On: 05 April 2013, At: 07:26
Publisher: Routledge
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Journal of the American College of Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uacn20

An Encapsulated Juice Powder Concentrate Improves Markers of Pulmonary Function and Cardiovascular Risk Factors in Heavy Smokers

Fabrizia Bamonti [a], Marco Pellegatta [b], Cristina Novembrino [a], Luisella Vigna [b], Rachele De Giuseppe [c], Federica de Liso [a], Dario Gregori [d], Cinzia Della Noce [e], Lorenzo Patrini [b], Gianfranco Schiraldi [f], Paola Bonara [g], Laura Calvelli [h], Rita Maiavacca [i] & Giuliana Cighetti [j]

[a] Dipartimento Scienze Mediche, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, ITALY

[b] Dipartimento Medicina Preventiva Clinica e del Lavoro, U.O. Medicina del Lavoro I, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, ITALY

[c] Fondazione Fratelli Confalonieri, Dipartimento Scienze Mediche, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, ITALY

[d] Dipartimento Medicina Ambientale e Sanità Pubblica, Università degli Studi di Padova, Padua, ITALY

[e] Istituto di Fisiologia Clinica CNR, Ospedale Niguarda Ca' Granda, Milan, ITALY

[f] Dipartimento Pneumologia, Ospedale Niguarda Ca' Granda, Milan, ITALY

[g] Dipartimento di Medicina Interna, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, ITALY

[h] NYMIG, Ospedale Niguarda Ca' Granda, Milan, ITALY

[i] Dipartimento Area Servizi Diagnostici, Laboratorio di Patologia Clinica, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, ITALY

[j] Dipartimento Scienze Cliniche "Luigi Sacco", Università degli Studi di Milano, Milan, ITALY

To cite this article: Fabrizia Bamonti , Marco Pellegatta , Cristina Novembrino , Luisella Vigna , Rachele De Giuseppe , Federica de Liso , Dario Gregori , Cinzia Della Noce , Lorenzo Patrini , Gianfranco Schiraldi , Paola Bonara , Laura Calvelli , Rita Maiavacca & Giuliana Cighetti (2013): An Encapsulated Juice Powder Concentrate Improves Markers of Pulmonary Function and Cardiovascular Risk Factors in Heavy Smokers, Journal of the American College of Nutrition, 32:1, 18-25

To link to this article: http://dx.doi.org/10.1080/07315724.2013.767652

Original Research

An Encapsulated Juice Powder Concentrate Improves Markers of Pulmonary Function and Cardiovascular Risk Factors in Heavy Smokers

Fabrizia Bamonti, PhD, Marco Pellegatta, MD, Cristina Novembrino, PhD, Luisella Vigna, MD, Rachele De Giuseppe, PhD, Federica de Liso, BS, Dario Gregori, PhD, Cinzia Della Noce, Lorenzo Patrini, MD, Gianfranco Schiraldi, MD, Paola Bonara, MD, Laura Calvelli, Rita Maiavacca, MD, Giuliana Cighetti, PhD

Dipartimento Scienze Mediche, Università degli Studi di Milano (F.B., C.N., F.d.L.), and Dipartimento Medicina Preventiva Clinica e del Lavoro, U.O. Medicina del Lavoro I (M.P., L.V., L.P.), and Fondazione Fratelli Confalonieri, Dipartimento Scienze Mediche, Università degli Studi di Milano (R.d.G.), Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, ITALY; Dipartimento Medicina Ambientale e Sanità Pubblica, Università degli Studi di Padova, Padua, ITALY (D.G.); Istituto di Fisiologia Clinica CNR (C.D.N.), and Dipartimento Pneumologia (G.S.), Ospedale Niguarda Ca' Granda, Milan, ITALY; Dipartimento di Medicina Interna, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, ITALY (P.B.); NYMIG, Ospedale Niguarda Ca' Granda, Milan, ITALY (L.C.); Dipartimento Area Servizi Diagnostici, Laboratorio di Patologia Clinica, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, ITALY (R.M.); Dipartimento Scienze Cliniche "Luigi Sacco", Università degli Studi di Milano, Milan, ITALY (G.C.)

Key words: tobacco, homocysteine, cysteine, folate, nutraceutical

Objective: Cigarette smoking is associated with reduced pulmonary function and increased risk factors for cardiovascular disease. This randomized placebo-controlled double-blind study evaluated the effects of two different combinations of mixed fruit and vegetable juice powder concentrate (Juice Plus+, NSA, Collierville, TN) on heavy smokers.

Methods: At baseline (T_0) and after 3 months' supplementation (T_1), pulmonary function parameters and cardiovascular risk factors—that is, plasma total homocysteine (tHcy) with related B vitamins and cysteine (tCys) concentrations—were assessed in 75 apparently healthy smokers (aged 49.2 $\pm$ 10.6 years, >20 cigarettes/d, duration $\geq$10 years) randomized into 3 groups: placebo (P), fruit/vegetable (FV) and fruit/vegetable/berry (FVB).

Results: T_0: most smokers showed abnormalities in tHcy and tCys concentrations. T_1: respiratory function was unchanged in P and slightly, but not significantly, improved in FV, whereas FVB showed a significant improvement in forced expiratory flow at 25% (FEF_{25}; $p < 0.0001$ vs P and FV) and significant improvement in CO diffusion lung/alveolar volume (DLCO/VA). FV and FVB (50%) showed significant reduction in tHcy and tCys compared to T_0 ($p < 0.0001$) and P ($p < 0.0001$).

Conclusions: At T_1, both supplemented groups, but to a greater extent the FVB group, showed improvements in some pulmonary parameters, cardiovascular risk factors, and folate status. The beneficial effects of Juice Plus+ supplementation could potentially help smokers, even if smoking cessation is advisable.

INTRODUCTION

Smokers are self-exposed to inhaled toxic molecules contained in tobacco smoke [1]. Habitual smoking is independently associated with hyperhomocysteinemia [2] and oxidative stress [3] and is related to the progression of atherosclerotic lesions [2]. Moreover, a relationship between smoking and a high risk of developing reduced pulmonary function and other

Address correspondence to: Fabrizia Bamonti, Dipartimento Scienze Mediche, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, via F. Sforza 35, 20122 Milan, ITALY. E-mail: fabrizia.bamonti@unimi.it

The study was supported in part by a grant from NSA, LLC.

