ERJ Express. **Published on February 11, 2010 as doi: 10.1183/09031936.00113809**

Impact of dietary shift to higher antioxidant foods in COPD: A Randomized Trial

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The work was performed at Health Center of Argalasti, Achilopoulion General Hospital, Pelion, Volos Greece under supervision of the Department of Respiratory

Medicine, School of Medicine, University of Thessaly, Larisa, Greece. The work was supported by the University of Thessaly, Greece.

Abstract word count 200, Text word count 3558

E. Keranis has no conflicts of interest to disclose. D. Makris has no conflicts of interest to disclose, P. Rodopoulou has no conflicts of interest to disclose, H. **Martinou has no conflicts of interest to disclose. G. Papamakarios has no conflicts of interest to disclose. H. Z. Daniil has no conflicts of interest to disclose**, **E. Zintzaras has no conflicts of interest to disclose**, **K.I. Gourgoulianis has no conflicts of interest to disclose.**

Abstract

Chronic obstructive pulmonary disease (COPD) is characterized by increased oxidative stress. Dietary factors such as ample consumption of foods rich in antioxidants such as fruits and vegetables, might have beneficial effects in COPD patients. We investigated the association between dietary shift to foods rich in antioxidants and lung function in COPD in a 3-year prospective study. 120 COPD patients were randomized either to follow a diet based on increased consumption of fresh fruits and vegetables (intervention group-IG), or to follow a free diet (control group-CG). The mean consumption of foods containing antioxidants was higher in IG than in CG throughout the study period ($p<0.05$); The relationship between consumption of foods rich in antioxidants and FEV_1(%pred) was assessed by using a general linear model for repeated measures: the two groups overall were different in time ($p=0.03$) with IG showing a better outcome. In investigating the effect of several confounders (gender/age/smoking-status/comorbidities/exacerbation) in group response over time, non-significant interactions were found between confounders, group and time. Our findings suggest that a dietary shift to higher antioxidant foods intake may be associated with improvement in lung function and in this respect, dietary interventions might be considered in COPD management.

(NCT00884299 – ClinicalTrials.gov)

Keywords: Chronic Obstructive Pulmonary Disease, diet antioxidants, epidemiology.

Abbreviation list

BMI = body mass index

CG = control group

COPD = Chronic Obstructive Pulmonary Disease

FEV_1 = forced expiratory volume in 1 second

FVC = forced vital capacity

IG = intervention group

GOLD = Global initiative for Chronic Obstructive Lung Disease

Introduction

The course of Chronic Obstructive Pulmonary Disease (COPD) is characterized by progressive and irreversible airflow obstruction, impaired quality of life and increased mortality [1]. COPD prevalence is increasing worldwide and thus, the management of the disease is currently considered a major health issue. Several therapeutic strategies including smoking cessation, pharmacological interventions and rehabilitation programmes are used aiming to improve the quality of life and to decelerate the lung function decline in COPD patients.

There is increasing evidence that COPD is characterized by increased airway and systemic inflammation which partially is triggered by the aggravated oxidative stress that is prevalent in COPD patients compared to healthy subjects. Thus, it has been hypothesized that balancing oxidative stress might be protective for the development of COPD and other airway diseases. Previous studies [2,3,4,5] investigated the protective role of dietary interventions (based on adequate intake of antioxidants and avoidance of increasing girth) against oxidative stress. These studies showed that foods rich in antioxidants (e.g. fruits, vegetables) may protect the lungs from oxidative damage [6,7] resulting from smoking or air pollution [8,9,] because antioxidants are able to modulate the acute harmful effects of oxidative air pollution and smoking on the lungs [10,11,12,13]. Notably, a beneficial association between fruit intake and lung function was observed in cross-sectional surveys where FEV_1 for subjects with increased fruit intake was about 100 ml higher than that for subjects with decreased intake of fruits [2,14,15].

However, the impact of dietary supplementation on lung function has not been demonstrated in prospective longitudinal studies in COPD. In this setting, questions still remain and the role of dietary interventions based on high intake of antioxidants (i.e. diet rich in fruits and vegetables) in COPD management, is not yet clear.

Our primary aim was therefore to investigate prospectively whether a nutritional intervention consisting of diet rich in antioxidants such as raw fresh fruits and vegetables, would significantly affect lung function decline in COPD patients compared to a free diet. We randomized a population of COPD patients of varying severity to receive either a diet rich in fruits and vegetables or a free diet and we studied the relationship between that dietary intervention and lung function decline in 3 years.

Methods

Patients

This investigation was a 3-year prospective study, incorporating a recruitment period of two months, a run-in period and outpatient clinic visits, scheduled every 6 months. Consecutive sampling was used to recruit patients between 2004 and 2007, from a community based outpatient primary medical clinic of Achilopoulion General Hospital. The clinic was based at Argalasti, a rural area in South Pelion, Greece. In order to be included in the study, patients should have a diagnosis of COPD based on medical history, clinical signs and spirometry according to the GOLD definition [1], to be free of a COPD exacerbation[1] for at least 4 weeks. Patients with a favourable response to bronchodilators (FEV_1 change post-bronchodilator >15%), history of Asthma, atopy, allergic rhinitis, continuous use of systemic steroids for more than 30 days in the previous year or history of lung cancer or other respiratory disease were excluded from the study. One thousand four hundred fifty five subjects who visited the outpatient clinic during the recruitment period were assessed for eligibility. One hundred twenty of them who were eligible, gave their consent and entered the study (Figure 1). The study was approved by the Internal Review Board and Ethics Comitee of University of Thessaly and Achilopoulion General Hospital.

