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Effect of fruit and vegetable intake on oxidative stress and inflammation in COPD: a RCT.

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ABSTRACT

Epidemiological evidence supports a positive relationship between fruit and vegetable (FV) intake, lung function and chronic obstructive pulmonary disease (COPD). Increasing FV intake may attenuate the oxidative stress and inflammation associated with COPD.

An exploratory randomised controlled trial to examine the effect of increased consumption of FV on oxidative stress and inflammation in moderate to severe COPD was conducted. Eighty-one symptomatically stable patients with a habitually low FV intake (≤2 portions FV/day) were randomised to the intervention group (≥5 portions FV/day) or the control group (≤2 portions FV/day). Each participant received self-selected weekly home deliveries of FV for 12 weeks.

Seventy-five participants completed the intervention. There was a significant between-group change in self-reported FV intake and biomarkers of FV intake (zeaxanthin, P=0.034 and β-cyptoxanthin, P=0.015) indicating good compliance; post-intervention intakes in intervention and control groups were 6.1 and 1.9 portions FV/day respectively. There were no significant changes in biomarkers of airway inflammation (interleukin-8, myeloperoxidase) and systemic inflammation (C-reactive protein) or airway and systemic oxidative stress (8-isoprostane).

This exploratory study demonstrated that patients with moderate to severe COPD were able to comply with an intervention to increase FV intake, however, this had no significant effect on airway or systemic oxidative stress and inflammation.

KEYWORDS: Chronic obstructive pulmonary disease, fruit, inflammation, oxidative stress, vegetables

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Trial registration number: NCT00435708; www.clinicaltrials.gov

INTRODUCTION

The World Health Organisation (WHO) have predicted that, between 1990 and 2020, COPD will move from the sixth- to the third-leading cause of death worldwide [1]. Systemic and airway inflammation and increased levels of oxidative stress are key pathophysiological features of COPD. Thus, factors that can ameliorate these underlying inflammatory processes and/or help to redress the oxidant/antioxidant balance are of therapeutic interest.

A number of dietary constituents possess anti-inflammatory and antioxidant properties and, hence, are of interest with respect to the pathogenesis of COPD. Many cross-sectional studies have demonstrated a significant positive association between fruit and vegetable (FV) intake and forced expiratory volume in one second (FEV_1), with evidence particularly strong for fruit [2-3] but also evident for vegetables [4-5]. Other research has indicated an inverse association between FV intake and COPD symptoms [2, 4] and Carey *et al.* [6] demonstrated that a lower fruit intake was associated with a decline in FEV_1 over 5-7 years follow-up. Furthermore, fruit intake has also been inversely associated with 20-25 year incidence of chronic non-specific lung disease and COPD mortality [2, 7]. A recent systematic review on vitamins and COPD, found that the intake of many vitamins commonly found in fruits and vegetables, are associated with features of COPD and highlighted the need for prospective randomised controlled trials in this field [8].

It is possible that dietary interventions may attenuate the oxidative stress and inflammation associated with COPD, which may, in turn, translate into beneficial outcomes for patients. Few food-based interventions in COPD have been published to date [9-10]. Cerdá *et al*. [9] randomised 30 stable COPD patients to consume 400ml of pomegranate juice or 400ml of a synthetic orange-flavoured drink daily for five weeks. They found no significant differences between the groups in lung function or clinical symptoms of COPD. Although juice was provided to participants on a weekly basis, nutritional biomarker compliance data was not reported. To date, there has only been one broad FV intervention in COPD patients. Keranis *et al*. [10] reported that a dietary shift from low to modest consumption of FV (based on patients' self-reported intake, objective biomarkers of FV intake were not reported) was associated with an increase in FEV_1 in a 3-year randomised prospective study in COPD patients. Participants were randomised to receive either advice at 6-monthly intervals to increase FV intake (n=60) or to freely choose their own diet (n=60).

