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Consumption of gold kiwifruit reduces severity and duration of selected upper respiratory tract infection symptoms and increases plasma vitamin C concentration in healthy older adults

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Abstract

In the elderly, immunosenescence and malnourishment can contribute to increased risk and severity of upper respiratory tract infections (URTI). Gold kiwifruit (*Actinidia chinensis* 'Hort16A') contains nutrients important for immune function and mitigation of symptoms of infection, including vitamins C and E, folate, polyphenols and carotenoids. The objective of the present study was to evaluate whether regular consumption of gold kiwifruit reduces symptoms of URTI in older people, and determine the effect it has on plasma antioxidants, and markers of oxidative stress, inflammation and immune function. A total of thirty-two community-dwelling people (≥65 years) participated in a randomised crossover study, consuming the equivalent of four kiwifruit or two bananas daily for 4 weeks, with treatments separated by a 4-week washout period. Participants completed the Wisconsin Upper Respiratory Symptom Survey-21 daily, and blood samples were collected at baseline and at the end of each treatment and washout period. Gold kiwifruit did not significantly reduce the overall incidence of URTI compared with banana, but significantly reduced the severity and duration of head congestion, and the duration of sore throat. Gold kiwifruit significantly increased plasma vitamin C, α-tocopherol and lutein/zeaxanthin concentrations, and erythrocyte folate concentrations, and significantly reduced plasma lipid peroxidation. No changes to innate immune function (natural killer cell activity, phagocytosis) or inflammation markers (high-sensitivity C-reactive protein, homocysteine) were detected. Consumption of gold kiwifruit enhanced the concentrations of several dietary plasma analytes, which may contribute to reduced duration and severity of selected URTI symptoms, offering a novel tool for reducing the burden of URTI in older individuals.

Key words: ***Actinidia chinensis*: Gold kiwifruit: Common cold: Influenza**

Symptoms of cold and influenza are one of the most common afflictions in humans, resulting in misery, loss of productivity, and absence from work and school[1]. Symptoms of upper respiratory tract infection (URTI), which may be caused by cold or influenza (flu) viruses, include scratchy or sore throat, runny or plugged nose, sneezing, coughing, hoarseness, head congestion, chest congestion and malaise. There is currently no specific antiviral therapy or vaccination available to prevent or treat the common cold[2]; vaccination against influenza only has modest efficacy in healthy, community-dwelling older adults[3]; and resistance to anti-influenza drugs has been reported[4–6]. Therefore, alternative prophylactic or treatment options for the common cold and flu are of importance to public health. Clinical trials on any new antiviral treatment for URTI would ideally need to demonstrate an overall reduction in symptoms or alternatively a reduction in severity or duration of symptoms, since both these parameters provide key benefits[7].

Undernutrition is greatest in developing countries, but is also prevalent in some elderly people of developed countries[8]. Nutritional status is an important factor for maintaining optimal immune function; both deficient and excessive intakes can have negative consequences on immune status and susceptibility to a variety of pathogens[9]. Nutrients that

Abbreviations: DiO, 3,3′-dioctadecyloxacarbocyanine perchlorate; Hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NK, natural killer; PH, phenylhydrazine; RPMI, Roswell Park Memorial Institute; URTI, upper respiratory tract infection; WURSS, Wisconsin Upper Respiratory Symptom Survey; ZINB, zero inflated negative binomial.

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are required for the immune system to function efficiently include essential amino acids, the essential fatty acid linolenic acid, folic acid, vitamins A, B_6, B_{12}, C and E, and minerals such as Zn, Cu, Fe and Se[8]. Furthermore, an increased oxidative stress load coupled with nutrient deficiency, specifically Se, has been shown to lead to enhanced virulence of selected viruses, including influenza[10].

The essential nutrients required for optimal immune function can usually be obtained through food. Recent studies have shown that an increase in fruit and vegetable consumption by elderly people improves their antibody response to vaccination, and reduces self-reported symptoms and illness[11], suggesting that nutritional intervention may be a valuable tool for preventing cold and flu infections, and for reducing the severity and duration of symptoms of URTI. *In vitro* studies suggest that a number of individual fruits inhibit infection by the influenza virus[12–15]. To our knowledge, the translation of these findings into improved health in humans has been reported only with a standardised elderberry extract, which significantly reduced the amount of time required to achieve a 'pronounced improvement' of symptoms and reduced the usage of conventional medication (nasal decongestant and painkillers)[16].

Kiwifruit can be described as nutritious, providing an excellent source of vitamin C and a good source of folate and potassium[17]. One fruit provides approximately 10 % of the recommended daily requirement of dietary fibre[17]. Kiwifruit also contains vitamin E and a range of polyphenols and carotenoids that might also be beneficial to health. Evidence is accumulating that gold kiwifruit (*Actinidia chinensis* Planch. 'Hort16A') may influence immune function. For example, gold kiwifruit pasteurised purée stimulated a mild gut-associated immune response in mice orally immunised with ovalbumin, a model protein, stimulating antigen-specific antibody production (total Ig and IgG) and antigen-specific proliferation of mesenteric lymph node cells[18]. Using a water extract prepared from the gold kiwifruit pasteurised purée, it was subsequently demonstrated that the purée modulated markers of innate immune function (phagocytosis, oxidative burst and natural killer (NK) cell activity) and adaptive immune function (T cell activation, and cytokine production in response to a recall antigen) of human blood cells *in vitro*[19]. The influence of consumption of gold kiwifruit on immune function in human subjects has not yet been reported.

Hence, the aim of this pilot study was to determine the effect of regular consumption of gold kiwifruit on the overall incidence, duration and severity of symptoms of URTI in community-dwelling older people, as well as on markers of oxidative stress, inflammation and innate immune function.

Materials and methods

Subjects

Healthy (n 37) community-dwelling people aged 65 years and older were recruited from the Palmerston North (New Zealand) area, to participate in a 20-week randomised crossover study. Screening for the study occurred by telephone and/or personal interviews, including a general health screening questionnaire. Exclusion criteria consisted of the following: diagnosis of any form of cancer, vascular disease, diabetes mellitus, or mental illness; major CVD; hepatitis; endocrine disease or inflammatory diseases such as rheumatoid arthritis; anaemia; malnutrition from malabsorption (vitamins B_{12} and B_6, folate, Fe); renal impairment; compromised immune status (AIDS, multiple myeloma, chronic lymphatic leukaemias). Also excluded were smokers, and those who regularly consumed more than two units of alcohol per day, vitamin supplements, anti-inflammatory drugs (non-steroidal anti-inflammatory drugs, ibuprofen, cyclo-oxygenase 2 inhibitors), tranquillizers, anti-depressants or anticonvulsants. Subjects were required to have a BMI of between 18 and 30 kg/m^2, and to undergo a health screening including kidney and liver function, and haematology (blood glucose and complete blood count; Medlab Central). The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and the study protocol was approved by the Human Ethics Committee Southern A (Application 08/16). Written informed consent was obtained from each subject.