At baseline all patients underwent physical examination that included measurements of height, weight, body mass index (BMI calculated as weight/heigth^2), they were questioned for smoking habits, alcohol intake, dietary habits, physical activity while lung function was assessed by pre- and post bronchodilation spirometry, and comorbidities by using the Charlson index. Respiratory symptoms such as dyspnea (duration and severity), sputum production, cough, wheezing, were assessed and used as additional confirmatory measurements regarding COPD diagnosis and assessment of disease severity. Baseline assessment was based on accepted diagnostic tools [16][17][18][19].

Spirometry

Every 6 months post-bronchodilation spirometry was performed following premedication with 200 μg salbutamol via metre-dose inhaler, according to standardized guidelines [1], using a computerized spirometer (Spirolab II, Italy). Spirometry was performed by laboratory technicians who were not aware of the study design, group allocation or study outcomes. Attention was taken on the day that the spirometry was performed; the subject was free of an exacerbation for at least 4 weeks.

Fruit and vegetables consumption assessment

In this study we evaluated the change in the nutritional status by using a questionnaire regarding food intake and by assessing BMI. Food intake assessment was based on previously accepted food questionnaire modified according to antioxidant food sources and the local dietary habits [20][21][22]. It contained questions for 38 different items regarding fresh salads or raw vegetables (including olives, spinach, beet, mushrooms, cruciferous, carrots, tomatoes, potatoes, broccoli, dark-green leafy), fresh fruits and pure fruit juice (including strawberries, plum, apples, citrus, oranges, mandarin, kiwi, nuts). Most of these nutrients are produced in this rural

Mediterranean region and all of them are available all year in this area. For each food item, patients were asked to rate their consumption in a scale as: once or more than once a day, most days, once or twice a week, less than once a week, or never. These responses were assigned a score from 5 to 0 accordingly, following the methods reported previously [4,23]. An overall fresh fruit consumption score was calculated by taking the mean of consumption levels. This was then divided into five categories for the purposes of presentation (0-1, 1.01-2, 2.01-3, 3.01-4, and 4.01-5), with the two extreme groups representing "never" and "more than once a day" on an annual basis. Change in fresh fruit consumption was calculated by the absolute difference in scores. Food categories such as overall meat, overall poultry, overall fish and overall cereals intake was assessed as well in order to identify whether the shift towards a diet rich in fruits and vegetables was associated with a decrease of other food groups.

Study groups and interventions

Patients were randomized to follow for three years either, a diet with increased consumption of foods containing antioxidants such as fresh fruits, fruit juices and vegetables (intervention group or IG, n=60) or, a free diet (control group or CG, n=60). Randomization was performed with the use of tables of random order [24] by a person (GP) who was not involved in the study monitoring or outcome assessment. Two attending physicians (EK, EM) and two specialist nurses (HM, GK) were responsible for seen and monitoring the patients in both groups and were blind to outcome assessment. It was scheduled for both groups to be seen equally by all physicians/nurses. IG patients were seen regularly in the outpatient clinic (scheduled outpatient visits every six months) where they were informed for the potential beneficial effects of fruits and vegetables in their health status by two members of the study team (one of the two attending physicians and one of the two specialist nurses). At baseline and at each visit it was clearly explained to them that the dietary goal was to increase fresh fruit /fruit juices/vegetable consumption of at least one portion per day compared to baseline based on results of the MORGEN study and of local dietary habits and to maintain this regime throughout the study period [15,23]. Patients in CG were also seen regularly (scheduled outpatient visits every six months) in the outpatient clinic by two members of the study team (one of the two attending physicians/nurses) receiving the standard care for COPD and dietary issues were not discussed. The dietary questionnaire was repeated in each outpatient visit. All patients were allowed to contact the study team each time they experienced deterioration of their clinical condition. COPD exacerbations were treated according to published guidelines [1] and recorded at each visit. Outcome was assessed by senior members of the research team (KG, DM).

Statistical analysis

In analysis, the dependant variable was change in post-bronchodilation forced expiratory volume in 1 sec (FEV_1) expressed as a percentage of the predicted value as previously described [19]. The two groups (control and intervention group) were compared using a general linear model for repeated measures with Bonferonni's adjustment. The model uses as response the FEV_1 and includes the following sources: group effect, patients within group effect, follow-up (time) effect, interaction between follow-up and group, and error. The effect of possible confounders (gender, age (cut-off 65yrs), smoking status (ever/never), morbidities (by using Charlson Index values) and exacerbations (frequent exacerbators or not according to median value [19]) was also assessed by including each confounding effect (in turn) and its interaction with group and time in the above model. The power for detecting the overall observed mean difference in FEV_1 between the two groups based on the sample estimated standard deviations at significant level 5%, given that 60 patients were allocated in each group, was calculated. The association between excess of fruits/vegetables consumption and improvement in FEV_1 was tested using the Fisher's exact test. The normality of the response variable FEV1 at each time point was tested using the Kolmogorov-Smirnov test. A result was considered significant when $p<0.05$. Data were analysed using the SPSS, v12 and StatsDirect software packages.