Given the epidemiological evidence to date, there is a need to further explore the relationship between FV intake and COPD in randomised controlled trials (RCTs). The aim of this study was to conduct an exploratory RCT to examine the effect of increased consumption of FV on oxidative stress and inflammation in people with moderate to severe COPD. The study design incorporated strategies to maximise adherence to the intervention and assessed objective biomarkers of compliance.

METHODS

Ethics and participants

Ethical approval was received from The Office for Research Ethics Committees Northern Ireland (ORECNI). All volunteers gave written informed consent. Participants were recruited between February 2007 and February 2009 from respiratory outpatient clinics at four Belfast hospitals. Inclusion criteria were: symptomatically stable, moderate to severe COPD (FEV_1 <80%) and a habitually low FV intake (≤2 portions FV/day; assessed by diet history, see below). Exclusion criteria included a history of diabetes mellitus, a regular intake of high dose antioxidant vitamin supplements and an arterial oxygen tension (PaO_2) <8kPa.

Study design

This was a 12-week open-label randomised controlled intervention study. A 12-week period was chosen for this intervention study as this has been shown to be sufficient time to ensure an increase in micronutrient status [11] in response to FV interventions, and also to induce changes in plasma C-reactive protein (CRP) and urinary isoprostanes [12-13]. IL-8 has recently been shown to be responsive to 10-week dietary interventions (with bread [14] and cheese [15]). After obtaining consent, participants were randomly assigned, in blocks of four using a random-number generator (www.randomization.com), to the intervention group (≥5 portions FV/day) or the control group (≤2 portions FV/day). A portion was as defined by the Food Standards Agency, i.e. an 80g serving of FV or 150ml fruit juice. During the 12-week intervention, each participant received self-selected weekly home deliveries of FV from a local retailer. Participants were advised on suitable storage and cooking methods and how best to incorporate the FV into their diet. Participants were also contacted weekly by the study researcher (FB) to encourage compliance, record self-reported exacerbations and to discuss any difficulties they were experiencing. Throughout the intervention, participants were advised to maintain their usual level of physical activity and keep other lifestyle factors unchanged.

Study assessments

Participants attended the Regional Respiratory Centre, Belfast City Hospital, for study assessments at baseline (week 0) and post-intervention (week 12). The same assessments were performed at each visit. A questionnaire was administered to assess alcohol intake and smoking status. Anthropometric measurements (weight, height, waist and hip circumference) were taken with participants in light indoor clothing and without shoes using standard protocols. A non-fasting blood sample (participants were advised to consume a standard breakfast of tea and toast at home before attending for study assessments), a spot urine sample and an induced sputum sample (see below) were collected. All biological samples were processed and frozen at –80ºC within 2 hours of collection. Blood pressure was recorded using a WelchAllyn automated sphygmomanometer (Nimed Ltd, Belfast) on the right arm in a seated position after resting for 10-15 minutes. The average of three readings was recorded. A pulmonary function test, without the use of a bronchodilator, was carried out to assess FEV_1 and forced vital capacity (FVC) according to the American Thoracic Society/European Respiratory Society Task Force recommendations [16]. Knudson 83 predicted references were used to calculate lung functions [17]. A series of 7-day diet histories were completed by each participant at weeks 0, 6 and 12 in order to assess baseline diet and self-reported compliance with the intervention; average daily FV intake was then hand-counted from these records.

Sputum induction and processing

4ml of a 4.5% saline solution was nebulised using an EASYneb II nebuliser (Nebuliser Flaem Nuova S.p.A., Italy, 2002) by the participant. After 5 minutes, participants were encouraged to cough and clear a sample. This procedure was repeated until the saline was finished (approximately 15-20 min). Sputum was processed and treated according to the method of Kelly *et al.* [18].