Study protocol

The study was conducted from July 2008 (mid-winter) to November 2008 (end of spring). Subjects underwent a 4-week washout period, during which time they were asked to refrain from consumption of vitamin C supplements, kiwifruit and kiwifruit products, and provide a blood sample at the end of the washout period. Subjects were then randomly assigned to consume the equivalent of either two freeze-dried bananas, or two fresh ZESPRI® GOLD kiwifruit and two freeze-dried ZESPRI® GOLD kiwifruit per day for 4 weeks, after which time a blood sample was taken. Subjects underwent another 4-week washout period, and a blood sample was again collected at the end of the period. Subjects then consumed either the kiwifruit or banana for 4 weeks, the alternative to what they had consumed during the first intervention period, after which time a blood sample was taken, followed by a final 4-week washout period and collection of a blood sample at the end of that washout period. At each sampling point, fasting venous blood samples were collected into tubes containing either heparin or EDTA. Subjects were asked to complete the Wisconsin Upper Respiratory Symptom Survey (WURSS 21, used under WARF license agreement #08-0329)[20] and record their kiwifruit consumption daily. The rationale for providing the equivalent of four kiwifruit per day was based on what was regarded as a daily amount that people could eat, particularly if it provided a health benefit. The basis for providing two fresh fruit and two freeze-dried fruit was to give participants the opportunity to consume the equivalent dose in a variety of ways; for example, to sprinkle the freeze-dried powder on their cereal or make it into a smoothie. Banana was provided as an alternative fruit during the 'placebo' treatment period in an effort to determine whether changes to symptoms of URTI and physiological markers measured were due to

a healthier lifestyle through the incorporation of fruit into the diet, or as a specific result from gold kiwifruit consumption. Banana was chosen as the alternative fruit as it has a relatively similar energy content, but lower nutritional value than gold kiwifruit with respect to most minerals, vitamins and carotenoids, as shown in Table 1. Compliance was assessed from differences in vitamin C and carotenoid plasma levels, which reflects the differences of these micronutrients in kiwifruit compared to banana.

Outcome variables

The primary variables were the duration and severity of symptoms of URTI recorded by subjects. Possible symptoms included runny nose, plugged nose, sneezing, sore throat, scratchy throat, cough, hoarseness, head congestion, chest congestion and feeling tired. The WURSS 21 survey records 'functional' domains, as described previously, and also 'quality of life' domains, including ability to think clearly, sleep well, breathe easily, walk/climb stairs/exercise, accomplish daily activities, work outside the home, work inside the home, interact with others, and live your personal life. The duration and severity to which URTI symptoms interfered with these domains were analysed.

The secondary variables were plasma antioxidant status (vitamin C concentration, selected carotenoid concentration, antioxidant capacity determined by the oxygen radical absorbance capacity and ferric reducing ability of plasma assays, lipid peroxidation (malondialdehyde (MDA)), protein carbonyl content, glutathione concentration, glutathione peroxidase activity and superoxide dismutase activity), and immune function and inflammatory markers (neutrophil and monocyte phagocytosis, NK cell activity, peripheral erythrocyte folate, plasma high-sensitivity C-reactive protein (Hs-CRP) and plasma homocysteine).

Table 1. Nutritional content of the intervention fruit (four gold kiwifruit* (344 g) or two bananas† (236 g))

Nutrient	Gold kiwifruit (344 g)	Banana (236 g)
Water (g)	286·3	149·8
Energy (kJ)	864·0	742·0
Protein (g)	4·2	1·2
Total lipid (fat) (g)	1·9	0·7
Carbohydrate (g)	49·0	45·7
Fibre, total dietary (g)	6·8	5·2
Minerals		
K (mg)	1088·0	716·0
Se (μg)	10·8	2·0
Vitamins		
Vitamin C, total ascorbic acid (mg)	362·4	17·4
Folate, total (μg)	116·0	40·0
β-Carotene (μg)	148	52·0
Lutein + zeaxanthin (μg)	392	44·0
Vitamin E, tocopherol (μg)	5·12	0·20
Vitamin K (μg)	18·8	1·00

* Kiwifruit, gold, raw; refuse: 26 % (skin); scientific name: *Actinidia chinensis;* NDB no: 9445 (nutrient values and weights are for edible portion); samples grown in New Zealand; cultivar is Hort 16A.

† Banana, raw; refuse: 36 % (skin); scientific name: *Musa acuminate Colla* NDB no: 09 040 (nutrient values and weights are for edible portion). USDA National Nutrient Database for Standard Reference, Release 23 (2010).

Vitamin C (ascorbic acid). Plasma, collected in evacuated tubes containing the anticoagulant EDTA, was mixed with an equal volume of cold 10 % metaphosphoric acid/2 mM-Na_2EDTA solution, and then centrifuged at 16 000 ***g***, 4°C. The supernatant was collected and stored at −80°C until analysis. Vitamin C was measured as ascorbic acid using HPLC combined with electrochemical detection. Prepared plasma samples were gently thawed and immediately diluted with 5× volume 5 % metaphosphoric acid/EDTA and analysed by HPLC. The HPLC system comprised a Water 2690 Solvent Delivery system, and a CoulArray® detector operating at 350 mV. The separation column was a Synergi Hydro 4 μ 250 × 4·6 mm from Phenomenex and the mobile phase was 50 mM-lithium acetate (adjusted to pH 2·5 with formic acid) at a flow of 1·0 ml/min. Injection volume was 80 μl. A calibration curve was prepared using an authentic standard of ascorbic acid and the concentration in samples calculated by interpolation of the standard curve. Detection limit for the method was 4 μM-ascorbic acid.

Plasma carotenoid content. The extraction and analysis of individual carotenoids (lycopene, lutein, zeaxanthin, α- and β-carotene), retinol and toco (α- and γ-tocopherol) were based on the method described by Su *et al.*[21] with minor changes. Briefly, 200 μl of plasma were extracted twice with 1 ml of hexane containing 0·01 % butylated hydroxytoluene. For quantification, an internal standard of echinenone 0·167 μg/ml was added to all samples before the extraction. The extract was dried under N_2 at room temperature, then reconstituted in 100 μl of mixture $CHCl_3$–MeOH–CH_3CN (30:35:35). Then, 50 μl were injected into the HPLC instrument (Shimadzu HPLC machine equipped with an SPD-M20A photodiode array detector and a NovoPak C^{18} column) with absorbance detection at 292 nm for the tocopherols, 325 nm for retinol and 450 nm for the carotenoids. The carotenoids, retinol and tocopherols were eluted from the column using a mobile phase of 0·0125 % ammonium acetate in MeOH (A), 100 % $CHCl_3$ (B) and CH_3CN with 0·1 % triethylamine (C) in three linear gradient steps: from 0 to 5 min, A 50 %, C decreased from 50 % to 44 % and B increased to 6 %; from 5 to 16 min, A increased to 55 %, C decreased from 44 % to 30 % and B increased from 6 % to 15 %. Wash with A–C 50:50 mixture for 3 min.