Results

Baseline characteristics of participants are presented in Table 1. Participants mean (SE) age was 68.1(1.4) years, 105 out of 120 (87.5%) were men. According to the GOLD severity of COPD 22 (18.5%) patients were stage I, 79 (65.8%) were stage II, 15 (12.5%) were stage III, 4 (3.3%) were stage IV. Table 1 demonstrates that there were no statistically significant differences between the two groups, intervention group (IG) and control group (CG), at baseline with respect to demographic, smoking and dietary habits clinical characteristics and spirometry. Participants have attended 794 out of 840 6-monthly scheduled visits (394 and 400 visits in IG and CG respectively). There was no difference between IG and CG in respect of the frequency seen by each attending physician (chi-square test, p=0.38). Twenty three patients (19.1%) stopped smoking during the study. There was no significant difference between the proportion of patients who quitted smoking in IG and CG. There were no significant changes in BMI and exercise between the two groups. There was also no significant difference regarding alcohol consumption between the two groups throughout the study. The mean (SE) annual exacerbation rates in the year preceding the study were 0.45 (0.1) and 0.51(0.1) in IG and CG respectively (p=0.1) and the mean annual rates during the study were 0.18(0.09) and 0.34(0.07) respectively (p=0.001). Three deaths occurred during the last semester of the study, all in IG (p=0.24).

Fresh fruit and vegetables consumption

Figures 2 and 3 show that patients in the IG group increased significantly the mean annual consumption of fresh fruits and vegetables compared to the control group. There was no significant difference in compliance regarding the suggested and the actual intake of fresh fruits and vegetables (Mann-Whitney U-test, $p=0.72$). In IG meat consumption score was reduced at the end of the study compared to baseline [1.90(0.3) vs 1.71(0.3) respectively, $p=0.45$, Wilcoxon signed rank test] whereas in CG no significant difference was found [1.87(0.4) to 1.91(0.3), $p=0.7$).

Lung function

The response variable FEV_1 was normally distributed at each time point ($p>0.20$). The mean annual rate of FEV_1(%pred) change of the entire population was 0.35 (95% CI -0.22 to 0.55) corresponding to an absolute value of 63 (-41 to 93) ml/year. Spirometric values in IG patients (FEV_1, FVC, and FEV_1/ FVC) were found significantly higher at the end of the study. The association between the frequency of different fruits and vegetables rich in antioxidants and average FEV_1(%pred) change per six months are shown in Table 2. Excess of antioxidant consumption and improvement in FEV_1 was statistically significant associated ($p<0.01$).

General linear model for repeated measures analysis revealed that the two groups (IG and CG) overall were different in time ($p=0.03$) with the IG showing a better outcome (Figure 4). However, the differences between the two groups were not consistent in time, i.e. there was an interaction between group effect and time and the difference in FEV_1 between groups tended to increase overtime ($p<0.01$). In investigating the effect of confounders (gender, age, smoking status, comorbidities and exacerbations) in group response over time, non-significant interactions were found between the confounders, the group and time ($p=0.81$, $p=0.16$, $p=0.77$, $p=0.48$ and $p=0.43$, respectively).

Discussion

In the present prospective randomized study, we evaluated the association between lung function decline and dietary shift to foods rich in antioxidants in a population consisting of 120 patients with COPD, for a total of 3-year follow-up period. The main finding of this study is that COPD patients who followed a diet with high intake of fruits and vegetables presented increase of annual FEV_1%pred while patients who were on standard diet exhibited a decrease in FEV_1 within 3 years (General linear model for repeated measures, p=0.03). Our results are consistent with the results of previous studies [2,6,15,25] which investigated the effect of antioxidant intake in lung function, despite the differences in design and study population between the present and former studies.

In the present investigation we found that dietary shift to a nutricion richer in antioxidants foods may have a significant impact on lung function. Patients who changed their diet from low to modest consumption of fruit and vegetables (intervention group-IG) exhibited an increase in FEV_1 which is a parameter of lung function that characterizes the course of COPD. In contrast patients in the control group (CG) exhibited a decline in lung function. The impact of dietary shift to higher antioxidants food intake, may have positively affected lung function in our COPD patients by balancing oxidative stress and reducing lung inflammation.

Fruits and vegetables contain significant amounts of antioxidant Vitamins such as C, E and beta Carotene which may protect the lungs from oxidative damage by smoking and air pollution. There is evidence from previous studies that those vitamins present antioxidant and antinflammatory properties [26]. Vitamin C, is present in intracellular and extra cellular lung fluids and supports the normal hydration of airway surfaces. Its deficiency may lead to dry, sticky mucus membranes lining the airway and thus, low levels of vitamin C may play a role in the progression of inflammation in airway diseases. Vitamin E is present in extracellular lung fluid and lipid membranes where it converts oxygen radicals and lipid peroxyl radicals to less-reactive forms. Beta-Carotene is a free- radical scavenger present in tissue membranes.

In this respect, previous studies [7,9,12,14] investigated whether the consumption of antioxidants affects lung function. However, these studies had observational design and used populations with respiratory symptoms and not particularly COPD patients. In addition, they used end points such as bronchial reactivity, which are applicable rather in Asthma than COPD. Thus, firm conclusions were difficult to be drawn. In the present prospective randomized study, we included COPD patients among 1455 subjects who visited the outpatient clinic during the recruitment period and according to a recent Greek study this population might represent the general COPD population in this region [27]. Thus, our findings which showed relationship between the consumption of food rich in antioxidants and lung function in COPD patients might be considered in the management of COPD.

One might argue that lung function in IG might have been positively affected by other factors which are not related to dietary shift to higher antioxidant foods such as fruits and vegetables. We certainly cannot exclude that other dietary, environmental, genetic factors or the presence of comorbidities may have affect the course of lung function in IG patients. For example, the addition of fruits and vegetables in the regime of IG patients resulted in decreased consumption of meat which is known to increase the burden of oxidative stress [26,28]. In addition, persons who initiate a preventive therapy (such as a dietary regime based on fruits and vegetables that might be considered as healthy) may also adhere to other social health behaviours that may also decrease the risk of adverse outcomes. Almost 47% of patients in our study had cardiovascular problems and a diet rich in fruits and vegetables might have been helpful in the status of these patients by improving muscle mass and performance[29][30]. However, in this study we assessed several factors that could have acted as confounding variables biases in the study such as changes in physical activity, alcohol intake, comorbidities, exacerbation frequency and no significant difference was found between the two groups.