Laboratory analysis

All laboratory analysis was performed on a blinded basis. Plasma ascorbic acid concentrations were measured on a Cobas FARA centrifugal analyser with a fluorescent attachment [19]. Serum concentrations of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene were determined by reverse-phase high-performance liquid chromatography (HPLC) with diode array detection [20]. Assays were standardised against appropriate National Institute of Standards and Technology (NIST) reference materials. Sputum myeloperoxidase

(MPO), interleukin-8 (IL-8) and serum cotinine were analysed by ELISAs on a Triturus® EIA Analyser system (Grifols S.A. Barcelona, Spain). Plasma CRP was measured with standard enzymatic assays (Randox, Crumlin, Northern Ireland) on an automated ILab-600 biochemical analyser (Instrumentation Laboratories, United Kingdom). Sputum neutrophil elastase was measured using an in-house continuous activity assay [21]. Sputum and urinary 8-isoprostanes were measured using an AutoDelfia (Dissociation Enhanced Lanthanide Fluoro ImmunoAssay) assay [22].

Statistical methods

All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) for Windows version 17.0 (SPSS Inc, Chicago, IL). Results are expressed as mean ± SD for normally distributed continuous variables. Skewed variables were logarithmically transformed for parametric analysis and presented as geometric mean and interquartile range. An independent samples t-test was used to compare the two groups at baseline and to examine differences in mean change between groups. Within group changes in endpoints were assessed using paired samples t-tests. Analysis was performed on the intention-to-treat principle; six participants (four in the control group and two in the intervention group) dropped out of the study (reasons given in results section below) and did not attend for the week 12 assessments so could not be included in an intention-to-treat analysis. The Chi-Square test was used to examine differences in nominal or categorical variables between groups.

A secondary analysis was also conducted removing data for participants who had experienced an exacerbation close to completion of the study as this may have had an acute effect on the outcome measures, thus masking the chronic effects of the intervention. Participants were excluded from this analysis if they had an elevated CRP level (>20 mg/L) at the end of the study as well as a self-reported exacerbation within the two weeks [23] prior to the study end. For the purposes of this study, we defined an exacerbation as "a sustained worsening of respiratory symptoms that is acute in onset and usually requires a patient to seek medical help or alter treatment [24]."

RESULTS

Summary of participant recruitment (Figure 1)

In total, 454 patients from four hospitals in Belfast were eligible to take part in this study; 373 (82.2%) declined. Reasons for declining participation included being unwilling to give up

time or to travel for study assessments (27.6%), a dislike of giving blood samples (4%), discouragement from other family members (8%), feeling too unwell (47%), and being uninterested in research (13.4%).

A total of 81 participants completed the baseline assessments and 75 participants completed the intervention. There were six dropouts in total following randomisation: four from the control group (one due to back injury, two who were admitted to hospital with other medical complications and one individual was unable to continue to participate owing to personal reasons) and two from the intervention group (one suffered a cardiovascular event and one died due to another medical complication). The intervention was implemented as intended and there were no adverse events associated with the intervention. The availability of paired data is indicated in the tables; it varies for the individual endpoints studied according to successful collection of biological material.

Baseline characteristics

Participants included 46 males (57%) and 35 females (43%). Forty-one percent of participants (n=33) had moderate COPD (FEV_1 <80% predicted) and 59% (n=48) had severe COPD (FEV_1 <50% predicted). Two (2.5%) participants were underweight, n=31 (38.3%) were normal weight, n=20 (24.7%) were overweight and n=28 (34.5%) were obese according to WHO criteria for body mass index. Seventy-five percent suffered from comorbidities. There were no significant differences in baseline characteristics between the two randomised groups (Table 1). Overall, 94% of the sample had low serum α-carotene status and 93% had low serum β-carotene status using cut-offs defined by Gey (1998) as being indicative of increased risk of cardiovascular disease and cancer [25]. There was evidence of biochemical depletion of vitamin C (status <11 μmol/L) in 21% of the sample (data not shown). There was no significant difference at baseline in inflammatory or oxidative stress biomarkers in participants with moderate *versus* severe COPD (data not shown).

