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

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

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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 (T0) and after 3 months’ supplementation (T1), 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 ± 10.6 years, >20 cigarettes/d, duration ≥10 years) randomized into 3 groups: placebo (P), fruit/vegetable (FV) and fruit/vegetable/berry (FVB).

Results: T0: most smokers showed abnormalities in tHcy and tCys concentrations. T1: 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% (FEF25; p < 0.0001 vs P and FV) and significant improvement in CO diffusion lung/alveolar volume (DLCO/V A). FV and FVB (50%) showed significant reduction in tHcy and tCys compared to T0 (p < 0.0001) and P (p < 0.0001).

Conclusions: At T1, 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
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-Khairly 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 foods 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, Juicy 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 deuterated 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 B12, 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/m², 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 ± 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...
Nutraceutics’ Effects on Cigarette Smoking Damage

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 ± 12 years)
- Blended fruit and vegetable juice concentrate powder (FV: 26 subjects, 16 men, 10 women, mean age 46.6 ± 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 ± 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 (T0) 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 (T1).

Compliance with lifestyle and study protocol was checked by weekly phone calls and by counting returned capsules at T1.

Spirometric Parameters

At T0 and at T1 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 (FEV1); forced expiratory flow at 25%, 50%, 75% forced vital capacity (FEF25, FEF50, FEF75); 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 FEV1/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 T0 and T1 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 T0 and T1, blood specimens from fasting subjects were collected in light-protected tubes, either without additives for serum folate (S-Fol), vitamin B12 (B12), 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. T0 and T1 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 B12 and HoloTC concentrations were determined
Nutraceutics’ Effects on Cigarette Smoking Damage

Table 1. Demographic and Baseline (T₀) Biochemical Characteristics of 75 Compliant Smokers

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Reference Interval or Cut-Off Values</th>
<th>P Group (n = 25)</th>
<th>FV Group (n = 26)</th>
<th>FVB Group (n = 24)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men/women</td>
<td>—</td>
<td>15/10</td>
<td>16/10</td>
<td>15/9</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>51.4 ± 12</td>
<td>46.6 ± 7.9</td>
<td>49.9 ± 11.4</td>
<td>NS</td>
</tr>
<tr>
<td>Number of cigarettes (daily)</td>
<td>—</td>
<td>31.2 ± 15.3</td>
<td>25.9 ± 8.7</td>
<td>25 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of smoking (years)</td>
<td>—</td>
<td>24.4 ± 3.9</td>
<td>23.5 ± 2.4</td>
<td>25.7 ± 11.1</td>
<td>NS</td>
</tr>
<tr>
<td>Analyte</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (18–25 Kg/m²)</td>
<td>23.0 ± 3.2</td>
<td>23.3 ± 1.9</td>
<td>22.7 ± 2.5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WBC (4–10 10⁹/L)</td>
<td>7.9 ± 1.2</td>
<td>8.9 ± 2.5</td>
<td>8.2 ± 2.3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>RBC (4.1–5.1 10¹²/L)</td>
<td>4.9 ± 0.5</td>
<td>5.2 ± 0.8</td>
<td>4.9 ± 0.5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Hb (12–16 g/dL)</td>
<td>15.1 ± 1.4</td>
<td>14.8 ± 1.9</td>
<td>15.0 ± 1.8</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>MCV (78–99 FL)</td>
<td>87.6 ± 19.4</td>
<td>87.0 ± 9.6</td>
<td>91.7 ± 6.9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>tHcy (&lt;10 μmol/L)</td>
<td>11.5 ± 6.6 (52%)</td>
<td>12.3 ± 7.8 (57%)</td>
<td>10.8 ± 7.4 (38%)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>tCys (250–275 μmol/L)</td>
<td>280 ± 63.8 (76%)</td>
<td>245.3 ± 65.1 (92%)</td>
<td>247.9 ± 75.9 (76%)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>S-Fol (7–28 nmol/L)</td>
<td>13.9 ± 5.4 (0%)</td>
<td>13.7 ± 6.7 (1%)</td>
<td>15.7 ± 7.7 (3.1%)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Ery-Fol (421–1462 mol/L)</td>
<td>471.2 ± 117.2 (33%)</td>
<td>534.5 ± 170.1 (35%)</td>
<td>603.5 ± 341.6 (25%)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Vitamin B₁₂ (164–835 pmol/L)</td>
<td>269 ± 85.4 (3%)</td>
<td>281 ± 84.6 (8%)</td>
<td>289 ± 95.1 (7%)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>HoloTC (≥40 pmol/L)</td>
<td>60.2 ± 23.0 (22%)</td>
<td>63.9 ± 18.9 (15%)</td>
<td>64.1 ± 23.1 (10%)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

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.

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 ± 10.3 vs 24.0 ± 1.5 kg/m², 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 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 FEF₂₅ differences between T₁ and T₀ values (expressed as mean delta) improved slightly compared to the P group’s, whereas the FVB group’s FEF₂₅ differences improved significantly compared to the P group (p < 0.0001) and the FV group.

by commercial immunoenzymatic assays (AxSYM B₁₂ and AxSYM Active-B₁₂, 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-diazele-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).
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’ FEV₁ mean delta values improved, even if not significantly so, whereas the P group’s FEV₁ 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 T₀ 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 T₁ both nutraceutical groups’ S-Fol and Ery-Fol concentrations increased significantly, whereas, as expected, vitamin B₁₂ 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]. FEV₁, 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.