Furthermore, the population studied represented mainly COPD patients followed at the same health centre and both population groups were in many terms homogenous (white, mediteraneans, inhabitants of a rural peninsula, similar baseline spirometric indices and clinical status). Thus, we assume that all of the subjects were affected by the same environmental factors and comorbidities have not affected our results.

Another alternative explanation for the relationship that was found between dietary shift to higher antioxidant foods and lung function in our study, it could be that patients who were marginally malnourished recovered with additional energy input and therefore their respiratory performance improved. However, baseline dietary status was similar in both groups in this study (Figures 2 and 3) and neither changes in BMI, smoking status nor social activities support this alternative explanation.

We certainly acknowledge that the beneficial impact of fruit and vegetable intake on lung function might be due to another bioactive nutrient and not due to the presence of antioxidants in fruits and vegetables only. A limitation of this study is that we have not assessed oxidative stress by using blood, urine or exited breath condensate marker that would provide further insight in our hypothesis. This could be certainly a point of interest that could be adequately assessed in the future.

Nineteen percent of patients stopped smoking during the present study. Kanner et al [30] analyzed the data of Lung Health Study and reported that patients who permanently quit smoking showed a deceleration in lung function decline or, even an improvement in lung function within years. However, it is not likely that smoking cessation may have affected our results. There was no significant difference between the proportion of patients who quitted smoking in IG and CG in our study and therefore, the effect of smoking in lung function may have affected both groups similarly.

Patients in IG group presented fewer exacerbations compared to controls during the study. In this respect one might argue that exacerbation frequency might have a significant impact on lung function changes in the two groups. However, in investigating the effect of confounders in group response over time, significant interactions were not found between exacerbations, patient group and time. Nevertheless, we should underline that exacerbations have been assessed by symptoms questioning alone in this study and exacerbation rates were lower compared to previous studies where more rigorous methodology based on diary cards data were used [19]. Therefore, patients might have underreported exacerbations in this study might and this limitation has to be considered in the interpretation of results.

In this prospective longitudinal investigation, data were collected by interviewing patients and by using questionnaires and accordingly, compliance in the suggested dietary regime was assessed. We certainly acknowledge that although we have assessed the intake of different fruits and vegetables, other food groups such as meat, poultry, fish and cereals have been assessed only as groups (i.e. overall meat intake). This fact did not permit us to evaluate further parameters such as the total energy intake that could provide further information for our population. However, our primary aim was to augment the consumption of foods rich in antioxidants, such as fruits and vegetables, which are easily available and less costly than meat or fish in this particular geographic area.

One might argue that using frequency questionnaires to assess consumption of food might not be as precise as a 24h recall or a 7-day recall that may permit better assessment of the adopted diet. In addition, apart from the questionnaire used another formal evaluation (i.e. home visits or 24h diary cards) that could further verify regarding compliance in diet advised has not been done. Nevertheless, frequency questionnaires are an accepted methodology that has been widely applied in previous COPD longitudinal studies [30] and easier to be applied in this setting than 24h recall method.

A final point that should be also noted is that the present is an open label study. Despite that the persons who gave the advice regarding fruits and vegetables consumption, were not involved in outcome assessment, they were aware of group allocation and this fact has to be considered in the interpretation of our findings.

In conclusion, in the present prospective randomised trial we demonstrated by using long term follow-up and adequate control subjects that the increase intake of fruit and vegetables may have a protective effect on FEV_1 in patients with COPD. We certainly acknowledge that the effects of a high fruit and vegetable intake on FEV_1 might be small to result in changes in clinically-manifest disease. Future investigations should assess whether a dietary shift to higher antioxidant foods has also an impact on other than lung function outcome measures which are important to patients' perception of their disease, such as quality of life, function or perceived dyspnoea. Nevertheless, our findings suggest that a diet rich in antioxidants such as fruits and vegetables may be associated with favourable outcomes in COPD and in this respect, the place of dietary interventions in the management of COPD warrants consideration.

Acknowledgements

E. K. coordinated the study and participated in the monitoring of the study, D.M. drafted the manuscript, H.M., G.P. and Z.D. performed monitoring of the study, P.R. assisted in data management and statistical analysis, E.Z. performed statistical analysis and draft part of the revised manuscript, K.I.G. motivated the study.

The authors wish to thank N. Tzanakis for his valuable contribution in the design of the study, and G. Kotopouli and E. Meli and all medical, nursing personnel of A.HC.C. for their participation in this study.