Oxygen radical absorbance capacity. This modified method was based on that of Prior *et al.*[22] and Huang *et al.*[23]. Briefly, 10 μl of diluted (1:100) plasma in 10 mM, pH 7·4 phosphate buffer and 160 μl of 0·5 μM-fluorescein were added to wells in a ninety-six well microplate, and incubated at 37°C for 30 min. Then, 30 μl of freshly prepared 0·25 M-2,2′-azobis(2-amidino-propane) dihydrochloride in phosphate buffer were added to the wells. The fluorescence was recorded (excitation wavelength 490 nm, emission wavelength 515 nm) every 1·5 min for 90 min. The results were calculated using the differences of areas under the fluorescein decay curves between the blank and samples. A standard curve was prepared using 25–400 μM of Trolox™. The final results were corrected for dilution, and expressed as μM Trolox™ equivalents. All determinations were performed in triplicate.

Ferric-reducing antioxidant potential. This modified method was based on that of Benzie & Strain[24]. Briefly, 25 μl of diluted (1:5) plasma in 300 mM, pH 3·6 acetate buffer and 140 μl of acetate buffer were added to wells in a ninety-six well microplate, and heated to 37°C. Then, 30 μl of freshly prepared ferric-reducing antioxidant potential reagent (1:1 10 mM-2,4,6-tripyridyl-*S*-triazine in 40 mM-hydrochloric acid and 20 mM-ferric chloride) were added to the wells and incubated at 37°C. Absorbance readings were taken after 15 min at 593 nm. A standard curve was prepared using 25–400 μM of Trolox™. The final results were corrected for dilution, and expressed as μM Trolox™ equivalents. All determinations were performed in triplicate.

Lipid peroxidation (malondialdehyde). The MDA standard was prepared by dissolving Na-MDA salt in dilute hydrochloric acid. The d2-MDA internal standard was prepared by acid hydrolysis of 1,3-dideutero-1-butoxy-1,3,3-triethoxypropane in dilute hydrochloric acid. The MDA standard and EDTA plasma samples were spiked with d2-MDA internal standard, butylated hydroxytoluene and aqueous acetic acid were then added and the standard/sample heated at 60°C for 30 min. After this time, the standards/samples were derivatised with phenylhydrazine (PH) for 60 min, and protected from the light. The PH-MDA adduct was then extracted into hexane and analysed by GC-MS. GC-MS was run in selected ion monitoring mode and Shimadzu software was used to calculate the peak area of the *m/z* 144 ion (PH-MDA) and the peak area of the *m/z* 146 ion (d2-PH-MDA). The ratio of the two peak areas was calculated in Microsoft® Excel and the final MDA concentration calculated from a six-point calibration curve.

Protein carbonyls. EDTA plasma was centrifuged at 16 000 ***g***, 4°C. The supernatant was collected and stored at 4°C. Protein carbonyls were measured by ELISA according to the manufacturer's instructions (Zenith Technology, Zentech PC test).

Glutathione. Blood was collected in heparin tubes and centrifuged at 1000 ***g***, 10 min, 4°C. Plasma and the leucocytes were removed; and the remaining erythrocytes were lysed in four times their volume of ice-cold HPLC grade water and centrifuged at 10 000 ***g***, 15 min, 4°C. The supernatant was collected and stored at −80°C. Samples were thawed at room temperature and deproteinated by adding an equal volume of 10 % (w/v) metaphosphoric acid, mixed by vortexing, incubated at room temperature for 5 min, and then centrifuged at 2000 ***g*** for 2 min. The supernatant was collected and 50 μl of 4 M-triethanolamine were added per ml of the supernatant. The remainder of the assay was performed according to the manufacturer's instructions using the glutathione assay kit (Cayman Chemical Company #703002) at a sample dilution of 1:20.

Glutathione peroxidase and superoxide dismutase enzyme activity. Blood was collected in heparin tubes and centrifuged at 1000 ***g***, 10 min, 4°C. Plasma and the leucocytes were removed; and the remaining erythrocytes were lysed in four times their volume of ice-cold HPLC grade water. The lysate was centrifuged at 10 000 ***g***, 15 min, 4°C. The supernatant was collected, aliquoted into two tubes and stored at −80°C. Glutathione peroxidase activity was determined using an assay kit (Cayman Chemical Company #703102), and the assay was performed according to the manufacturer's instructions using a sample dilution of 1:10. Superoxide dismutase activity was determined using an assay kit (Cayman Chemical Company #706002), and the assay was performed according to the manufacturer's instructions using a sample dilution of 1:100. Hb concentrations were determined in parallel on the same erythrocyte lysate samples prepared for the enzyme assays. Hb was measured using the Hb assay kit (Quantichrom #DIHB-250) according to the manufacturer's instructions at a sample dilution of 1:100. Enzyme activity was expressed as U/g Hb.

Neutrophil and monocyte phagocytosis. Heparinised blood (100 μl) was mixed with non-viable *Escherichia coli* (4×10^6) pre-labelled with fluorescein isothiocyanate. The mean complete blood counts for all participants were $2{\cdot}6 \times 10^5$ neutrophils and $0{\cdot}44 \times 10^5$ monocytes per 100 μl of whole blood; thus, the neutrophil:*E. coli* and monocyte:*E. coli* ratios were 1:15 and 1:91, respectively. As phagocytosis targets (*E. coli*) were at all times present in excess for both phagocytic cell types, time was the only limiting factor in the assay.

Aliquots of whole blood were pre-cooled in assay tubes for 45 min at 5°C. Upon addition of *E. coli*, the samples were promptly placed in a 37°C water bath. After 10 min, the samples were removed and treated with FACSLyse (Becton Dickinson) as per the manufacturer's instructions, and the cells washed twice with PBS (2 ml). The cells were resuspended in PBS containing trypan blue (0·3 % w/v) to quench extracellular fluorescence. Duplicate samples were acquired on an FACSCalibur within 30 min of assay completion and analysed using CellQuest software (BD Biosciences). Neutrophils and monocytes were gated individually by scatter characteristics. The cells were identified as positive for phagocytosis if they had a fluorescence level higher than that of control samples held on ice (% cells positive). The median fluorescence intensity of the positive cell population was calculated individually for monocytes and neutrophils.