Abbreviations: BMI = body mass index, B_{12} = vitamin B_{12}, CBC = complete blood count, Cys = cysteine, DLCO/VA = CO diffusion lung per unit alveolar volume, EDTA = ethylenediaminetetraacetic acid, Ery-Fol = erythrocyte folate, FEF_{25} = forced expiratory flow at 25% of forced vital capacity, FEF_{50} = forced expiratory flow at 50% of forced vital capacity, FEF_{75} = forced expiratory flow at 75% of forced vital capacity, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, Hcy = homocysteine, HoloTC = holotranscobalamin, NAC = N-acetylcysteine, PEF = peak expiratory flow, RONS = reactive oxygen and nitrogen species, S-Fol = serum folate.

chronic obstructive pulmonary conditions has been reported [4].

Lung exposure to reactive oxygen and nitrogen species (RONS) production is greatly increased by smoking, with a resulting antioxidant imbalance, cellular biochemical changes, damage to lung parenchyma, and increasing need for antioxidant nutrients [5]. Airway epithelial mucus cell hyperplasia and decreased cilia and ciliary beat frequency precede changes in pulmonary function [6]. Tobacco smoking may influence plasma total homocysteine (tHcy) and total cysteine (tCys) concentrations [7, 8]; redox changes in these aminothiols may partially explain the adverse influence of tobacco on long-term health [9]. Hyperhomocysteinemia and associated changes in cysteine have been reported in patients with cardiovascular disease [10]. El-Khairy et al. demonstrated a U-shaped relationship between plasma tCys and cardiovascular disease (with the highest risk at the lowest and highest values and the desirable reference interval as mid-range of values) [11]. The interplay between different aminothiols may have an important role in protecting and repairing oxidative damage [12].

Smokers' lifestyles are different from nonsmokers' lifestyles in that smokers consume less fruit and vegetables and therefore could have lower antioxidant capacity from diet alone [2, 13]. Many fruit and vegetable components function as antioxidants and neutralize RONS [14]. Moreover, whole fruits and/or vegetables may be biologically more effective than synthetic compounds given as supplements [5].

If diet-derived antioxidants are protective against oxidative damage, smokers' low dietary intake of plant foods could contribute to suboptimal lung function and increase respiratory morbidity [13]. Because lasting dietary change has proven difficult to achieve, a supplement designed to provide natural nutrients from fruits and vegetables, such as Juice Plus+ (NSA, Collierville, TN) may help control RONS activity when added to the habitual diet of heavy smokers.

Juice Plus+ supplement, composed primarily of fruit and vegetable juice powder concentrate, contains several antioxidant compounds (vitamins C and E, folate, and flavonoids). Walda et al. [15] have shown the protective effects of fruit containing polyphenols and vitamin E against chronic inflammatory diseases. The beneficial effects of Juice Plus+ intervention were reported by Samman et al. [16] and Kiefer et al. [17] using different regimens (dosage, duration, subjects). Increased plasma levels of important antioxidant nutrients were found in two crossover trials: 2 periods of 6 weeks separated by a 3-week wash-out with smokers and nonsmokers [16] and a total period of 14 weeks (crossover week 7) with healthy men and women [17].

Moreover, as previously reported, Juice Plus+ supplement reduces tHcy levels [16, 18, 19] and oxidative stress markers [20–22] and increases beta-carotene [21] and folate concentrations [18].

The first Juice Plus+ commercial product, tested previously [16, 17, 20], was supplied by the factory with a newer berry product (Juice Plus+ Vineyard) and any added effects were thereafter investigated. In our first paper [20], we evaluated the beneficial effects of one month Juice Plus+ on light smokers; in our second study [23] we evaluated the effects of 3 months of Juice Plus+ alone or with added Juice Plus+ Vineyard compared to placebo on heavy smokers without known respiratory complications. In both studies we observed a significant decrease in plasma free malondialdehyde (f-MDA, an index of recent lipid peroxidation) concentrations using the gas chromatography–mass spectrometry reference method (with dideuterated MDA as an isotopic internal standard) [24]. After consumption of both Juice Plus+ formulations, Jin et al. [25] reported increased superoxide dismutase concentrations and reduced markers of chronic systemic inflammation in healthy adults.

In our second randomized double-blind placebo-controlled study, after 3 months' nutraceutical supplementation, we found a significant improvement in some oxidative alterations attributed to long-term cigarette smoking [23]. Therefore, the aim of this ancillary study was to evaluate the effects of 3 months' supplementation with the two different formulations (Juice Plus+ and Juice Plus+ Vineyard) on heavy smokers' pulmonary function and cardiovascular risk factors (i.e., plasma tHcy and tCys). Moreover, the status of the Hcy metabolically related vitamins—that is, serum and erythrocyte folate, vitamin B_{12}, and its biologically active form holotranscobalamin—was assessed.

METHODS

Subjects

Eligible participants were apparently healthy current heavy smokers who reported a smoking history of 20 or more cigarettes per day for at least 10 years without respiratory complications detected on spirometric examination. Exclusion criteria included a history of chronic or current health conditions or unstable psychiatric disorders and alcohol abuse, body mass index (BMI) $<$ 19 or BMI $>$ 25 kg/m^2, pregnancy, lactation, use of long term medication, or regular vitamin supplementation. During an initial screening visit, all participants provided written informed consent and were then interviewed about general health, habitual dietary intake, lifestyle, and smoking habits. This report is of a subpopulation of the most compliant smokers reported in our previous study [23] (75 volunteers: 46 men, 29 women, mean age 49.2 $\pm$ 10.6 years). They were recruited at two Milan hospitals (Dipartimento Pneumologia, Ospedale Niguarda and Clinica del Lavoro "Devoto", Ospedale Maggiore Policlinico). Eligibility was evaluated by standard routine examinations including complete blood count (CBC) and lipid panel. This study was conducted according to the Declaration of Helsinki guidelines for Research on Human Subjects and was approved by both Human Ethic Committees of the "San Giuseppe e Sacra Famiglia" Ospedale, Erba, Italy (Registration number 27/05/CE/smc), and

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy (Registration number 2552). All participants were asked to maintain the same lifestyle, diet, and daily smoking habits for the 3-month study period.