References

1. Pauwels RA, Buist AS, Peter M, Calverley A, Jenkins CR, Hurd SS . Global strategy for the diagnosis, management and prevention of Chronic Obstructive Pulmonary disease. NHLBI/WHO Global initiative for Chronic Obstructive Lung Disease (GOLD). Workshop summary. Am J Respir Crit Care Med 2001; 163(5):1256-1276.
2. Tabak C, Smit HA, Rasanen L, Fidanza F. Dietary factors and pulmonary function : a cross sectional study in middle aged men from three European countries. Thorax 1999; 54:1021-1026.
3. Britton JR, Pavord ID, Richards KA, Knox AJ, Wisniewski AF, Lewis SA, Tattersfield AE, Weiss ST. Dietary antioxidant vitamin intake and lung function in the general population. Amer J Respir Crit Care Med.1995; 151:1383-1387.
4. Ness AR, Khaw KT, Bingham S, Day NE. Vitamin C status and respiratory function. Eur J Clin Nutr 1996; 50:573-579.
5. Dow L, Tracey M, Villar A, Coggon D, Margetts BM, Campbell MJ, Holgate ST. Does dietary intake of vitamins C and E influence lung function in old people? Am J Respir Crit Care Med 1996;154 :1401-1404.
6. Grievink L, Smit HA, Ocke MC, van't Veer P, Kromhout D . Dietary intake of antioxidant (pro)-vitamins, respiratory symptoms and pulmonary function: the MORGEN study. Thorax 1998; 53:166-171.
7. Schwartz J, Weiss ST. Dietary factors and their relation to respiratory symptoms: the second national health and nutrition examination survey. Am J Epidemiol 1990; 132:67-76.

8. Heffner JE, Repine JE. Pulmonary strategies of antioxidant defense. Am Rev Respir Dis 1989; 140: 531-554.
9. Chatham MD, Eppler JH Jr, Saunder LR, Green D, Kulle TJ. Evaluation of the effects of vitamin C on ozone induced bronchoconstriction in normal subjects. Annals New York Acad Scien 1987; 498: 269-279.
10. Mohensin V. Effect of vitamin C on NO_2- induced airway hyper responsiveness in normal subjects. Am Rev Respir Dis 1987; 136: 1408-1411.
11. Mohensin V. Lipid peroxidation and antielastase activity in the lung under oxidant stress: role of antioxidant defenses. J Appl Physiol 1991; 70: 1456-1462.
12. Bucca C, Rolla G, Farina JC. Effect of vitamin C on transient increase of bronchial responsiveness in conditions affecting the airways. Annals New York Acad Scien 1992; 669: 175-187.
13. Romieu I, Meneses F, Ramirez M, Ruiz S, Perez Padilla R, Siena JJ, Gerber M, Grievink L, Dekker R, Walda I, Brunekreef B. Antioxidant supplementation and respiratory functions among workers exposed to high levels of ozone. Am J Respir Crit Care Med 1998;158 : 226-232.
14. Strachan DP, Cox BD, Erzinclioglu SW, Walters DE, Whichelow MJ. Ventilatory function and winter fresh fruit consumption in a random sample of British adults. Thorax 1991; 46: 624-629.
15. Tabak C, Smit HA, Heederik D, Ocke' MC, Kromhout D. Diet and chronic obstructive pulmonary disease: independent beneficial effects of fruits, whole grains, and alcohol (the Morgen study). Clin Exp Allergy 2001; 31:747-755.
16. Martinez JA, Straccia L, Sobrani E, Silva GA, Vianna EO, Filho JT. Dyspnea scales in the assessment of illiterate patients with chronic obstructive pulmonary disease. Am J Med Sci 2000; 320:240-3.

17. Ferris BG. Epidemiology Standardization Project. II. Recommended respiratory disease questionnaires for use with adults and children in epidemiological research. Am Rev Respir Dis 1978; 118:7–57.
18. Charlson M, Szatrowski T, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol 1994;47:1245–1251.
19. Makris D, Moschandreas J, Damianaki A, Ntaoukakis E, Siafakas NM, Milic Emili J, Tzanakis N . Exacerbations and lung function decline in COPD: New insights in current and ex-smokers. Respir Med 2007; 101: 1305-1312.
20. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol. 1986;124(3):453-69.
21. Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, Brighenti F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. J Nutr 2003;133(9):2812-9.
22. Chatzi L, Apostolaki G, Bibakis I, Skypala I, Bibaki-Liakou V, Tzanakis N, Kogevinas M, Cullinan P. Protective effect of fruits, vegetables and the Mediterranean diet on asthma and allergies among children in Crete. Thorax. 2007;62(8):677-83.
23. Brug J, Schols A, Mesters I. Dietary change, nutrition education and chronic obstructive pulmonary disease. Patient Educ Couns 2004; 52; 3: 249-257.
24. R. Mead, R. N. Curnow. Statistical Methods in Agriculture and Experimental Biology (Texts in Statistical Science). Chapman and Hill/CRC, USA,1983: 34.
25. Watson L, Margetts B, Howarth P, Dorward M, Thompson R, Little P. The association between diet and chronic obstructive pulmonary disease in subjects selected from general practice. Eur Respir J 2002; 20:313-318.

26. Lippman RD. Free radical-induced lipoperoxidation and aging. In: Miquel J, Quintanilha AT, Weber H (eds) Handbook of free radicals and antioxidants in biomedicine. CRC Press, Boca Raton, FL, 1989:187-197.
27. Tzanakis N, Anagnostopoulou U, Filaditaki V, Christaki P, Siafacas N. COPD group of the Hellenic Thoracic Society. Prevalence of COPD in Greece. Chest 2004;125:892-900.
28. Balabanidis A. Free radicals and their role in biological systems. Beta Med Arts 2006 ;8:160-162.
29. Wouters EF. Eat well to get well. Thorax 2003; 58:739-740.
30. Kanner RE, Anthonisen NR, Connet JE. Lung Health Study Research Group. Lower respiratory illness promote FEV_1 decline in current smokers but not ex-smokers with mild chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001; 164:358-364.

Figure Legends

Figure 1. Flow diagram of the study.