Natural killer cell assay. K562 cells (ATCC) were cultured and passaged as per the supplier's instructions. Following this, 1 d before use, the cells were seeded in flasks at 10^6 per ml in Roswell Park Memorial Institute (RPMI) medium containing 10 % fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 292 μg/ml L-glutamine, 5×10^{-5} M-2-mercaptoethanol (Gibco BRL; complete medium 'R-10') in the presence of 0·1 % 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO; Sigma Aldrich) fluorescent dye. On the day of the assay, fluorescently labelled cells were washed twice to remove unincorporated DiO, counted, and resuspended in fresh R-10 at 10^6 per ml.

Heparinised blood samples were diluted 1:1 in RPMI medium and layered over Ficoll-Hypaque 1077. Tubes were centrifuged at 400 ***g*** for 30 min. The leucocyte-rich interface was recovered, washed twice, resuspended in RPMI medium, and the cells counted using a flow cytometer. Peripheral blood mononuclear cells were diluted to a final concentration of 10^6 cells/ml in R-10.

NK cells from blood were tested for their ability to kill tumour cells using an assay method derived from standard procedures[25–28]. Briefly, peripheral blood mononuclear cell preparations were mixed with labelled K562 target cells in a ratio of 40:1 in duplicate ninety-six well plates (final volume 250 μl), providing an effector:target cell ratio of 6:1 based on published NK cell frequencies[29,30]. Plates were centrifuged at 200 ***g*** for 1 min to enhance effector–target cell contact, and then incubated at 37°C (with 95% humidity and 5% CO_2, protected from light) for 4 h. Propidium iodide was added to each well at a final concentration of 25 μg/ml for the last 15 min of the assay. Control wells containing only peripheral blood mononuclear cells, or only K562 target cells, were similarly prepared.

Duplicate samples were acquired on an FACSCalibur flow cytometer using CellQuest software (>5000 events/sample) (BD Biosciences). Target cells were identified by scatter characteristics and DiO fluorescence. Viable and non-viable cells were distinguished by propidium iodide fluorescence.

Peripheral erythrocyte folate. Whole blood (100 μl) collected in an EDTA tube was lysed with Folate Lysis Reagent (1 ml; ABBOT Diagnostics Division). The lysate was stored frozen, and analysed for folate by Canterbury Health Laboratories using the Chemiflex Abbot Architect system.

Plasma high-sensitivity C-reactive protein. Assays on human plasma for Hs-CRP were performed as per the manufacturer's instructions (Dade Behring). Briefly, plasma samples were mixed with polystyrene particles coated with monoclonal antibodies that specifically recognise human Hs-CRP. This resulted in particle aggregation, which when passed through a beam of light scattered the beam. The intensity of the scattered light, which was proportional to the concentration of Hs-CRP in the sample, was measured and quantified based on comparison with a standard containing a known concentration of Hs-CRP; the concentration in the latter sample was determined by standardisation against the international reference preparation BCR-CRM 470.

Plasma homocysteine. Plasma homocysteine measurements were carried out by Canterbury Health Laboratories using a AxSYM fluorescence polarisation immunoassay kit (Abbot Laboratories) as per the manufacturer's instructions.

Statistical analysis

Statistical analysis was carried out in SAS 9.1 (SAS Institute Inc.) using the MIXED procedure. A mixed-effects model was fitted with time of sample, treatment sequence and treatment being the fixed effects, while participant was set as a random effect. The analysis of the immune health data set also included a sex factor, an age covariate and their interaction, as fixed effects in the analysis. Type 3 sums of squares were used to test for overall differences among the fixed effects. In addition, the superoxide dismutase and protein carbonyl analysis included a plate fixed effect to model differences between assay plates. An autoregressive covariance structure of order 1 was used to account for the possible carryover effects of the previous time period. The resulting residual plots for each model were checked for the validity of the normality assumption, with only the protein carbonyl, folate, lycopene and Hs-CRP data needing to be log-transformed to meet this assumption. One of the plates for the protein carbonyl data was removed from the analysis, as the standards had failed and therefore the resulting carbonyl concentrations were unreliable. This plate contained mostly the samples from the last washout period and so this period was also removed from the model.

The analysis of the individual WURSS-21 questionnaire data set was carried out using a zero inflated negative binomial (ZINB) model. Although the number of days with a particular symptom was bounded by a maximum of 28 d, the ZINB model did provide a good fit. This questionnaire gave rise to an extremely high number of zeros in the data set, representing people who did not have a given symptom; but when a symptom was present, the severity was scored reasonably high by participants; therefore a standard Poisson regression did not fit well. The ZINB model modelled the data in two parts. The first part models the excess zeros with a logistic regression, with participant as the predictor; while the second part models the non-zero values using the treatment, period and group as predictors (and some of the zeros) as a standard Poisson regression model, and allows a variance greater than the mean, thereby avoiding 'over dispersion' and the consequence of greatly reduced *P*-values. The ZINB model was fitted in *R* 2·90 using the pscl package. As the package only provided standard errors of the regression coefficients, the standard errors of the fitted means were calculated using the jack-knife method, with each sample leaving one participant out in turn. For all analyses, values of $P \leq 0{\cdot}05$ were considered significant.

Results

Subjects

Of the thirty-seven people enrolled in the study, data from five people were excluded; two owing to non-compliance and three did not complete the study because of unrelated medical reasons. A description of sex, age, BMI, height and weight of the study population is presented in Table 2. There were no adverse comments from the participants about consuming the equivalent of four kiwifruit/d.

Influence of regular consumption of gold kiwifruit on symptoms of upper respiratory tract infection

Irrespective of the order of intervention treatment (kiwifruit and then banana, or banana and then kiwifruit), there was a

Table 2. Description of the study population (*n* 32) by sex
(Number of participants, mean values with their standard errors)

	Female		Male	
	Mean	SEM	Mean	SEM
Participants (*n*)	20		12	
Age (years)	71·3	1·3	72·0	1·7
BMI (kg/m^2)	26·9	1·2	26·5	1·1
Weight (kg)	70·0	3·6	77·7	3·5
Height (cm)	160·9	1·5	171·3	2·4

gradual decline in the proportion of subjects reporting one or more symptoms of URTI during each treatment or washout period as the trial progressed (data not shown), suggesting a seasonal effect. Similarly, symptoms also tended to be less severe in spring, compared with winter (data not shown).

The influence of regular consumption of gold kiwifruit on the duration (number of days) of symptoms of URTI was assessed (Table 3). When participants experienced a sore throat or head congestion within the 28 d treatment period, the duration of this symptom was significantly reduced when gold kiwifruit was consumed compared with when banana was consumed. A sore throat was present for an average of 5·42 d when participants were consuming banana, but this was significantly reduced to an average of 2·01 d when kiwifruit was consumed ($P=0·024$). Head congestion was present for an average of 4·69 d when participants were consuming banana, but this was significantly reduced to an average of 0·88 d when kiwifruit was consumed ($P=0·029$). Of the remaining physiological domain symptoms, four of eight tended to be reduced in duration but not significantly so. Kiwifruit consumption did not significantly reduce the duration of any of the functional symptoms.