Study Design

As described previously [23], subjects were randomly divided into 3 groups. In this double-blind placebo-controlled ancillary study the number of subjects was uneven: some initially enrolled and then chose not to participate and therefore were not included in the analysis.

- Placebo (P: 25 subjects, 15 men, 10 women, mean age 51.4 $\pm$ 12 years)
- Blended fruit and vegetable juice concentrate powder (FV: 26 subjects, 16 men, 10 women, mean age 46.6 $\pm$ 7.9 years)
- FV as described above with additional grape and berry juice concentrate powder ingredients (FVB: 24 subjects, 15 men, 9 women, mean age 49.9 $\pm$ 11.4 years)

The FV capsules contained primarily fruit and vegetable juice powder concentrate from apple, beet, broccoli, cabbage, carrot, cherry (acerola), cranberry, kale, orange, peach, papaya, parsley, pineapple, spinach, tomato and provided 7.5 mg beta-carotene, 234 mg vitamin C, 32 mg vitamin E, and 420 μg folate (42 kJ/day) [21, 22]. The FVB capsules additionally contained berry juice powder from bilberry, blackberry, black currant, blueberry, cranberry, elderberry, grape (Concord), raspberry, red currant and provided 7.5 mg beta-carotene, 200 mg vitamin C, 60 mg vitamin E, and 600 μg folate (63 kJ/day) [22]. All subjects were instructed to take their assigned capsules twice daily with meals (3 in the morning and 3 in the evening) for 3 months while following their habitual diet and lifestyle.

These capsules contained all placebo powder; a blend of the equivalent of 2 fruit capsules, 2 vegetable capsules, and 2 placebo capsules; or a blend equivalent to 2 fruit, 2 vegetable, and 2 berry capsules. To keep the study blinded, the placebo capsules were identical in appearance and contained primarily microcrystalline cellulose. Capsules were provided in opaque gelatin shells by the study sponsor and packaged in identical and unlabeled bottles. All of the capsules were tested for accurate potency by capsule assignment group. All capsules were replaced with a fresh supply prior to the 2-year expiration date. The sealed bottles of capsules were marked with assigned random numbers at the factory. All subjects regularly consumed a Mediterranean diet. Dietary intake assessment was checked at baseline (T_0) using the "Nutrition Status Assessment Score" questionnaire [26], which listed a number of items, such as number of cigarettes, use of alcohol, physical activity, and intake of ordinary Italian foods [23, 26]. The protocol directions were assessed by all participants by a daily diary returned to clinicians after 3 months' supplementation (T_1).

Compliance with lifestyle and study protocol was checked by weekly phone calls and by counting returned capsules at T_1.

Spirometric Parameters

At T_0 and at T_1 pulmonary function was measured with an electronic flow volume spirometer V-max 22 with Autobox (SensorMedics, Milan, Italy) according to European Respiratory Society/American Thoracic Society guidelines (ERS ATS 2005) [27]. The following spirometry indexes were measured: forced expiratory volume in 1 second (FEV_1); forced expiratory flow at 25%, 50%, 75% forced vital capacity (FEF_{25}, FEF_{50}, FEF_{75}); CO diffusion lung per unit of alveolar volume (DLCO/VA), forced vital capacity (FVC); all parameters are reported as Actual/Expected %. The actual Tiffeneau index is the FEV_1/FVC ratio, where FVC is the maximum amount of air anyone can expel from the lungs after maximum inspiration. According to Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines [28], subjects were not eligible for this study if their actual Tiffeneau index was less than 0.7 and/or less than 88% of expected value (expected normal values are calculated by spirometer, accounting for several factors; i.e., age, sex, height, weight, ethnicity). The use of the Tiffeneau index is recommended by GOLD guidelines [28] as an early sensitive indicator of airway obstruction and helps to differentiate air flow limitations from restrictive abnormalities. All subjects were without respiratory complications and had at least 3 consecutive forced expiratory tests (5% intra-individual variation among the 3 values is accepted): the best peak expiratory flow was chosen as each subject's personal index according to ERS ATS 2005 guidelines [27]. The highest peak expiratory flow value is indicative of the highest forced expiration. Smoking status was not assessed by measuring urinary cotinine concentrations at T_0 and T_1 because several factors affecting the cotinine results of the smokers might lead to inaccurate evaluations, especially when the assessment of personal exposure to tobacco smoke is based on self-reports.

Blood Samples

At T_0 and T_1, blood specimens from fasting subjects were collected in light-protected tubes, either without additives for serum folate (S-Fol), vitamin B_{12} (B_{12}), and holotranscobalamin (HoloTC) measurements or with EDTA to prevent coagulation for CBC, erythrocyte folate (Ery-Fol), and tHcy and tCys analysis. A specimen of whole blood with EDTA was immediately centrifuged for total Hcy and Cys measurement, aliquoted, and immediately frozen on liquid nitrogen and stored at -80°C. T_0 and T_1 serum and whole blood samples were frozen and stored at -80°C for batch analysis at the end of the study.