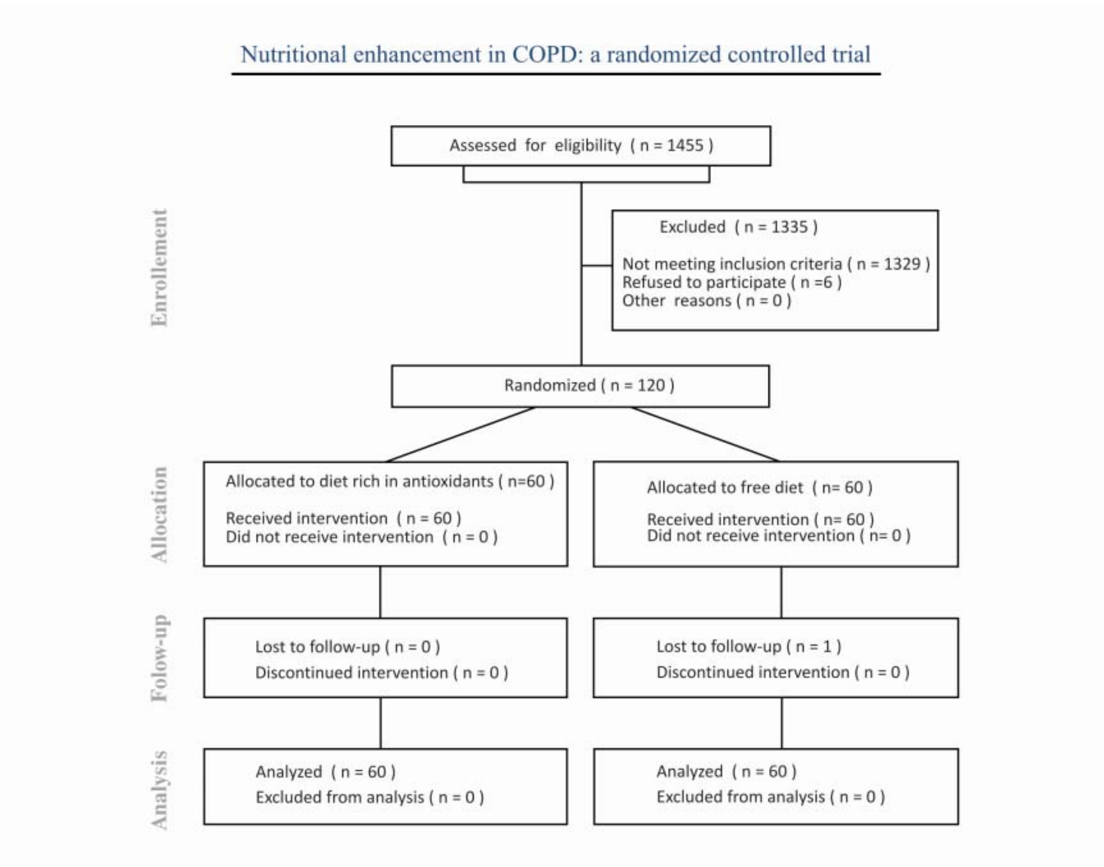

Figure 2. Annual consumption score of vegetables in patients who followed a diet rich in fruits and vegetables [intervention group(IG)] and in patients who followed a free diet during the study [control group (CG)]. Bars represent mean values.

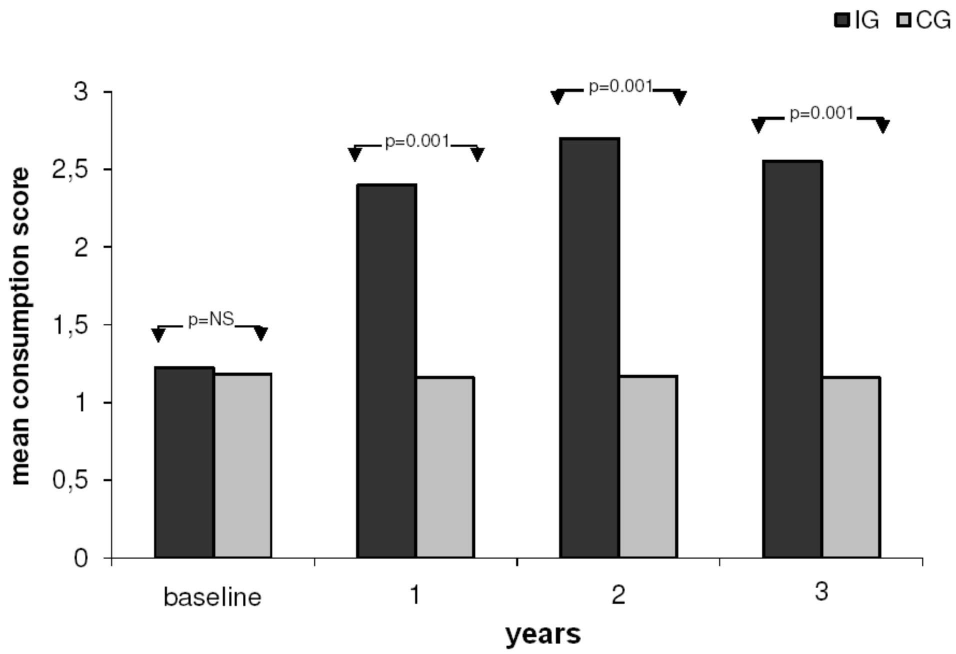

Figure 3. Annual consumption score of fresh fruits in patients who followed a diet rich in fruits and vegetables [intervention group(IG)] and in patients who followed a free diet during the study [control group (CG)]. Bars represent mean values.