The influence of regular consumption of gold kiwifruit on the severity of symptoms of URTI was also assessed. The severity scores were calculated as the sum of the daily symptom ratings for each treatment period. Regular consumption of kiwifruit compared with banana significantly reduced the severity of head congestion ($P=0·015$, Table 4). The severity of a further five of nine physiological domain symptoms and one of ten functional domain symptoms tended to be reduced in severity but not significantly so.

Dietary analytes in plasma and erythrocytes

Consumption of gold kiwifruit led to significantly higher concentrations of plasma vitamin C, α-tocopherol and lutein/zeaxanthin, and significantly lower concentrations of plasma γ-tocopherol and α-carotene, compared with consumption of banana ($P<0·05$, Table 5). Consumption of gold kiwifruit significantly enhanced the concentration of folate in erythrocytes, compared with consumption of banana ($P=0·037$, Table 5).

Antioxidant activity and markers of oxidative stress, CVD and inflammation, and immune function

Consumption of kiwifruit did not significantly influence plasma antioxidant capacity (oxygen radical absorbance capacity and ferric-reducing antioxidant potental), erythrocyte glutathione concentration and erythrocyte glutathione peroxidase activity (Table 6). Nevertheless, kiwifruit consumption significantly reduced plasma MDA concentrations ($P=0·035$), a marker of lipid peroxidation, compared with consumption of banana.

Elevated plasma homocysteine and Hs-CRP concentrations are markers of inflammation and risk factors for CVD. Consumption of kiwifruit did not significantly reduce the concentrations of either factor (Table 6); however, the trial

Table 3. Comparison of the duration of upper respiratory tract infection symptoms recorded by study participants (subjects) during regular consumption of the equivalent of four gold kiwifruit or two bananas daily for 4 weeks

(Number of subjects with symptoms, mean values with their standard errors)

		No. of days when symptom present, when consuming kiwifruit		No. of days when symptom present, when consuming banana		
Symptom	No. of subjects with symptom	Mean	SEM	Mean	SEM	*P*
How sick do you feel today?	22	3·57	1·62	6·17	4·41	0·290
Physiological domain						
Runny nose	24	7·19	2·29	11·85	7·31	0·275
Plugged nose	16	3·70	2·15	6·97	7·11	0·474
Sneezing	19	7·17	5·33	5·87	4·14	0·768
Sore throat	20	2·01	0·56	5·42	1·17	0·024
Scratchy throat	18	3·60	3·21	1·73	1·03	0·379
Cough	23	5·93	2·75	5·12	2·22	0·726
Hoarseness	13	1·22	0·99	3·03	3·72	0·271
Head congestion	16	0·88	1·42	4·69	5·29	0·029
Chest congestion	13	0·34	0·32	1·46	0·65	0·083
Feeling tired	19	5·30	2·51	4·20	3·39	0·712
Functional domain						
Thinking clearly	19	2·80	2·89	2·02	2·06	0·670
Sleep well	15	6·94	4·29	6·81	2·54	0·975
Breathe easily	17	4·01	3·41	5·72	6·01	0·671
Walk, climb stairs, exercise	11	2·14	0·91	3·38	1·16	0·282
Accomplish daily activities	9	3·18	1·76	3·06	2·38	0·945
Working outside the home	10	2·10	3·60	1·90	1·96	0·896
Working inside the home	10	2·56	2·19	2·73	2·26	0·911
Interact with others	10	1·99	1·66	2·65	2·47	0·608
Live your personal life	12	1·58	0·867	2·40	2·13	0·483

Table 4. Comparison of the severity of upper respiratory tract infection symptoms recorded by study participants (subjects) during regular consumption of the equivalent of four gold kiwifruit or two bananas daily for 4 weeks
(Number of subjects with symptoms, mean values with their standard errors)

Symptom	No. of subjects with symptom	Severity of symptom when symptom present, when consuming kiwifruit		Severity of symptom when symptom present, when consuming banana		*P*
		Mean	SEM	Mean	SEM	
How sick do you feel today?	22	7·47	3·95	10·21	8·27	0·572
Physiological domain						
Runny nose	24	8·95	2·68	14·21	8·16	0·331
Plugged nose	16	5·38	3·52	12·73	12·94	0·388
Sneezing	19	11·36	8·58	7·41	5·18	0·530
Sore throat	20	3·68	1·58	7·88	3·89	0·256
Scratchy throat	18	7·35	7·05	2·49	2·25	0·251
Cough	23	8·58	3·58	6·28	2·53	0·492
Hoarseness	13	1·95	1·26	3·38	3·96	0·527
Head congestion	16	1·29	2·06	6·72	7·23	0·015
Chest congestion	13	0·45	0·53	1·89	0·75	0·114
Feeling tired	19	13·12	7·45	7·01	6·27	0·379
Functional domain						
Thinking clearly	19	3·36	3·23	2·36	2·39	0·664
Sleep well	15	15·56	11·20	12·49	3·74	0·733
Breathe easily	17	5·67	4·75	7·25	7·32	0·778
Walk, climb stairs, exercise	11	3·59	1·90	3·28	2·80	0·899
Accomplish daily activities	9	6·30	5·01	3·15	3·01	0·364
Working outside the home	10	7·32	7·11	2·83	2·85	0·237
Working inside the home	10	3·81	3·89	3·20	2·94	0·804
Interact with others	10	3·66	3·13	2·65	0·92	0·675
Live your personal life	12	3·08	2·04	2·56	2·39	0·798

population did not appear to have elevated concentrations of either of these markers (>15 μmol/l homocysteine[31], >3 mg/l Hs-CRP[32]).

NK cell activity and phagocytosis are measures of innate immune function. There was no significant difference in NK cell activity following regular consumption of gold kiwifruit compared with banana (Table 6). Phagocytic activity was measured in polymorphonuclear neutrophils and monocytes. Compared with banana, gold kiwifruit did not significantly enhance phagocytosis in either cell population (Table 6).

Discussion

The purpose of the present study was to determine whether regular consumption of gold kiwifruit by community-dwelling people aged 65 years or older reduces the incidence, duration

Table 5. Comparison of plasma analytes following consumption of the equivalent of four gold kiwifruit or two bananas daily for 4 weeks
(Mean values with their standard errors or standard error ratios)

Analyte	Plasma concentration when consuming kiwifruit		Plasma concentration when consuming banana		*P*
	Mean	SEM	Mean	SEM	
Vitamin C* (μM)	72·87	3·68	53·73	3·71	<0·0001
γ-Tocopherol (μM)	1·83	0·16	2·25	0·16	0·0002
α-Tocopherol (μM)	35·61	0·97	32·28	0·97	<0·0001
Retinol (μM)	2·29	0·09	2·25	0·09	0·5066
Lutein/zeaxanthin (nM)	600·90	44·81	497·82	44·81	0·0003
β-Cryptoxanthin (nM)	213·01	33·15	225·42	33·15	0·5254
Lycopene† (nM)					0·8931
Mean	345·09		342·51		
SER	1·11		1·11		
α-Carotene (nM)	104·67	9·01	121·31	9·01	0·0006
β-Carotene (nM)	733·82	62·86	704·55	62·86	0·2711
Erythrocyte folate† (nmol/l)					0·0369
Mean	611·43		559·20		
SER	1·08		1·08		

SER, standard error ratio.
* The df for each analyte are 120–123, but are reduced for vitamin C (df = 114) owing to missing values in the data set.
† The values for lycopene and erythrocyte folate have been log-transformed, but the means and SER presented have been back-transformed. To obtain the mean values and standard errors, the mean should be multiplied or divided by the SER and the respective values added or subtracted.