Analytical and Biochemical Analysis

The CBC was performed as routine samples at the study hospitals. Serum B_{12} and HoloTC concentrations were determined

Table 1. Demographic and Baseline (T_0) Biochemical Characteristics of 75 Compliant Smokers

	Reference Interval or Cut-Off Values	P Group ($n = 25$)	FV Group ($n = 26$)	FVB Group ($n = 24$)	p
Men/women	—	15/10	16/10	15/9	NS
Age (years)	—	51.4 ± 12	46.6 ± 7.9	49.9 ± 11.4	NS
Number of cigarettes (daily)	—	31.2 ± 15.3	25.9 ± 8.7	25 ± 12	NS
Duration of smoking (years)	—	24.4 ± 3.9	23.5 ± 2.4	25.7 ± 11.1	NS
Analyte					
BMI	(18–25 Kg/m^2)	23.0 ± 3.2	23.3 ± 1.9	22.7 ± 2.5	NS
WBC	(4–10 109/L)	7.9 ± 1.2	8.9 ± 2.5	8.2 ± 2.3	NS
RBC	(4.1–5.1 1012/L)	4.9 ± 0.5	5.2 ± 0.8	4.9 ± 0.5	NS
Hb	(12–16 g/dL)	15.1 ± 1.4	14.8 ± 1.9	15.0 ± 1.8	NS
MCV	(78–99 fL)	87.6 ± 19.4	87.0 ± 9.6	91.7 ± 6.9	NS
tHcy	(<10 μmol/L)	11.5 ± 6.6 (52%)	12.3 ± 7.8 (57%)	10.8 ± 7.4 (38%)	NS
tCys	(250–275 μmol/L)	280 ± 63.8 (76%)	245.3 ± 65.1 (92%)	247.9 ± 75.9 (76%)	NS
S-Fol	(7–28 nmol/L)	13.9 ± 5.4 (0%)	13.7 ± 6.7 (1%)	15.7 ± 7.7 (3.1%)	NS
Ery-Fol	(421–1462 mol/L)	471.2 ± 117.2 (33%)	534.5 ± 170.1 (35%)	603.5 ± 341.6 (25%)	NS
Vitamin B_{12}	(164–835 pmol/L)	269 ± 85.4 (3%)	281 ± 84.6 (8%)	289 ± 95.1 (7%)	NS
HoloTC	(>40 pmol/L)	60.2 ± 23.0 (22%)	63.9 ± 18.9 (15%)	64.1 ± 23.1 (10%)	NS

Data are expressed as mean ± SD. (%) = % of subjects with values outside of the reference interval or cutoff.
P = placebo, FV = fruit/vegetable, FVB = fruit/vegetable/berry, NS = nonsignificant, BMI = body mass index, WBC = white blood cells, RBC = red blood cells, Hb = hemoglobin, MCV = mean corpuscular volume, tHcy = total homocysteine, tCys = total cysteine, S-Fol = serum folate, Ery-Fol = erythrocyte folate, HoloTC = holotranscobalamin.

by commercial immunoenzymatic assays (AxSYM B_{12} and AxSYM Active-B_{12}, respectively) on the automated AxSYM analyzer (Abbott Diagnostics, Abbott Park, IL), as previously reported [29, 30]. S-Fol and Ery-Fol concentrations were determined by commercial immunoenzymatic assay (AxSYM Folate) as previously described [31]. Plasma tHcy and tCys concentrations were measured using high-performance liquid chromatography with fluorescence detection (ProStar, Varian, Surrey, UK) as previously reported [32]. Briefly, EDTA plasma (100 μL) was treated with a 10% tri-*n*-butylphosphine in dimethylformamide solution (10 μL) for 30 minutes at 4°C in order to reduce the oxidized thiols and release them from plasma proteins. One hundred microliters of 10% trichloroacetic acid containing 1 mmol/L EDTA was added, mixed, and centrifuged (10,000 *g*, 2 minutes) to remove precipitated proteins. The clear supernatant (100 μL) was mixed with dimethylformamide (5 μL), 1 mol/L borate buffer (100 μL, pH 11) containing 4 mmol/L EDTA, 1.55 mol/L NaOH (10 μL), and ammonium-7-fluoro-benzo-2-oxa-1,3-diazole-4-sulfonate (10 μL) as a derivatizing reagent. The mixture was incubated for 60 minutes at 60°C before high-performance liquid chromatography analysis. Both tHcy and tCys concentrations include oxidized (disulfides), conjugated (protein-bound), and reduced free Hcy and Cys.

Statistical Analysis

Continuous variables are reported as mean ± SD. The differences between groups were evaluated by *t* test for independent samples and by analysis of variance. Follow-up differences in each group were evaluated by *t* test for paired samples. Statistical significance was assigned as a p value < 0.05. Statistical analyses were performed using R statistical Software (Revolution Analytics, Palo Alto, CA).

RESULTS

This ancillary project included 75 participants (46 men, 29 women, mean age 49.2 ± 10.6) who were >95% protocol compliant and completed the 3-month study. As shown in Table 1, at baseline no statistical differences in demographic and biochemical characteristics were found between the 3 randomized groups. Women had approximately the same mean age as men (49.0 ± 10.3 vs 49.4 ± 11.0 years, respectively; p = NS) but a significantly lower BMI (22.0 ± 3.0 vs 24.0 ± 1.5 kg/m^2, respectively; $p = 0.001$) than men. At baseline, all groups had a normal hematological status and similar levels of tHcy and tCys. However, on average 50% of smokers had mild hyperhomocysteinemia and most subjects had tCys concentrations either above or below the reference interval. With regard to folate, on average 31% of subjects had Ery-Fol concentrations below the reference interval, although few subjects had S-Fol levels below the reference interval. With regard to B_{12} status, on average 6% of subjects' B_{12} concentrations were below the reference interval, and on average 15% of subjects' HoloTC levels were below the cutoff. As shown in Table 2, at baseline there were no significant differences in respiratory parameters between the 3 randomized groups. After 3 months' supplementation (Table 3), the FV group's FEF_{25} differences between T_1 and T_0 values (expressed as mean delta) improved slightly compared to the P group's, whereas the FVB group's FEF_{25} differences improved significantly compared to the P group ($p < 0.0001$) and the FV

Table 2. Baseline (T_0) Pulmonary Parameters of 75 Compliant Smokers

Pulmonary function	P group ($n = 25$)	FV group ($n = 26$)	FVB group ($n = 24$)	p
FEV_1 (%)	98.2 ± 12.3	102.2 ± 13.2	99.2 ± 14.0	NS
FEF_{25} (%)	96.4 ± 20.2	104.4 ± 26.1	97.1 ± 29.4	NS
DLCO/VA (%)	79.5 ± 14.3	82.0 ± 18.1	75.4 ± 10.7	NS
FVC (%)	102 ± 24.9	109 ± 16.2	106.2 ± 12.6	NS
Tiffeneau index (%)	91.7 ± 9.1	95.2 ± 7.3	93 ± 9	NS