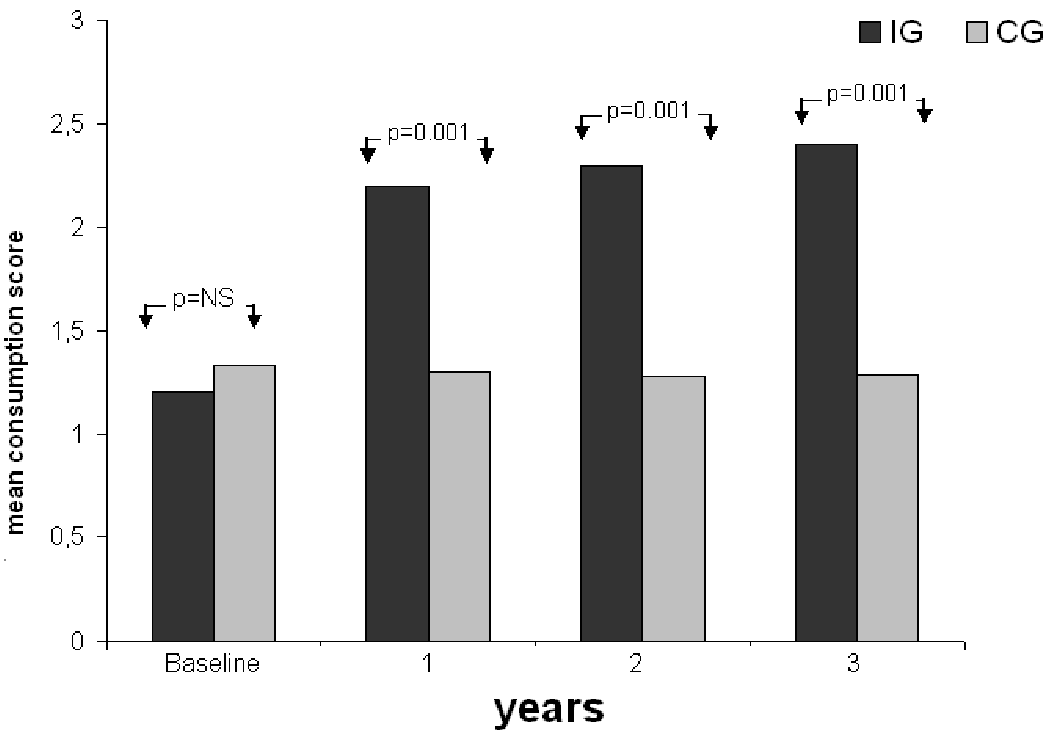

Figure 4. FEV_1(%pred) change from baseline over the 3-year study period by dietitic intervention. Open squares represent patients who followed a diet rich in fruits and vegetables (IG), closed squares represent patients who followed a free diet (CG); values are means with bars representing SE. P value represents the difference in average annual decline rate in FEV_1(%pred) between IG and CG as obtained by a general linear model for repeated measures with Bonferonni's adjustment.