Table 6. Comparison of plasma antioxidant activity, antioxidant enzyme activity, and markers of oxidative stress, CVD, and innate immune function following consumption of the equivalent of four gold kiwifruit or two bananas daily for 4 weeks
(Mean values with their standard errors or standard error ratios)

	Plasma concentration when consuming kiwifruit		Plasma concentration when consuming banana		
Biomarker	Mean	SEM	Mean	SEM	*P*
ORAC (μM-TE)	12 054	385·78	11 363	385·78	0·1640
FRAP (μM-TE)	580·79	20·41	565·71	20·41	0·1848
MDA (μM)	5·37	0·31	6·26	0·31	0·0351
Protein carbonyl† (nmol/mg protein)					0·9550
Mean	0·18		0·18		
SER	1·05		1·05		
Glutathione (μM)	145·07	4·10	139·37	4·10	0·1668
Glutathione peroxidase (U/g Hb)	12 589	421·76	12 253	421·76	0·1852
Superoxide dismutase (U/g Hb)	3927·41	184·10	3965·21	184·10	0·8421
Homocysteine (μmol/l)	11·62	0·45	11·52	0·45	0·6367
Hs-CRP† (ng/l)					0·3175
Mean	1·20		1·38		
SER	1·22		1·22		
NK cell activity (% target cells killed)	62·42	2·28	63·63	2·29	0·5794
PMN phagocytosis (% FITC positive cells)	60·96	2·19	65·73	2·21	0·1198
Monocyte phagocytosis (% FITC positive cells)	67·96	2·00	69·78	2·01	0·3691

ORAC, oxygen radical absorbance capacity; TE, Trolox™ equivalent; FRAP, ferric-reducing antioxidant potential; MDA, malondialdehyde; SER, standard error ratio; Hs-CRP, high-sensitivity C-reactive protein; NK, natural killer; PMN, polymorphonuclear neutrophil; FITC, fluorescein isothiocyanate.
*The df for each assay are 120–123, but are reduced (df=84) for protein carbonyl because experimental error during analysis of samples from the final washout resulted in the data from that period being discarded.
†The values for protein carbonyl and Hs-CRP have been log-transformed, but the means and SER presented have been back-transformed. To obtain the mean values and standard errors, the mean should be multiplied or divided by the SER and the respective values added or subtracted.

and severity of symptoms of URTI in comparison to banana. Daily consumption of the equivalent of four gold kiwifruit was associated with a significant reduction in the duration of a sore throat ($P=0·024$), and the duration and severity of head congestion ($P=0·029$ and $P=0·015$, respectively) (Tables 3 and 4). Importantly, a reduction in the duration of sore throat from 5·42 d to 2·01 d, and head congestion from 4·69 d to 0·88 d, in a 28 d period, could be considered a clinically relevant improvement. Furthermore, sore throat is an early symptom of the common cold, and head congestion tends to be experienced later as the cold progresses[7]; therefore, gold kiwifruit consumption may lessen the burden of symptoms at all stages of infection. Similarly, a recent parallel study (n 529) in 18–65-year-olds reported significantly fewer days with moderate or severe symptoms of common cold in subjects consuming an encapsulated juice powder concentrate prepared from fruits and vegetables, compared with placebo, although the number of days with any cold symptom was similar between the groups[33].

Significant increases in plasma vitamin C ($P<0·0001$), α-tocopherol ($P<0·0001$), lutein/zeaxanthin ($P=0·0003$) and erythrocyte folate ($P=0·0396$) were achieved following consumption of gold kiwifruit (Table 5). Conversely, significantly higher concentrations of plasma γ-tocopherol ($P=0·0002$) and α-carotene ($P=0·0006$) were achieved following consumption of banana. This suggests that participants complied with the study protocol, as the fruits contain comparatively high concentrations of these vitamins and phytochemicals, respectively (http://www.nal.usda.gov/fnic/foodcomp/search/; accessed August 2010). An increase in plasma lutein and decrease in β-cryptoxanthin following kiwifruit consumption (species not specified) has also recently been reported by Bøhn *et al.*[34]. The micronutrients provided by kiwifruit appear to be particularly important to immune function and prevention of respiratory infections. In a recent study of elderly, low-income, community-dwelling Ecuadorians, the high incidence of respiratory infection or colds reported in 1 month was significantly associated with micronutrient deficiency[35], and deficiencies of vitamins C, D, B_6 and B_{12}, folic acid and Zn were particularly common in this population. Furthermore, lower age and higher serum concentrations of total protein, albumin, vitamin E and folate were associated with an intact immune response to the influenza vaccine in elderly nursing home residents and healthcare workers[36]. As these micronutrients are present in gold kiwifruit, the findings reported here support the hypothesis that regular consumption of this fruit type might have a prophylactic effect, particularly in elderly populations.

Interdependence between optimal immune function and regulation of oxidative stress is well recognised. Several immune cells produce reactive oxygen species as part of the body's defence against infection, but oxidative damage to cell membranes can lead to a loss of membrane integrity and altered membrane fluidity, resulting in alterations in signalling within and between immune cells[37]. Decline in immune function in older people may be attributed, at least in part, to an increase in oxidative stress[37]. Therefore, increases in dietary antioxidants, endogenous antioxidant compounds and antioxidant enzyme activity may contribute to improved immune function in older people. In this study, significantly less lipid peroxidation (MDA) was present when people consumed gold kiwifruit ($P=0·0351$, Table 6). This suggests that significantly increased concentrations of diet-derived antioxidant analytes, and

possibly some limited (non-significant) improvements in a number of endogenous mechanisms that serve to control reactive oxygen species, may have led to reduced oxidative damage. Tissue damage seen in lungs after influenza virus infection is due to the reactive oxygen species produced during the processes of phagocytosis and oxidative burst[38]. Furthermore, influenza infection results in a decrease in the total concentration of glutathione, vitamin C and vitamin E from lung tissue[39]. Therefore, the enhanced concentrations of plasma antioxidant analytes derived from gold kiwifruit could reduce tissue damage as a result of respiratory infections.