Data are expressed as mean ± SD and refer to Actual/Expected %. P = placebo, FV = fruit/vegetable, FVB = fruit/vegetable/berry, NS = nonsignificant, FEV_1 = forced expiratory volume in 1 second, FEF_{25} = forced expiratory flow at 25% of forced vital capacity, DLCO/VA = CO diffusion lung per unit alveolar volume, FVC = forced volume capacity, Tiffeneau Index (FEV_1/FVC)% = index of air-flow obstruction.

group ($p < 0.001$). The FV group's ($p < 0.05$) and FVB group's ($p < 0.001$) DLCO/VA values improved significantly compared to the P group's. Both supplemented groups' FEV_1 mean delta values improved, even if not significantly so, whereas the P group's FEV_1 values were negative. By comparison, the P group's respiratory function deteriorated slightly. As shown in Table 3, both FV and FVB groups', but not P group's, tHcy levels decreased significantly ($p < 0.0001$ vs T_0 and $p < 0.001$ vs P). Both FV and FVB groups', but not P group's, tCys concentrations improved significantly. On average, 50% of supplemented smokers' tCys concentrations normalized ($p < 0.0001$ vs P). At T_1 both nutraceutical groups' S-Fol and Ery-Fol concentrations increased significantly, whereas, as expected, vitamin B_{12} and HoloTC levels were unchanged (data not shown). Analysis of variance, used to compare the differences between groups, showed the same p values as the t test.

DISCUSSION

After only 3 months' supplementation with two different formulations of encapsulated fruit and vegetable juice powder concentrate, heavy smokers without existing respiratory complications showed significant improvement in some respiratory function markers and in folate status, in addition to normalized values of the cardiovascular risk factors (i.e., plasma tHcy and tCys). Placebo subjects did not show any change from baseline values. Smokers' smallest airways are the first impacted; inflammation at first can reduce respiratory function and then lead to chronic obstructive pulmonary changes [6, 33]. FEV_1, an index predominantly related to the function of middle size and larger airways, has been reported to take one year to develop (depending on factors such as intensity and duration of cigarette smoking) and is indicative of deep pulmonary exposure and damage

Table 3. Pulmonary and Biochemical Parameters of 75 Compliant Smokers at the End of the Study (T_1) and Differences (Mean Δ) between T_1 and T_0 Values after 3 Months' Supplementation

	P group ($n = 25$)		FV group ($n = 26$)		FVB group ($n = 24$)	
Parameter	T_1	T_1-T_0	T_1	T_1-T_0	T_1	T_1-T_0
FEV_1 (%)	97.7 ± 17.1	−0.67 ± 3.46	102.8 ± 13.2	0.41 ± 4.66	99.4 ± 12.1	0.2 ± 4.15
FEF_{25} (%)	89.5 ± 10.9	−6.50 ± 4.2	101.1 ± 15.2	−3.2 ± 5.6$^{\#}$	98.6 ± 4.9	1.9 ± 3.6$^{\blacksquare}$
DLCO/VA (%)	75.2 ± 8.62	−5.22 ± 12.9	80.3 ± 2.4*	0.46 ± 3.10*	80.9 ± 1.79*	5.1 ± 4.96$^{\blacktriangle}$
FVC (%)	101.6 ± 19.3	−0.7 ± 8.4	110 ± 13.4	1.1 ± 5.8	107.1 ± 11.3	1.2 ± 6.7
Tiffeneau index (%)	89.3 ± 17.3	−2.17 ± 10.2	96.2 ± 13.1	0.9 ± 7.4	93.8 ± 12.4	0.6 ± 6.2
tHcy (μmol/L)	11.4 ± 5.9	−0.07 ± 1.5 (48%)	9.4 ± 6.3$^{\blacktriangle}$	−2.8 ± 2.3$^{\blacksquare}$ (26%)	8.1 ± 3.5$^{\blacktriangle}$	−2.9 ± 5.4$^{\blacksquare}$ (24%)
tCys (μmol/L)	244.9 ± 56.7)	−39.5 ± 36.2 (66%)	260.6 ± 40.5	16.2 ± 38.4$^{\blacksquare}$ (32%)	258.3 ± 62.8	11.3 ± 42.3$^{\blacksquare}$ (29%)
S-Fol (nmol/L)	16.0 ± 5.6	2.1 ± 5.6 (4%)	27.8 ± 8.3$^{\blacktriangle}$	14.1 ± 8.6$^{\blacktriangle}$ (0%)	31.8 ± 8.7$^{\blacktriangle}$	16.7 ± 8.8$^{\blacktriangle}$ (0%)
Ery-Fol (nmol/L)	625 ± 107	153 ± 112 (4%) (4%)	1062 ± 331$^{\blacktriangle}$	527 ± 356$^{\blacksquare}$ (0%) (0%)	1199 ± 395$^{\blacktriangle}$	622 ± 321$^{\blacksquare}$ (0%) (0%)

Data are expressed as mean ± SD and refer to Actual/Expected %. (%) = Percentage of subjects with values out of reference interval or cutoff.

*$p < 0.05$ vs P, $^{\blacktriangle}p < 0.001$ vs P, $^{\blacksquare}p < 0.0001$ vs P, $^{\#}p < 0.001$ vs FVB.

P = placebo, FV = fruit/vegetable, FVB = fruit/vegetable/berry, FEV_1 = forced expiratory volume in 1 second, FEF_{25} = forced expiratory flow at 25% of forced vital capacity, DLCO/VA = CO diffusion lung per unit alveolar volume, FVC = forced volume capacity, tHcy = total homocysteine, tCys = total cysteine, S-Fol = serum folate, Ery-Fol = erythrocyte folate, Tiffeneau Index (FEV_1/FVC)% = index of air-flow obstruction.