TABLE 1

General baseline characteristics[1] of 81 participants with moderate to severe COPD according to randomisation to a low or high FV group

Variable	Low FV group ($n=41_{max}$)	High FV group ($n=40_{max}$)
Age (y)	61.2 ± 8.3	63.2 ± 9.1
Men	25 (61%)	21 (53%)
Height (m)	1.66 ± 0.10	1.66 ± 0.10
Weight (kg)	74.3 ± 15.5	77.3 ± 16.7
BMI (kg/m^2)	27.2 ± 5.7	28.2 ± 6.0
Systolic blood pressure (mmHg)	134.7 ± 20.1	139.6 ± 19.5
Diastolic blood pressure (mmHg)	78.7 ± 8.8	81.1 ± 9.7
Current smokers	22 (54%)	14 (35%)
Former smokers	18 (44%)	26 (65%)
Number of pack years	34.4 (20.9-59.9)	40.5 (19.9-60.0)
Education (years)	10.7 ± 2.1	11.3 ± 2.2
FEV_1 (% predicted):	50.4 ± 17.6	45.2 ± 17.0
- Moderate COPD: n (FEV_1range)	19 (52-85%)	14 (51-88%)
- Severe COPD: n (FEV_1 range)	22 (20-49%)	26 (20-50%)
FVC (% predicted)	77.4 ± 20.9	73.9 ± 16.7
FEV_1/FVC (% predicted)	65.6 ± 16.8	60.4 ± 15.7
FV intake (portions/day)	1.4 ± 0.6	1.5 ± 0.7

[1]Continuous variables are summarised as mean ± SD for normally distributed data and geometric mean (interquartile range) for skewed data. FV: fruit and vegetables; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; FEV_1/FVC: forced expiratory volume in one second to forced vital capacity ratio.

Change in self-reported FV intake (Figure 2)

At the study end, there was a significant between-group difference ($P<0.001$) in change in self-reported FV intake as a result of the intervention; the intervention group increased intake by 4.6 portions per day (from 1.5±0.7 portions/day to 6.1±1.8 portions/day). The control group also had a marginal increase in FV intake of 0.5 portions per day (from 1.4±0.6

portions/day to 1.9±0.5 portions/day), but were still consuming the target intake for this group of <2 portions FV/day at the end of the intervention.

Change in biomarkers of FV intake

Table 2 reports the change in micronutrient status according to study group. There was a significant between-group difference in change in zeaxanthin (P=0.034) and β-cryptoxanthin (P=0.015) status at 12 weeks. There was also a trend for a between-group difference in change in lutein status (P=0.076).

At baseline 92.5% of the sample had low serum α-carotene status and low serum β-carotene status using cut-offs defined by Gey (1998) as being indicative of increased risk of cardiovascular disease and cancer [25]. At the end of the intervention period, 79% had low serum α-carotene status and 84% had low serum β-carotene status. Likewise, for vitamin C, 12.5% of the intervention group had evidence of biochemical depletion of vitamin C (status <11 μmol/L) at baseline compared to 0% at the end of the intervention.

Change in other lifestyle factors

There was no significant change in alcohol intake, smoking status or serum cotinine concentrations during the intervention for participants in either the intervention or control group (data not shown).

TABLE 2

Changes in biochemical markers of micronutrient status according to FV allocation in moderate to severe COPD patients

Variable (µmol/L)	Low FV diet (n=36)				High FV diet (n=38)				Between-group comparison P value[3]
	Week 0[1]	Week 12	% change	Within group P value[2]	Week 0	Week 12	% change	Within group P value[2]	
Vitamin C	30.664 ± 26.975[4]	33.439 ± 21.193	9	0.344	35.676 ± 19.953	47.642 ± 19.231	34	0.001	0.040
Lutein	0.145 (0.100-0.228)[5]	0.158 (0.122-0.218)	9	0.046	0.154 (0.117-0.208)	0.187 (0.140-0.247)	21	<0.001	0.076
Zeaxanthin	0.026 (0.021-0.033)	0.028 (0.020-0.042)	8	0.152	0.023 (0.017-0.031)	0.029 (0.021-0.041)	26	<0.001	0.034
β-cryptoxanthin	0.033 (0.023-0.048)	0.043 (0.027-0.061)	30	0.004	0.041 (0.029-0.057)	0.067 (0.039-0.108)	63	<0.001	0.015
α-carotene	0.040 (0.032-0.069)	0.048 (0.031-0.085)	20	0.019	0.048 (0.037-0.066)	0.064 (0.046-0.093)	33	0.001	0.431
β-carotene	0.148 (0.100-0.256)	0.179 (0.126-0.310)	21	0.016	0.191 (0.136-0.273)	0.229 (0.160-0.288)	20	0.003	0.948
Lycopene	0.250 (0.165-0.415)	0.301 (0.189-0.515)	20	0.217	0.212 (0.137-0.432)	0.266 (0.166-0.541)	25	0.185	0.489