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**Table 2. Baseline (T₀) Pulmonary Parameters of 75 Compliant Smokers**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P group (n = 25)</th>
<th>FV group (n = 26)</th>
<th>FVB group (n = 24)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ (%)</td>
<td>98.2 ± 12.3</td>
<td>102.2 ± 13.2</td>
<td>99.2 ± 14.0</td>
<td>NS</td>
</tr>
<tr>
<td>FEF25 (%)</td>
<td>96.4 ± 20.2</td>
<td>104.4 ± 26.1</td>
<td>97.1 ± 29.4</td>
<td>NS</td>
</tr>
<tr>
<td>DLCO/VA (%)</td>
<td>79.5 ± 14.3</td>
<td>82.0 ± 18.1</td>
<td>75.4 ± 10.7</td>
<td>NS</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>102 ± 24.9</td>
<td>109 ± 16.2</td>
<td>106.2 ± 12.6</td>
<td>NS</td>
</tr>
<tr>
<td>Tiffeneau index (%)</td>
<td>91.7 ± 9.1</td>
<td>95.2 ± 7.3</td>
<td>93 ± 9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD and refer to Actual/Expected %, P = placebo, FV = fruit/vegetable, FVB = fruit/vegetable/berry, NS = nonsignificant, FEV₁ = forced expiratory volume in 1 second, FEF₂₅ = forced expiratory flow at 25% of forced vital capacity, DLCO/VA = CO diffusion lung per unit alveolar volume, FVC = forced volume capacity, Tiffeneau Index (FEV₁/FVC)% = index of air-flow obstruction.

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**Table 3. Pulmonary and Biochemical Parameters of 75 Compliant Smokers at the End of the Study (T₁) and Differences (Mean Δ) between T₁ and T₀ Values after 3 Months’ Supplementation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P group (n = 25)</th>
<th>FV group (n = 26)</th>
<th>FVB group (n = 24)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ (%)</td>
<td>97.7 ± 17.1</td>
<td>102.8 ± 13.2</td>
<td>99.4 ± 12.1</td>
<td>0.2 ± 4.15</td>
</tr>
<tr>
<td>FEF25 (%)</td>
<td>89.5 ± 10.9</td>
<td>101.1 ± 15.2</td>
<td>89.6 ± 4.9</td>
<td>1.9 ± 3.6</td>
</tr>
<tr>
<td>DLCO/VA (%)</td>
<td>75.2 ± 8.62</td>
<td>80.3 ± 2.4*</td>
<td>80.9 ± 1.79*</td>
<td>5.1 ± 4.96*</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>101.6 ± 19.3</td>
<td>110 ± 13.4</td>
<td>107.1 ± 11.3</td>
<td>1.2 ± 6.7</td>
</tr>
<tr>
<td>Tiffeneau index (%)</td>
<td>89.3 ± 17.3</td>
<td>96.2 ± 13.1</td>
<td>93.8 ± 12.4</td>
<td>0.6 ± 6.2</td>
</tr>
<tr>
<td>tHcy (μmol/L)</td>
<td>11.4 ± 5.9</td>
<td>9.4 ± 6.3*</td>
<td>8.1 ± 3.5A</td>
<td>2.9 ± 5.4</td>
</tr>
<tr>
<td>tCys (μmol/L)</td>
<td>244.9 ± 56.7</td>
<td>260.6 ± 40.5</td>
<td>258.3 ± 62.8</td>
<td>11.3 ± 42.3</td>
</tr>
<tr>
<td>S-Fol (nmol/L)</td>
<td>16.0 ± 5.6</td>
<td>27.8 ± 8.3*</td>
<td>31.8 ± 8.7A</td>
<td>16.7 ± 8.8</td>
</tr>
<tr>
<td>Ery-Fol (nmol/L)</td>
<td>625 ± 107</td>
<td>1062 ± 331A</td>
<td>1199 ± 395A</td>
<td>622 ± 32I</td>
</tr>
</tbody>
</table>

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, ** p = 0.001 vs P, *** p = 0.0001 vs P. ▲ p < 0.001 vs FV, ▲ p < 0.001 vs FVB.

P = placebo, FV = fruit/vegetable, FVB = fruit/vegetable/berry, FEV₁ = forced expiratory volume in 1 second, FEF₂₅ = forced expiratory flow at 25% of forced vital capacity, DLCO/VA = CO diffusion lung per unit alveolar volume, FVC = forced volume capacity, tHcy = total homocystine, tCys = total cysteine, S-Fol = serum folate, Ery-Fol = erythrocyte folate, Tiffeneau Index (FEV₁/FVC)% = index of air-flow obstruction.
Nutraceutics’ Effects on Cigarette Smoking Damage

In our subjects, not all pulmonary markers improved after the interventions. In fact, FEV1 and the Tiffeneau index did not significantly improve in either phytonutrient group, whereas FEF25 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 T1, 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 FEF25) 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 lipooxygenase and cyclooxygenase, 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 FEV1 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 B12, is considered the earliest and most sensitive marker of vitamin B12 deficiency [30]. Adequate folate and vitamin B12 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 mucolytic 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.

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REFERENCES