In addition to their direct antioxidant capacity, dietary vitamins and phytochemicals also modulate humoral and cellular immune function, and this might influence the severity of infection-related inflammation. For example, a stable ascorbic acid derivative induced an increase in IgM production from stimulated mouse spleen B cells[40]. The mucosal tissues are the main portal entry for many pathogens, and secretory IgA and to some extent IgM are the major neutralising antibodies directed against mucosal pathogens to prevent their entry and can function intracellularly to inhibit virus replication[41]. Further, folate is an essential nucleic acid providing a building block for antibodies and cytokines, and therefore is important for both humoral and cellular immunity[42]. It follows therefore, that increased plasma vitamin C and erythrocyte folate concentrations achieved as a result of regular consumption of gold kiwifruit might promote antibody production, enhancing the detection and clearance of infection.

The symptoms of viral respiratory infections relate to viral replication and are associated with a cascade of inflammatory mediators[43]. Airway inflammation, as a result of rhinovirus infection, for example, occurs because epithelial cells produce various inflammatory mediators[44]. Using *in vitro* respiratory models, carotenoids and polyphenols have been shown to reduce inflammatory cytokine production[44,45], and inhibit the replication of rhinovirus 1B[44]. Further, a mixed dietary treatment of ubiquinone Q_9, α-tocopherol and β-carotene reduced the production of TNF-α, IL-1α, IL-1β, IL-2 and interferon-γ in plasma in a mouse model of acute inflammation, but did not reduce concentrations of IL-6 or the anti-inflammatory cytokine IL-10[46]. These studies and others like them suggest that dietary compounds, including minerals, vitamins and phytochemicals can influence cytokine production under conditions of infection and/or inflammation. While this may in part be due to an antioxidant effect that preserves immune and mucosal cell integrity, other mechanisms that directly influence the production of antibodies and cytokines may be at play. For example, Bøhn *et al.*[34] recently demonstrated that daily consumption of three kiwifruit (species not specified) resulted in altered expression (up- and down-regulation) of gene sets for immune-related processes and responses, and up-regulation of expression of DNA and repair gene sets. Irrespective of the mechanism, sufficient intake of fruits and vegetables, including gold kiwifruit, is clearly important for the prevention and clearance of respiratory infections.

Interestingly, consumption of gold kiwifruit did not result in enhanced innate immune function (NK cell activity, polymorphonuclear neutrophil phagocytosis and monocyte phagocytosis), when determined *ex vivo* (Table 6). Previous studies have reported varying effects of nutritional intervention on immune function. For example, a fruit-and-vegetable juice powder concentrate enhanced plasma vitamin C and selected carotenoid concentrations, including lutein, and supplementation significantly enhanced the numbers of circulating γδ T cells[47]. Conversely, daily consumption of a specific *Camellia sinensis* (green tea) preparation resulted in fewer cold and flu symptoms, but did not enhance the numbers of γδ T cells[1]. However, when isolated γδ T cells were stimulated with an antigenic challenge *ex vivo*, cells from participants consuming green tea were primed to respond more vigorously[1]. γδ T cells may be important in the progression of URTI, because they are proposed to carry out immunosurveillance against virally infected cells[47]. The effect of gold kiwifruit consumption on γδ T cell number or 'priming' was not determined in this study, but may be worthy of consideration in the future.

In conclusion, regular consumption of gold kiwifruit by community-dwelling older people has a positive and significant influence on a number of plasma antioxidants, including vitamins C and E (α-tocopherol), and lutein/zeaxanthin, and significantly reduces cellular damage from oxidative stress, namely lipid peroxidation. Furthermore, when participants consuming gold kiwifruit experienced symptoms of URTI, many symptoms tended to be shorter in duration and less severe; this was significant for duration and severity of head congestion, and duration of sore throat. Although the mechanisms behind the influence of enhanced plasma antioxidants and dietary analytes on improved symptoms of URTI after consumption of gold kiwifruit remain speculative, the reduction in duration and severity of selected URTI symptoms indicates that gold kiwifruit may provide an important and clinically relevant contribution to lessening the burden of respiratory infection in older individuals.

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References

1. Rowe CA, Nantz MP, Bukowski JF, *et al.* (2007) Specific formulation of *Camellia sinensis* prevents cold and flu symptoms and enhances gamma delta T cell function: a randomized, double-blind, placebo-controlled study. *J Am Coll Nutr* **26**, 445–452.