[6, 34]. In contrast, changes in FEF_{25} (an index predominantly related to small size pulmonary airways) can reveal an early bronchiolar damage and may improve faster than FEV_1. The FEV_1/FVC ratio, also called the Tiffeneau index, is a calculated ratio used in the diagnosis of obstructive and lung disease [35]. The significant increase in the DLCO/VA value may be due to a reduction of bronchiolar wall inflammation status and/or blood microcirculation leading to the increased FEF_{25} index. In our subjects, not all pulmonary markers improved after the interventions. In fact, FEV_1 and the Tiffeneau index did not significantly improve in either phytonutrient group, whereas FEF_{25} and DLCO/VA significantly improved in both of these groups and to a greater degree in the FVB group. This may be an indicative result of increased dietary antioxidants provided by the FV and (even more) the FVB capsules during the relatively short study period. The placebo group did not show any improvement. Cigarette smoke is known to induce mucus hypersecretion and nonproductive cough. Interestingly, at T_1, an increase in productive morning cough was subjectively reported by 19 FV and FVB subjects. The reduction of mucus viscosity and the widening of the smaller airways (documented by improved FEF_{25}) may explain increased expectoration and hence increased productive cough. No difference in respiratory function was observed in the P group but 50% showed worsening respiratory function, possibly due to seasonal factors (i.e., bronchitis, allergies). Our findings were in agreement with the study by Roll et al., which reported a reduction in common cold symptoms using the same FV supplement for 8 months, including a 2-month run-in period [36]. The presence of biologically important thiols has been reported in several fruits and vegetables, with content depending on various storage and cooking conditions [37]. Bioflavonoids have both antioxidant and anti-inflammatory properties and may also contribute to the smokers' improved respiratory function. Flavonoids have reported anti-inflammatory effects attributed to inhibition of lipoxygenase and cyclooxigenase, which are involved in the formation of pro-inflammatory factors (such as prostaglandins and leukotrienes), leading to pulmonary pathogenesis [38]. A study by Tabak et al. showed a positive association between flavonoid intake and FEV_1 increase [39]. Our results are in agreement with the study by Walda et al., which reported the protective effect of fruit containing polyphenols and vitamin E against chronic inflammatory diseases [15], and the study by Jin et al., which showed reduced markers of chronic systemic inflammation in healthy adults taking the same FV and FVB encapsulated juice concentrate [25]. Moreover, the improvement in respiratory functions due to the antioxidant properties of the FV and FVB capsules can be related to both the decrease in plasma free malondialdehyde (an indicator of recent lipid peroxidation) and reduced oxidized low-density lipoproteins, as we have previously reported on these same heavy smokers [23].

Although following a Mediterranean diet, some subjects had a suboptimal baseline HoloTC and Ery-Fol status, in agreement with Tungtrongchitr et al. [40]. At baseline, on average 15% of subjects had low levels of HoloTC (Table 1). The determination of cobalamin status by measuring the HoloTC concentrations represents a new approach for diagnosing subtle cobalamin deficiency. HoloTC, the biologically active form of vitamin B_{12}, is considered the earliest and most sensitive marker of vitamin B_{12} deficiency [30]. Adequate folate and vitamin B_{12} levels are necessary for cellular metabolism, whereas insufficiency is associated with several disorders [41–43]. Ery-Fol concentration is a reliable indicator of long-term folate status and general dietary intake, and S-Fol indicates more recent intake [44]. A more comprehensive estimate of total folate status is provided by assessing both parameters, as we did in this and previous studies [44, 45]. Several mechanisms (decreased dietary intake, reduced absorption, diminished hepatic uptake, increased urinary excretion) may explain folate deficiency in smokers [40]. However, in agreement with the European Concerted Action Project, a possible interaction between chemical components of cigarette smoke and folate coenzymes [46] could lower folate status and promote hyperhomocysteinemia [40], increasing the risk of cardiovascular diseases [47]. In this study, though almost all the smokers had adequate S-Fol levels, about 30% of subjects had baseline suboptimal Ery-Fol concentrations (Table 1). After 3 months of either FV or FVB supplementation, S-Fol and Ery-Fol levels increased significantly ($p < 0.001$ and $p < 0.0001$, respectively) compared to subjects in the P group and, interestingly, the majority of Ery-Fol values were within the reference interval. This finding is in agreement with previous studies using the FV capsules [16, 18, 19]. Both formulations (Juice Plus+ and Juice Plus+ Vineyard) only influenced folate status, as expected. With regards to tHcy and tCys levels, at baseline more than 50% of our smokers suffered from mild hyperhomocysteinemia and most of smokers showed tCys levels outside the reference interval. After 3 months' supplementation, only FV and FVB groups' total homocysteine levels decreased significantly ($p < 0.0001$), and tCys levels normalized (reference interval, 250–275 μmol/L) in a higher percentage of smokers compared to baseline. According to other authors [10, 11], tHcy and tCys levels could be considered intercorrelated cardiovascular risk factors. Our findings support these hypotheses because, after a 3-month supplementation, most of the smokers in this study who were assigned the FV and FVB capsules showed an improvement in respiratory function and in both tHcy and tCys concentrations. Sobczak et al. [7] evaluated the influence of smoking on plasma tHcy and tCys levels and reported that this is a strong determinant only of plasma tHcy but not tCys levels.

Cysteine is thought to be the limiting amino acid for glutathione synthesis. Glutathione is an important component of the endogenous antioxidant system. We speculate that the improved cysteine levels may help explain the mucolitic effect reported by 19 of the FV and FVB subjects. In fact, N-acetylcysteine is a glutathione precursor and a mucolytic compound with antioxidant and anti-inflammatory properties. These N-acetylcysteine

mucolytic properties are due to disruption of disulfide bridges in mucoprotein macromolecules, thus decreasing mucus viscosity, and its biotransformation to cysteine is better absorbed when mixed with vitamins and flavonoids [48]. This is an interesting hypothesis; however, our study did not deal with the potential underlying mechanisms due to the number of components contained in fruit and vegetables. Additional research is necessary to confirm these findings.

In conclusion, the pulmonary function and biochemical parameters of the healthy heavy smokers assigned to the FV and FVB capsules seemed to have beneficial effects from the nutraceutical treatment. It appears that there was a partial reduction in some of the damaging effects of smoking cigarettes. These findings could be related to the significant improvement in some oxidative alterations previously reported [23] and could suggest a potential use of nutraceutical treatment to reduce some smoking-related complications.