[1] Baseline values (before diet) did not differ significantly between-groups (independent samples t-test). [2] Within-group comparisons analysed by paired samples t-test.

[3]Analysed by independent samples t-test. [4] Values are mean ± SD. [5] Values are geometric mean (interquartile range). FV: fruit and vegetables.

Change in lung function, oxidative stress and inflammation

There were no significant differences between the low versus high FV group for lung function tests or for any of the biomarkers of oxidative stress and inflammation (Table 3).

Exacerbations during the intervention

During the 12-week study, 15 participants in the control group had an exacerbation (of which 3 required hospitalisation) and 20 participants in the intervention group had an exacerbation (of which 3 required hospitalisation); exacerbation rate did not differ significantly between groups (Chi-square statistic: P=0.223). In total, 16 participants had a CRP level greater than 20 mg/L at week 12 and had experienced an exacerbation within the 14 days prior to the end of the study. When the analysis was conducted following removal of these 16 participants, again, there were no significant differences between the two groups for any of the inflammatory or oxidative stress markers measured (data not shown).

TABLE 3

Change in markers of lung function and biomarkers of oxidative stress and inflammation according to FV allocation in moderate to severe COPD patients

Variable	Low FV diet ($n=37_{max}$)				High FV diet ($n=38_{max}$)				Between-group comparison P value[3]
	Week 0[1]	Week 12	% change	P value[2]	Week 0	Week 12	% change	P value[2]	
FEV_1 (% predicted), (n_L=37, n_H=38)	50.3 ± 18.5[4]	51.8 ± 17.7	3	0.288	45.1 ± 17.3	45.8 ± 17.5	2	0.552	0.654
FVC (% predicted), (n_L=37, n_H=38)	77.2 ± 21.9	83.8 ± 19.8	9	0.032	74.3 ± 16.8	75.8 ± 17.1	2	0.356	0.142
FEV_1/FVC (% predicted), (n_L=37, n_H=38)	65.6 ± 17.5	62.1 ± 16.1	-5	0.103	60.1 ± 16.0	59.1 ± 15.8	-2	0.484	0.306
IL-8 - sputum (ng/mL), (n_L=20, n_H=21)	9.00 (4.75-20.49)	7.67 (4.43-15.88)	-15	0.538	11.68 (5.42-19.20)	9.99 (4.53-15.92)	-15	0.407	0.992
MPO - sputum (ng/mL), (n_L=29, n_H=29)	956 (498-1891)	947 (493-2084)	-1	0.945	1218 (590-2330)	1384 (836-1881)	14	0.427	0.500
NE - sputum (µg/mL), (n_L=16, n_H=20)	4.93 (0.18-17.20)	2.92 (0.68-4.61)	-41	0.348	2.94 (0.77-11.16)	2.33 (0.64-7.72)	-21	0.453	0.621
CRP - plasma (mg/L), (n_L=36, n_H=38)	4.15 (1.75-8.52)	3.39 (1.89-6.21)	-18	0.309	3.38 (1.63-6.26)	4.09 (1.98-5.99)	21	0.219	0.116
8-iso - urine (nM/mM creatinine), (n_L=34, n_H=35)	1.54 (1.08-1.90)	1.41 (1.04-1.74)	-8	0.104	1.76 (1.17-2.07)	1.65 (1.21-2.27)	-6	0.353	0.806
8-iso - sputum (ng/mL), (n_L=27, n_H=25)	1.84 (1.17-3.28)	1.70 (1.26-2.42)	-8	0.743	1.44 (0.9-3.04)	1.83 (1.18-2.63)	27	0.302	0.334