2. Saxena RC, Singh R, Kumar P, *et al.* (2010) A randomized double blind placebo controlled clinical evaluation of extract of *Andrographis paniculata* (KalmCold (TM)) in patients with uncomplicated upper respiratory tract infection. *Phytomedicine* **17**, 178–185.
3. Jefferson T, Rivetti D, Rivetti A, *et al.* (2005) Efficacy and effectiveness of influenza vaccines in elderly people: a systematic review. *Lancet* **366**, 1165–1174.
4. Fleming DM (2001) Managing influenza: amantadine, rimantadine and beyond. *Int J Clin Pract* **55**, 189–195.
5. Dapat C, Suzuki Y, Saito R, *et al.* (2010) Rare influenza A (H3N2) variants with reduced sensitivity to antiviral drugs. *Emerg Infect Dis* **16**, 493–496.
6. Le QM, Kiso M, Someya K, *et al.* (2005) Avian flu: isolation of drug-resistant H5N1 virus. *Nature* **437**, 1108.
7. Eccles R (2005) Understanding the symptoms of the common cold and influenza. *Lancet Infect Dis* **5**, 718–725.
8. Calder PC & Kew S (2002) The immune system: a target for functional foods? *Br J Nutr* **88**, S165–S176.
9. Field CJ, Johnson IR & Schley PD (2002) Nutrients and their role in host resistance to infection. *J Leukoc Biol* **71**, 16–32.
10. Beck MA, Handy J & Levander OA (2004) Host nutritional status: the neglected virulence factor. *Trends Microbiol* **12**, 417–423.
11. Sanderson P, Elsom RL, Kirkpatrick V, *et al.* (2010) UK food standards agency workshop report: diet and immune function. *Br J Nutr* **103**, 1684–1687.
12. Roschek B Jr, Fink RC, McMichael MD, *et al.* (2009) Elderberry flavonoids bind to and prevent H1N1 infection *in vitro*. *Phytochemistry* **70**, 1255–1261.
13. Yingsakmongkon S, Miyamoto D, Sriwilaijaroen N, *et al.* (2008) *In vitro* inhibition of human influenza A virus infection by fruit-juice concentrate of Japanese plum (*Prunus mume* SIEB. et ZUCC). *Biol Pharm Bull* **31**, 511–515.
14. Hamauzu Y, Yasui H, Inno T, *et al.* (2005) Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *J Agric Food Chem* **53**, 928–934.
15. Knox YM, Suzutani T, Yosida I, *et al.* (2003) Anti-influenza virus activity of crude extract of *Ribes nigrum* L. *Phytother Res* **17**, 120–122.
16. Zakay-Rones Z, Thom E, Wollan T, *et al.* (2004) Randomized study of the efficacy and safety of oral elderberry extract in the treatment of influenza A and B virus infections. *J Int Med Res* **32**, 132–140.
17. Ferguson AR & Ferguson LR (2003) Are kiwifruit really good for you? *Acta Hortic* **610**, 131–138.
18. Hunter DC, Denis M, Parlane NA, *et al.* (2008) Feeding ZESPRI™ GOLD Kiwifruit puree to mice enhances serum immunoglobulins specific for ovalbumin and stimulates ovalbumin-specific mesenteric lymph node cell proliferation in response to orally administered ovalbumin. *Nutr Res* **28**, 251–257.
19. Skinner MA, Loh JMS, Hunter DC, *et al.* (2011) Gold kiwifruit (*Actinidia chinensis* 'Hort16A') for immune support. *Proc Nutr Soc* **70**, 276–280.
20. Barrett B, Brown RL, Mundt MP, *et al.* (2009) Validation of a short form Wisconsin Upper Respiratory Symptom. *Health Qual Life Outcomes* **7**, 76.
21. Su Q, Rowley KG & O'Dea K (1999) Stability of individual carotenoids, retinol and tocopherols in human plasma during exposure to light and after extraction. *J Chromatogr B Biomed Appl* **729**, 191–198.
22. Prior RL, Hoang H, Gu LW, *et al.* (2003) Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. *J Agric Food Chem* **51**, 3273–3279.
23. Huang D, Ou B, Hampsch-Woodill M, *et al.* (2002) Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. *J Agric Food Chem* **50**, 1815–1821.
24. Benzie IFF & Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In *Methods Enzymology*, pp. 15–27 [P Lester, editor]. San Diego, CA: Academic Press.
25. Zamai L, Mariani AR, Zauli G, *et al.* (1998) Kinetics of *in vitro* natural killer activity against K562 cells as detected by flow cytometry. *Cytometry* **32**, 280–285.
26. Johann S, Blumel G, Lipp M, *et al.* (1995) A versatile flow cytometry-based assay for the determination of short-term and long-term natural killer cell activity. *J Immunol Methods* **185**, 209–216.
27. Kantakamalakul W, Jaroenpool H & Pattanapanyasat K (2003) A novel enhanced green fluorescent protein (EGFP) -K562 flow cytometric method for measuring natural killer (NK) cell cytotoxic activity. *J Immunol Methods* **272**, 189–197.
28. Bracher M, Gould HJ, Sutton BJ, *et al.* (2007) Three-colour flow cytometric method to measure antibody-dependent tumour cell killing by cytotoxicity and phagocytosis. *J Immunol Methods* **323**, 160–171.
29. Bruunsgaard H, Pedersen AN, Schroll M, *et al.* (2001) Decreased natural killer cell activity is associated with atherosclerosis in elderly humans. *Exp Gerontol* **37**, 127–136.
30. Gill HS, Rutherford KJ & Cross ML (2001) Dietary probiotic supplementation enhances natural killer cell activity in the elderly: an investigation of age-related immunological changes. *J Clin Immunol* **21**, 264–271.
31. Soinio M, Marniemi J, Laakso M, *et al.* (2004) Elevated plasma homocysteine level is an independent predictor of coronary heart disease events in patients with type 2 diabetes mellitus. *Ann Intern Med* **140**, 94–100.
32. Zacho J, Tybjaerg-Hansen A & Nordestgaard BG (2010) C-reactive protein and all-cause mortality-the Copenhagen City Heart Study. *Eur Heart J* **31**, 1624–1632.
33. Roll S, Nocon M & Willich SN (2010) Reduction of common cold symptoms by encapsulated juice powder concentrate of fruits and vegetables: a randomised, double-blind, placebo-controlled trial. *Br J Nutr* **105**, 118–122.
34. Bøhn SK, Myhrstad MC, Thoresen M, *et al.* (2010) Blood cell gene expression associated with cellular stress defense is modulated by antioxidant-rich food in a randomised controlled clinical trial of male smokers. *BMC Med* **8**, 54.
35. Hamer DH, Sempertegui F, Estrella B, *et al.* (2009) Micronutrient deficiencies are associated with impaired immune response and higher burden of respiratory infections in elderly Ecuadorians. *J Nutr* **139**, 113–119.
36. Hara M, Tanaka K & Hirota Y (2005) Immune response to influenza vaccine in healthy adults and the elderly: association with nutritional status. *Vaccine* **23**, 1457–1463.
37. Hughes DA (2000) Dietary antioxidants and human immune function. *Nutr Bull* **25**, 35–41.

38. Han SN, Meydani M, Wu DY, *et al.* (2000) Effect of long-term dietary antioxidant supplementation on influenza virus infection. *J Gerontol* **55**, B496–B503.
39. Hennet T, Peterhans E & Stocker R (1992) Alterations in antioxidant defences in lung and liver of mice infected with influenza A virus. *J Gen Virol* **73**, 39–46.
40. Ichiyama K, Mitsuzumi H, Zhong M, *et al.* (2009) Promotion of IL-4-and IL-5-dependent differentiation of anti-mu-primed B cells by ascorbic acid 2-glucoside. *Immunol Lett* **122**, 219–226.
41. Cox RJ, Brokstad KA & Ogra P (2004) Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines. *Scand J Immunol* **59**, 1–15.
42. Maggini S, Wintergerst ES, Beveridge S, *et al.* (2007) Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. *Br J Nutr* **98**, Suppl. 91, S29–S35.
43. Kaiser L, Fritz RS, Straus SE, *et al.* (2001) Symptom pathogenesis during acute influenza: interleukin-6 and other cytokine responses. *J Med Virol* **64**, 262–268.
44. Saedisomeolia A, Wood LG, Garg ML, *et al.* (2009) Lycopene enrichment of cultured airway epithelial cells decreases the inflammation induced by rhinovirus infection and lipopolysaccharide. *J Nutr Biochem* **20**, 577–585.
45. Gauliard B, Grieve D, Wilson R, *et al.* (2008) The effects of dietary phenolic compounds on cytokine and antioxidant production by a549 cells. *J Med Food* **11**, 382–384.
46. Novoselova EG, Lunin SM, Novoselova TV, *et al.* (2009) Naturally occurring antioxidant nutrients reduce inflammatory response in mice. *Eur J Pharmacol* **615**, 234–240.
47. Nantz MP, Rowe CA, Nieves C, *et al.* (2006) Immunity and antioxidant capacity in humans is enhanced by consumption of a dried, encapsulated fruit and vegetable juice concentrate. *J Nutr* **136**, 2606–2610.

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