FVs on the response to tetanus toxoid vaccination, it is possible that our study size was not adequate. We retrospectively estimate that our study had 80% power to detect as significant ($P < 0.05$; 2-tailed test) a difference in response to vaccination of a $\times 8.9$ increase on 2 portions/d compared with a $\times 24$ increase on 5 portions/d. A difference smaller than this could have been missed and would have required a larger study to reliably detect such a difference.

Although multivitamin supplementation has been shown to improve cell-mediated immune function (34) and delayed-type hypersensitivity response in older people (35), data on multivitamin



and mineral supplements in the prevention of infections in older people are weak and inconclusive (36, 37). The current study has highlighted the possibility that a broader, food-based approach, such as increasing FV consumption, might be more beneficial than taking a supplement. Some studies (7–9), although not all (10–12), have previously suggested immunomodulatory effects of specific fruit, vegetables, or FV products or a combination of FVs, but none of these studies have examined the vaccine response or infection rates. A recent study showed that a 6-mo intervention with a juice-powder concentrate from FVs reduced moderate or severe common cold symptom days by 20% in health care professionals exposed to patient contact (38), whereas a similar product tended to reduce work days lost to illness in trained men after 28 wk (39). To our knowledge, our study is the first to use a free choice of a mixture of whole FVs and, therefore, is directly relevant to the usual diet and dietary recommendations; hence, there is a need for additional studies with robust study designs and endpoint data collection, including infection rates.

In conclusion, in older people in the current study, there was a greater specific antibody response to Pneumovax II vaccination in subjects who consumed 5 portions FVs/d than in subjects who consumed 2 portions FVs/d; this increased response was confined to subjects who had never previously received the Pneumovax II vaccination. Therefore, increased FV intake may improve the antibody response to Pneumovax II vaccination in older people. This finding links an achievable dietary goal with potentially enhanced protective immunity. This is an encouraging endorsement of the 5-a-day message and could have important public health implications.

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The authors' responsibilities were as follows—AG: carried out all immune function analyses and prepared the initial draft of the manuscript; CEN: was responsible for coordinating and managing the day-to-day running of the study, including participant recruitment and study execution (dietary assessment, dietary analysis, nutrient status analysis, and data input); SECMG: assisted with participant recruitment and study execution and provided technical assistance with nutrient-status analysis; MCM: directed the dietary assessment and analysis; CCP: was the study statistician and supervised all statistical analysis; JVW: was the principal investigator, produced the final draft of the manuscript, had full access to all study data, and took responsibility for the integrity of the data and accuracy of the data analysis; ISY and JDE: were coinvestigators; ISY, JDE, and JVW: were responsible for the study conception, design, and management; and all authors: contributed to the drafts, revisions, and proofreading of the manuscript. None of the authors had a conflict of interest.

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