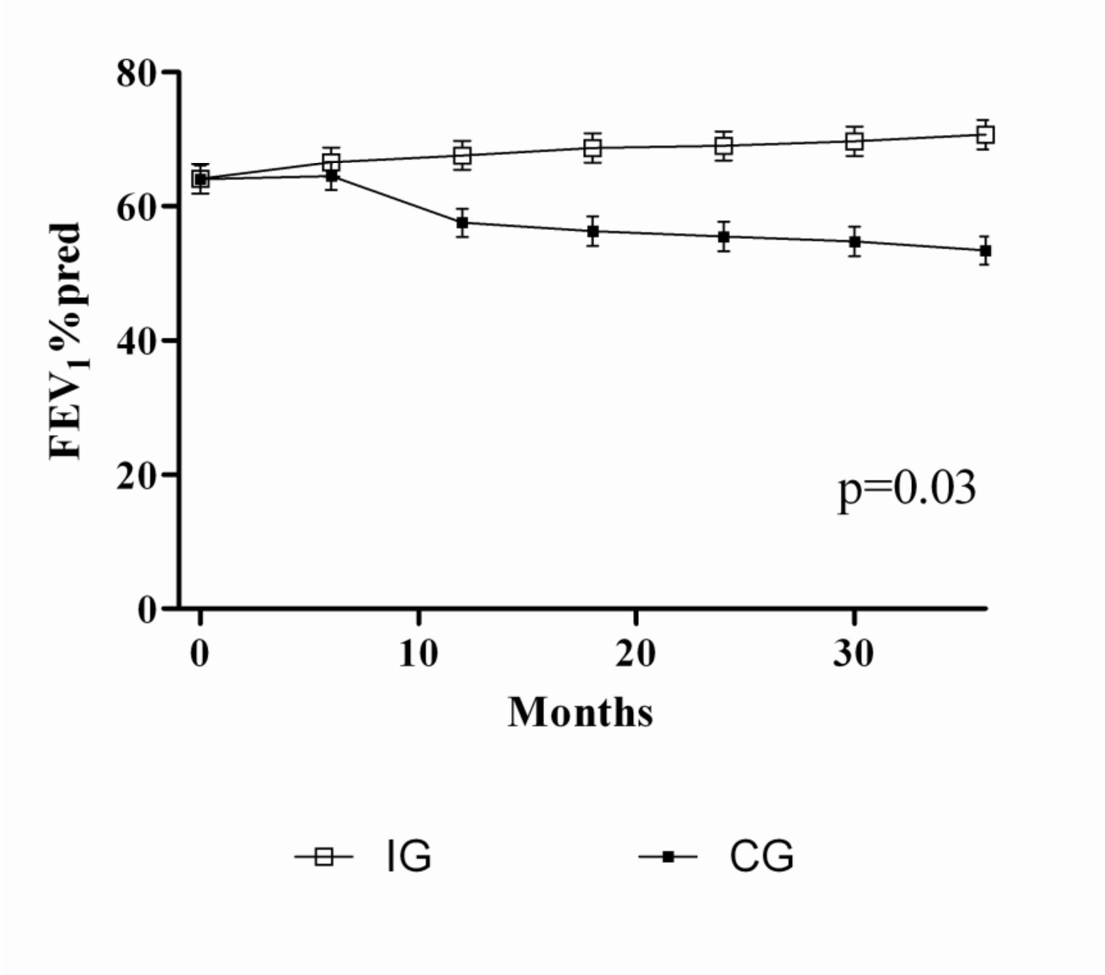

Table 1. Baseline characteristics of COPD patients overall and by dietary intervention

Variables	Overall n=120	Intervention group n=60	Control group N=60	p- value
Male / Female, n	105/15	54/6	51/9	0.58
Age, years	68.1 (1.4)	66.1 (1.46)	70.2 (1.15)	0.07
BMI (body mass index)	28.6 (0.41)	28.2 (0.60)	29.02 (0.66)	0.35
Current Smokers, n (%)	37 (30.8)	17 (28.3)	20 (33.3)	0.69
Ex-smokers, n (%)	76 (63.3)	39 (65)	37 (61.6)	0.84
Pack-years	68 (3.79)	62.4 (5.03)	73.6 (5.62)	0.14
Vegetable consumption score	1.18 (0.4)	1.75 (0.4)	1.46 (0.4)	0.35
Fresh fruits-juices consumption score	1.42 (0.4)	1.22 (0.4)	1.48 (0.5)	0.7

Meat consumption score	1.87 (0.2)	1.90 (0.3)	1.87 (0.4)	0.7
Fish consumption score	1.05 (0.2)	1.11 (0.3)	1.02 (0.4)	0.6
Alcohol consumption score	1.61 (0.3)	1.3 (0.3)	1.18 (0.3)	0.17
Chronic cough, n (%)	89 (74)	45 (75)	44 (72)	0.61
Chronic sputum , n (%)	86 (71)	39 (65)	47 (78)	0.39
Chronic dyspnea , n (%)	52 (56)	28 (46)	24 (40)	0.75
FEV_1 , L	1.82 (0.06)	1.93 (0.10)	1.71 (0.07)	0.09
FEV_1%pred	64.28 (1.58)	64.39 (2.32)	64.17 (2.19)	0.94
FVC, L	2.75 (0.09)	2.90 (0.14)	2.59 (0.11)	0.09
FVC%pred	74.64 (1.66)	75.71 (2.47)	73.50 (2.22)	0.52
FEV_1/FVC %pred	64.67 (0.96)	64.77 (1.14)	64.57 (1.56)	0.92
PEF, Lit/min	4.62 (0.17)	4.98 (0.28)	4.26 (0.21)	0.10
Saturation O2, %	95.58 (0.24)	95.20 (0.33)	95.96 (0.34)	0.81
Charlson Index	2.58 (0.12)	2.39 (0.16)	2.77 (0.17)	0.11
Arthritis, n (%)	1(0.8)	0(0.0)	1(1.7)	1
Cardiac failure, n (%)	25(20.8)	10(16.7)	15(25.0)	0.36
Coronary disease, n (%)	33(27.5)	16(26.7)	17(28.3)	1
Diabetes, n (%)	22(18.3)	8(13.3)	14(23.3)	0.23
Diseases of the liver, n (%)	2(1.7)	0(0.0)	2(3.3)	0.49
Hypertension, n (%)	69(57.5)	32(53.3)	37(61.7)	0.46
Nephropathy, n (%)	3(2.7)	2(1.7)	1(3.3)	1
Stroke, n (%)	8(6.7)	4(6.7)	4(6.7)	1

Continuous data are expressed as mean (SE) otherwise is indicated, Mann-Whitney test or Fisher's Exact test was used appropriately for comparisons between groups.

Table 2. Association between frequency of fruits and vegetables rich in antioxidants and average FEV_1%pred change per six-months, in COPD patients participated in the study.

	Consumption		
	several days per week	*once or less per week*	
	FEV_1%pred change		p values
Olive (black)	1.14 (0.93, 1.36)	-0.54 (-1.02, -0.43)	0.0001
Spinach	1.15 (0.95, 1.35)	-0.85 (-1.10, -0.60)	0.0001
Mushrooms	1.03 (0.68, 1.23)	-0.93 (-0.52, 0.65)	0.12
Beet	1.09 (0.86, 1.32)	-0.90 (-1.15, -0.66)	0.07
Broccoli	1.14 (0.89, 1.13)	-0.91 (-0.76, -0.21)	0.0005
Radichio	1.11 (0.91, 1.30)	-0.91 (-1.17, -0.66)	0.0001
Strawberry	1.19 (0.97, 1.14)	-0.64 (-0.9, -0.37)	0.005
Orange	1.00 (0.76, 1.23)	-1.01 (-1.12, -0.77)	0.0001
Plum	1.09 (0.88, 1.29)	-0.89 (-1.11, -0.64)	0.0001
Grapes (yellow)	0.57 (-0.29, 0.84)	-0.90 (-1.23, -0.57)	0.0003
Tangerine	0.35 (-0.38, 1.32)	-0.41 (-1.12, 0.32)	0.1

Data are presented as mean(95%CI).

p values represent differences (Mann Whitney-U test) between FEV_1%pred six-months change according to frequency of fruits and vegetables consumption.