Finally, even if the beneficial effects of intervention with nutraceutical formulations could encourage further investigations, this cannot substitute for smoking cessation.

ACKNOWLEDGMENTS

The sponsor was involved in discussions with all of the authors regarding the study design and provided all of the study capsules but did not have access to any data and was not involved in the interpretation of the results. The authors thank all of the volunteers for their participation and compliance. The authors are also grateful to Mary Coduri for linguistic consultation and to Anita Boddie and Elisabetta Scurati Manzoni for scientific and linguistic contributions.

REFERENCES

1. Carnevali S, Petruzzelli S, Longoni B, Vanacore R, Barale R, Cipollini M, Scatena F, Paggiaro P, Celi A, Giuntini C: Cigarette smoke extract induces oxidative stress and apoptosis in human lung fibroblasts. Am J Physiol Lung Cell 284:L955–L963, 2003.
2. Okumura K, Tsukamoto H: Folate in smokers. Clin Chim Acta 412:521–526, 2011.
3. Chavez J, Cano C, Souki A Bermúdez V, Medina M, Ciszek A, Amell A, Vargas ME, Reyna N, Toledo A, Cano R, Suárez G, Contreras F, Israili ZH, Hernández-Hernández R, Valasco M: Effect of cigarette smoking on the oxidant/antioxidant balance in healthy subjects. Am J Ther 14:189–193, 2007.
4. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF: Systemic effects of smoking. Chest 131:1557–1566, 2007.
5. Kelly G: The interaction of cigarette smoking and antioxidants. Part I: Diet and carotenoids. Altern Med Rev 7:370–388, 2002.
6. Unverdorben M, Mostert A, Munjal S, van der Bijl A, Potgieter L, Venter C, Liang Q, Meyer B, Roethig HJ: Acute effects of cigarette smoking on pulmonary function. Regul Toxicol Pharmacol 57:241–246, 2010.
7. Sobczak A, Wardas W, Zielinska-Danch W, Pawlicki K: The influence of smoking on plasma homocysteine and cysteine levels in passive and active smokers. Clin Chem Lab Med 42:408–414, 2004.
8. Cintra F, Tufik S, D'Almeida V, Calegare BF, de Paola A, Oliveira W, Rizzi C, Roizenblatt S, Poyares D: Cysteine: A potential biomarker for obstructive sleep apnea. Chest 139:246–252, 2011.
9. Pryor WA, Stone K: Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate and peroxynitrite. Ann N Y Acad Sci 686:12–29, 1993.
10. Ozkan Y, Ozkan E, Simsek B: Plasma total homocysteine and cysteine as cardiovascular risk factors in coronary heart disease. Int J Cardiol 82:269–277, 2002.
11. El-Khairy L, Ueland PM, Refsum H, Graham IM, Vollset SE: European Concerted Action Project: Plasma total cysteine as risk factor for vascular disease. Circulation 103:2544–2549, 2001.
12. Mansoor MA, Svardal AM, Ueland PM: Determination of the in vivo redox status of cysteine cysteinylglycine homocysteine and glutathione in human plasma. Anal Biochem 200:218–229, 1992.
13. Palaniappan U, Jacobs Starkey L, O'Loughlin J, Gray-Donald K: Fruit and vegetable consumption is lower and saturated fat intake is higher among Canadians reporting smoking. J Nutr 131:1952–1958, 2001.
14. Chang JL, Chen G, Ulrich CM, Bigler J, King IB, Schwarz Y, Li S, Li L, Potter JD, Lampe JW: DNA damage and repair: Fruit and vegetable effects in a feeding trial. Nutr Cancer 62:329–335, 2010.
15. Walda IC, Tabak C, Smit HA, Räsänen L, Fidanza F, Menotti A, Nissinen A, Feskens EJ, Kromhout D: Diet and 20-year chronic obstructive pulmonary disease mortality in middle-aged men from three European countries. Eur J Clin Nutr 56:638–643, 2002.
16. Samman S, Sivarajah G, Man JC, Ahmad ZI, Petocz P, Caterson ID: A mixed fruit and vegetable concentrate increases plasma antioxidant vitamins and folate and lowers plasma homocysteine in men. J Nutr 133:2188–2193, 2003.
17. Kiefer I, Prock P, Lawrence C, Wise J, Bieger W, Bayer P, Rathmanner T, Kunze M, Rieder A: Supplementation with mixed fruit and vegetable juice concentrates increased serum antioxidants and folate in healthy adults. J Am Coll Nutr 23:205–211, 2004.
18. Kawashima A, Madarame T, Koike H, Komatsu Y, Wise JA: Four week supplementation with mixed fruit and vegetable juice concentrates increased protective serum antioxidants and folate and decreased plasma homocysteine in Japanese subjects. Asia Pac J Clin Nutr 16:411–421, 2007.
19. Panunzio MF, Pisano A, Antoniciello A, Di Martino V, Frisoli L, Cipriani V, Mongelli MA, Bronzetti G: Supplementation with fruit and vegetable concentrate decrease plasma homocystine levels in a dietary controlled trial. Nutr Res 23:1221–1228, 2003.
20. Bamonti F, Novembrino C, Ippolito S, Soresi E, Ciani A, Lonati S, Scurati-Manzoni E, Cighetti G: Increased free malondialdehyde concentrations in smokers normalise with a mixed fruit and vegetable juice concentrate: A pilot study. Clin Chem Lab Med 44:391–395, 2006.
21. Nantz MP, Rowe CA, Nieves CJ, Percival SS: Immunity and antioxidant capacity in humans is enhanced by consumption of a dried, encapsulated fruit and vegetable juice concentrate. J Nutr 136:2606–2610, 2006.