[1] Baseline values (before diet) did not differ significantly between-groups (independent samples t-test). [2] Within-group comparisons analysed by paired samples t-test. [3] Analysed by independent samples t-test. [4] Continuous variables are summarised as mean ± SD for normally distributed data and geometric mean (interquartile range) for skewed data. n_L = number of participants in low FV diet. n_H = number of participants in high FV diet. FV: fruit and vegetables; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; FEV_1/FVC: forced expiratory volume in one second to forced vital capacity; IL-8: interleukin 8; MPO: myeloperoxidase; NE: neutrophil elastase; CRP: C-reactive protein; 8-iso: 8-isoprostane.

DISCUSSION

The health benefits of FV are well supported by observational epidemiology but it is only recently that data regarding the precise nature of their biological effects has started to emerge from randomised controlled trials [26]. Examination of these effects in both healthy and diseased groups will help to inform both public health messages and evidence-based clinical practice. This trial is the first to explore the effect of increased FV intake on biomarkers of inflammation and oxidative stress in patients with moderate to severe COPD. Participant retention rates were high and both self-reported FV intake and biomarkers of FV intake indicated that the intervention was implemented successfully in this group of patients; with the high FV group consuming a mean of six portions of FV per day by the end of the intervention from a baseline of less than two portions a day. Despite evidence of good compliance with the study protocol, there was, however, no significant difference between the two groups for markers of oxidative stress or inflammation.

The biomarkers chosen for this study, in particular IL-8 which has been most extensively studied, are reliable indicators of airway inflammation and oxidative stress in COPD populations [27-28]. IL-8 and systemic CRP correlate positively with disease severity, exacerbation rates and lung function decline in COPD [28]; the lack of a significant difference in disease biomarkers according to disease severity in this study is most likely owing to the large inter-individual variability that was encountered. Recently, food-based interventions with bread (n=20) and cheese (n=10) [14-15] for ten weeks in generally healthy participants have induced significant lowering of circulating IL-8 concentrations. CRP and isoprostanes are well regarded as biomarkers of inflammation and oxidative stress, respectively [29-30]. Cross-sectional studies generally support an association between higher FV intake and lower serum levels of CRP, however FV interventions in healthy populations have shown mixed results [31]. Watzl *et al.* [12] demonstrated a significant reduction in plasma CRP following 4-weeks on a high FV diet in healthy non-smoking men and a 2-year Mediterranean-style dietary intervention in patients with metabolic syndrome significantly lowered serum levels of CRP, IL-6, IL-7 and IL-18 [32]. Thompson *et al.* [13] reported a reduction in the excretion of 8-isoprostanes after an 8-week high FV diet in healthy women. However, two recent intervention studies, a 2-month Mediterranean diet intervention [33] and a 2-month FV intervention [26] have shown no effect of increased FV intake on circulating CRP, yet these studies have been able to demonstrate significant improvements in measures of vascular

function (flow-mediated dilatation and venous occlusion plethysmography, respectively), thus indicating that such biomarkers may be dissociated from true biological effects and, therefore may be of limited use in dietary studies [31]. Further novel biomarkers of COPD may prove to be more sensitive to FV or antioxidant interventions and assessment of a broader panel of biomarkers may be prudent in such work, however, costs often prohibit such explorations.

The assessment of airway inflammation and oxidative stress was limited, to some extent, by the success rate of induced sputum collection as paired samples were obtained for 77% of the sample; this should be considered when planning future similar studies. The large intra- and inter-study variability and lack of data on within-subject variability in inflammatory markers in COPD [34] makes it difficult to estimate the sample size for a definitive study; a retrospective power calculation based on the numbers of subjects actually included in the final analysis showed that, assuming no change in end-points in our control group, the study had in excess of 80% power to detect a 60% reduction on IL-8, a 45% reduction in MPO and a 50% reduction in plasma CRP in the intervention group. To give an indication of sample sizes required to detect smaller changes, based on the variability of changes in log-transformed IL-8 values observed in our data, a study of 400 COPD subjects (n=200/group) would be required to detect a 25% change in IL-8 values on intervention with 80% power. A larger sample size would, of course, have improved the power of the study; however, even trends towards significance were not apparent in the data.

Although circulating antioxidant status increased in this study it cannot be assumed that this, in turn, impacted on airway antioxidant status. Little is still known about how the antioxidant pool in the respiratory tract lining fluid is maintained and whether or not dietary antioxidant intake can influence this process [35]. A study in non-asthmatic children reported a significant correlation between ascorbate in bronchoalveolar lavage and ascorbate concentrations in serum ($r=0.297$, $P=0.018$) [36], indicating that dietary antioxidants may be able to influence antioxidant defences in the lung. An investigation of antioxidant levels in induced sputum samples from this study would help to elucidate the relationship between dietary antioxidant intake and antioxidant concentrations within the respiratory tract.

To our knowledge, there is only one other FV intervention in COPD patients to date. Keranis *et al.* [10] recently reported findings from a 3-year prospective study in which participants from COPD outpatient clinics in Greece, were randomised to consume a diet with increased

consumption of fresh FV (n=60) or to consume a diet of their own free choice (n=60). The intervention group were given advice to consume FV which was reinforced every 6 months at scheduled outpatient appointments. The study reported that the intervention group, who changed their diet from low to modest consumption of FV (change in terms of portions for fruit and vegetables not reported), displayed an increase in FEV_1, whereas control group patients exhibited a decline in lung function (P=0.03 for difference between groups). The lack of objective compliance data in this long-term study makes it difficult to confidently attribute the positive observations to increased consumption of FV. This study did, however, employ a long-term follow-up of participants and, it is possible that, given the chronic nature of COPD, such a lengthy follow-up may be necessary in order to examine the true biological effects of dietary interventions in this population. It is also encouraging that the patient group studied by Keranis *et al.* [10], approximately 78% of whom had moderate to severe COPD, were able to increase FV intake and sustain this for 3 years, however the caveat regarding objective biomarkers of FV intake discussed above remains a limiting issue.

In the present study there was a significant change in FV intake between the intervention and control group (difference in change in FV intake was 4.2 portions/day between the groups), and this was reflected in significant between-group differences in biomarkers of FV intake (zeaxanthin and β-cryptoxanthin). The control group did increase their intake of FV by, on average, 0.5 portions per day but their overall mean intake remained within the study target of <2 portions per day. This increase in intake in the control group was minimal and not unexpected given the fact that the intervention involved home delivery of FV to both groups, as well as weekly contact with the study researcher, both of which would heighten awareness of consumption.

The limitations of the present study must be acknowledged. This was, by necessity, an open-label study, however the laboratory analysis was performed on a blinded basis. It was also a short-term intervention and so results cannot be extrapolated to the situation of chronic consumption. Furthermore, two barriers (cost and access) to FV intake were removed in the study in order to enhance compliance. Participants were asked not to make any other lifestyle changes during the study and they reported no change in smoking behaviour or alcohol intake. However, physical activity was not assessed pre- and post-intervention. Whilst changes in physical activity may be unlikely in this population, it cannot be ruled out as a potential

confounder. Exacerbation assessment is also a limiting factor. Although exacerbation rate did not appear to differ between groups, it is important to note that exacerbations were self-reported and were not verified by medical records. Finally, the participants in this study may not be representative of all patients with moderate to severe COPD, 82% of eligible participants declined to participate and patients with lower BMI's were under-represented; it is likely that such individuals declined to participate owing to the perceived demands of the study.

Further work in this area should not be precluded based on the results of this initial study alone as many individual factors could affect the outcome of such work. Dietary interventions, such as this, may have differing effects in different sub-groups of COPD patients. The study duration and endpoints that are most likely to be responsive are also a critical consideration; it is possible that longer-term dietary interventions may be more effective in COPD. In terms of the nature of the intervention itself, there are many possible approaches, each with their own merits, for example, one could hypothesise that a broader dietary approach that focuses on several key foods or food groups, rather than focusing on one particular food group (FV) may be more likely to have a beneficial effect.