22. Lamprecht M, Oettl K, Schwaberger G, Hofmann P, Greilberger JF: Several indicators of oxidative stress, immunity, and illness improved in trained men consuming an encapsulated juice powder concentrate for 28 weeks. J Nutr 137:2737–2741, 2007.
23. Novembrino C, Cighetti G, De Giuseppe R, Vigna L, de Liso F, Pellegatta M, Gregori D, Maiavacca R, Bamonti F: Effects of encapsulated fruit and vegetable juice powder concentrates on oxidative status in heavy smokers. J Am Coll Nutr 30:49–56, 2011.
24. Cighetti G, Debiasi S, Paroni R, Allevi P: Free and total malondialdehyde assessment in biological matrices by gas chromatography–mass spectrometry: What is needed for an accurate detection. Anal Biochem 266:222–229, 1999.
25. Jin Y, Cui X, Singh UP, Chumanevich AA, Harmon B, Cavicchia P, Hofseth AB, Kotakadi V, Stroud B, Volate SR, Hurley TG, Hebert JR, Hofseth LJ: Systemic inflammatory load in humans is suppressed by consumption of two formulations of dried, encapsulated juice concentrate. Mol Nutr Food Res 54:1–9, 2010.
26. Calvelli L, Accinni R, Gregori D, Della Noce C, Bamonti F, Vigna L, Schiraldi G, Novembrino C, Cossovich A, Chiericozzi M, Scala E, Allegra L: A quick and effective questionnaire to value dietary intakes and oxidative stress related to food in pneumological patients. Progr Nutr 13:55–64, 2011.
27. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J: Standardisation of spirometry. Eur Respir J 26:319–338, 2005.
28. Franssen FME, Spruit MA, Wouters EFM: Determinants of polypharmacy and compliance with GOLD guidelines in patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 6:493–501, 2011.
29. Brady J, Wilson L, McGregor L, Valente E, Orning L: Active B_{12}: A rapid, automated assay for holotranscobalamin on the Abbott AxSYM analyzer. Clin Chem 54:567–573, 2008.
30. Bamonti F, Moscato GA, Novembrino C, Gregori D, Novi C, De Giuseppe R, Galli C, Uva V, Lonati S, Maiavacca R: Determination of serum holotranscobalamin concentrations with the AxSYM active B_{12} assay: Cut-off point evaluation in the clinical laboratory. Clin Chem Lab Med 48:249–253, 2010.
31. De Vecchi AF, Novembrino C, Patrosso C, Cresseri D, Ippolito S, Rosina M, Colucci P, Lando G, Bamonti-Catena F: Effect of incremental doses of folate on homocysteine and metabolically related vitamin concentrations in nondiabetic patients on peritoneal dialysis. ASAIO J 49:655–659, 2003.
32. Accinni R, Parodi O: Determination of plasma homocysteine. In Drew AF (ed): "Atherosclerosis: Experimental Methods and Protocols, Vol. 52." Totowa: Humana Press, pp 77–103, 2001.
33. Barnes PJ: Chronic obstructive pulmonary disease. N Engl J Med 343:269–280, 2000.
34. Simmons MS, Connett JE, Nides MA, Lindgren PG, Kleerup EC, Murray RP, Bjornson WM, Tashkin DP: Smoking reduction and the rate of decline in FEV1: results from the lung health study. Eur Respir J 25:1011–1017, 2005.
35. Swanney MP, Ruppel G, Enright PL, Pedersen OF, Crapo RO, Miller MR, Jensen RL, Falaschetti E, Schouten JP, Hankinson JL, Stocks J, Quanjer PH: Using the lower limit of normal for the FEV1/FVC ratio reduces the misclassification of airway obstruction. Thorax 63:1046–1051, 2008.
36. Roll S, Noco M, Willich N: 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, 2011.
37. Demirkol O, Adams C, Ercal N: Biologically important thiols in various vegetables and fruits. J Agric Food Chem 52:8151–8154, 2004.
38. Laughton MJ, Evans PJ, Moroney MA, Hoult JRS, Halliwell B: Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives. Biochem Pharmacol 42:1673–1681, 1991.
39. Tabak C, Arts ICW, Smit HA, Heederik D, Kromhout D: Chronic obstructive pulmonary disease and intake of catechins, flavonols, and flavones. Am J Respir Crit Care Med 164:61–64, 2001.
40. Tungtrongchitr R, Pongpaew P, Soonthornruengyot M, Viroonudomphol D, Vudhivai N, Tungtrongchitr A, Phonrat B, Pooudong S, Schelp FP: Relationship of tobacco smoking with serum vitamin B12, folic acid and haematological indices in healthy adults. Public Health Nutr 6:675–681, 2003.
41. Valera-Moreiras G, Murphy MM, Scott JM: Cobalamin, folic acid, and homocysteine. Nutr Rev 67:S69–S72, 2009.
42. Pitkin RM: Folate and neural tube defects. Am J Clin Nutr 85:285S–288S, 2007.
43. Kronenberg G, Colla M, Endres M: Folic acid, neurodegenerative and neuropsychiatric disease. Curr Mol Med 9:315–323, 2009.
44. Bamonti-Catena F, Buccianti G, Porcella A, Valenti G, Como G, Finazzi S, Maiolo AT: Folate measurements in patients on regular hemodialysis treatment. Am J Kidney Dis 33:492–497, 1999.
45. De Vecchi AF, Bamonti-Catena F, Finazzi S, Campolo J, Taioli E, Novembrino C, Colucci P, Accinni R, De Franceschi M, Fasano MA, Maiolo AT: Homocysteine, vitamin B12, serum and erythrocyte folate in peritoneal dialysis and hemodialysis patients. Perit Dial Int 20:169–173, 2000.
46. Abu Khaled M, Watkins CL, Krumdieck CL: Inactivation of B_{12} and folate coenzymes by butyl nitrite as observed by NMR: Implications on one-carbon transfer mechanism. Biochem Biophys Res Commun 135:201–207, 1986.
47. O'Callaghan P, Meleady R, Fitzgerald T, Graham I: European COMAC group. Smoking and plasma homocysteine. Eur Heart J 23:1580–1586, 2002.
48. Dekhuijzen PNR: Antioxidant properties of N-acetylcysteine: Their relevance in relation to chronic obstructive pulmonary disease. Eur Respir J 23:629–636, 2004.

Received February 20, 2012; revision accepted October 21, 2012.