In conclusion, this study demonstrated that patients with moderate to severe COPD were able to comply with an intervention to increase FV intake; however, this increased intake had no significant effect on airway or systemic oxidative stress and inflammation. Although no signal was apparent, a potentially beneficial effect of increased FV intake in COPD cannot be excluded based on this exploratory study alone as longer-term interventions, with different endpoints, may be required to demonstrate biological effects in this population. The experiences from this trial in terms of feasibility of recruitment, challenges of induced sputum sample collection and variance in the study endpoints should be considered when planning future studies in this area.

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Figure 1

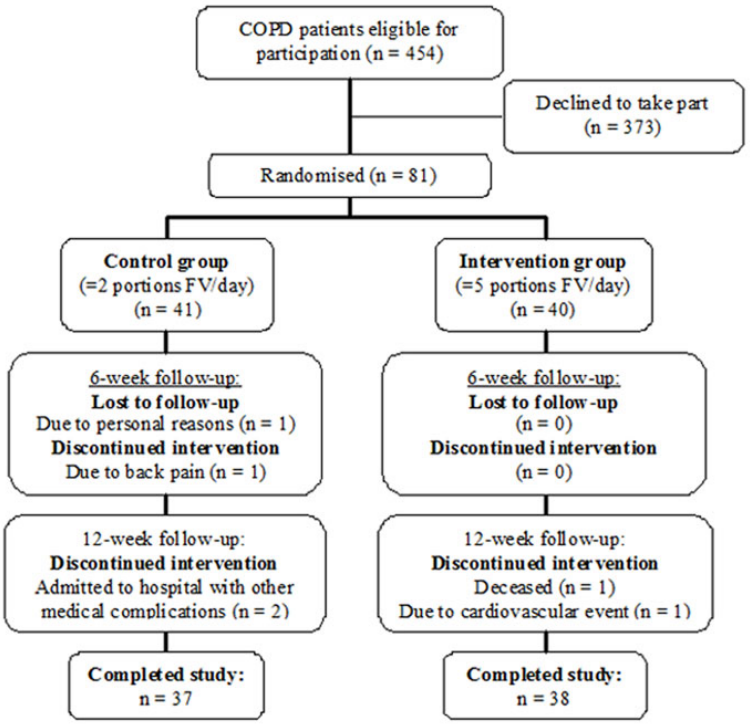

FIGURE1. Progress of participants through the FV intervention

Figure 2

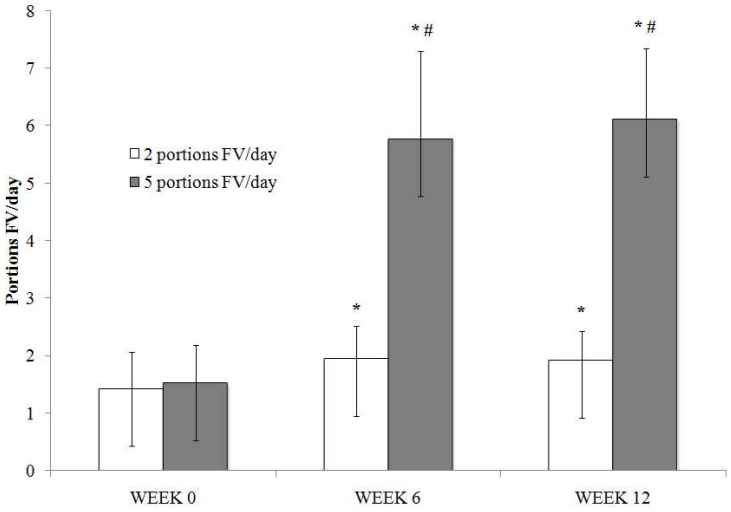

FIGURE 2. Self-reported intake of FV (portions/day) during the intervention period.
Data are mean (SD); *Within-group comparison - significantly different from baseline (P<0.001, paired samples t-test); #Between-group comparison significantly different (2 *vs.* 5 portions FV/day analysed by independent samples t-test; P<0.001).