Original Research

Effects of Encapsulated Fruit and Vegetable Juice Powder Concentrates on Oxidative Status in Heavy Smokers

Cristina Novembrino, PhD, Giuliana Cighetti, PhD, Rachele De Giuseppe, BS, Luisella Vigna, MD, Federica de Liso, BS, Marco Pellegatta, MD, Dario Gregori, PhD, Rita Maiavacca, MD, Fabrizia Bamonti, PhD

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

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Objective: Long-term cigarette smoking has negative effects on oxidative status, promoting low-density lipoprotein (LDL) oxidation and formation of lipid peroxides. We evaluated the effects of 2 different encapsulated formulas, consisting primarily of mixed juice powder concentrate, on oxidative status compared with placebo.

Methods: This randomized, double-blind, placebo-controlled study was performed on 101 apparently healthy heavy smokers (>20 cigarettes/d, duration >10 years; median age 47 years, range 41–57 years; 54 M) before and after 3 months' supplementation. Subjects were randomized into 3 groups, well matched for sex and age: (1) placebo; (2) fruit/vegetable (FV); and (3) fruit/vegetable/berry (FVB). Analysis of oxidative status was performed on 75 (46 M) compliant subjects (>95% of assigned capsules). Changes in lipid panel parameters, oxidative-INDEX (Oxy-I, calculated on the basis of serum hydroperoxides and total antioxidant capacity measured by spectrophotometric methods), oxidized-LDL (ox-LDL; enzyme-linked immunosorbent assay [ELISA] method), and malondialdehyde (MDA; gas chromatography-mass spectrometry method) in free (fMDA), bound (bMDA), and total (tMDA = fMDA + bMDA) forms are reported. Statistical analysis was performed with R statistical software.

Results: After supplementation, compared with placebo, both FV and FVB groups showed a significant decrease in total cholesterol ($p < 0.05$), ox-LDL ($p = 0.03$), and fMDA levels ($p = 0.004$) accompanied by a slight increase in bMDA concentrations, possibly as the result of fMDA conjugation. Moreover, a significant decrease in Oxy-I was found in both active groups compared with placebo ($p < 0.001$).

Conclusion: Intervention with both nutraceutical formulations resulted in improvement in some oxidative alterations attributed to long-term cigarette smoking.

INTRODUCTION

Tobacco use is the greatest preventable cause of mortality in developed countries, with a significant public health impact worldwide. Cigarette smoking causes 80%–90% of all lung cancer deaths, and the risk of dying from lung cancer is 23 times greater among male smokers, and 13 times greater among female smokers, than nonsmokers [1]. In addition, tobacco use increases risk for other cancers [2], for chronic lung disease (e.g., emphysema, bronchitis), and for cardiovascular disease [1,3–5]. Moreover, a positive correlation has been reported between severity of disease and the number of cigarettes smoked [6,7].

Address reprint requests to: Cristina Novembrino, PhD, Via F. Sforza 35, 20122 Milano, ITALY. E-mail: fabrizia.bamonti@unimi.it

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Abbreviations: AU = arbitrary unit, bMDA = bound malondialdehyde, BMI = body mass index, CBC = complete blood count, ECG = electrocardiogram, ELISA = enzyme-linked immunosorbent assay, fMDA = free malondialdehyde, FV = fruit and vegetable, FVB = fruit, vegetable, and berry, GC-MS = gas chromatography-mass spectrometry, HDL-c = high-density lipoprotein cholesterol, LDL = low-density lipoprotein, LDL-c = low-density lipoprotein cholesterol, MDA = malondialdehyde, NSAS = Nutrition Status Assessment Score, Oxy-I = oxidative-INDEX, ox-LDL = oxidized-LDL, ROS = reactive oxygen species, TAC = total antioxidant capacity, TBA = thiobarbituric acid, tChol = total cholesterol, tMDA = total malondialdehyde.

Underlying mechanisms of the adverse biological effects of cigarette smoking have not yet been completely explained but are reported to include oxidative damage to essential biological constituents [8,9] due to reactive radicals and/or species contained in the cigarette smoke [10,11]. Two lines of evidence can be cited to support a role for smoke-born radicals in smoking-induced pathologic conditions. First, free radicals are involved in many of the smoking-associated chronic diseases, such as emphysema, chemical carcinogenesis, and atherogenesis. Second, concentrations of radicals in inhaled smoke are so high (e.g., compared with smog) that radical-mediated reaction pathways appear logical.

It has been postulated that increased risk for disease among smokers with respect to nonsmokers may be due in part to differences in lifestyle behaviors, including dietary habits. In fact, smoking people tend to have higher intakes of saturated fatty acids and lower intakes of polyunsaturated fat, iron, beta-carotene, and vitamin E compared with nonsmoking people [12], together with lower consumption of fruits and/or vegetables [13] and lower serum dietary antioxidant concentrations.

Under physiologic conditions, metabolic reactions (e.g., mitochondrial energetic pathways) result in the formation of reactive oxygen species (ROS), unavoidable products of the aerobic lifestyle, which can be counteracted by an integrated endogenous antioxidant system (total antioxidant capacity [TAC]) with various defense mechanisms (endogenous and/or exogenous, enzymatic, hydrophilic and lipophilic, intracellular and extracellular) and interacting elements. Oxidative stress can occur when the endogenous antioxidant barrier is unable to neutralize an excessive pro-oxidant cascade, resulting in alterations in cell membrane lipids, proteins, and nucleic acids, thus impairing cell metabolism and viability. This high ROS production, mainly of superoxide anion, hydroxyl radical, peroxyl radicals, and hydrogen peroxide, has been observed in several pathologic conditions such as inflammation, hypoxia-reperfusion, and exposure to radiation and to exogenous substances [14,15].

The occurrence of impaired oxidative status is highlighted by several biomarkers, among them malondialdehyde (MDA), one of the terminal compounds of lipid peroxidation. This is commonly used as a marker of oxidative stress and oxidative damage [16,17]. In biological matrices, MDA exists in both free (fMDA) and bound (bMDA) forms to available thiolic and/or aminic groups of proteins, nucleic acids, and lipoproteins.

Total MDA (tMDA: free + bound MDA) represents an index of total injury and is the only MDA form detected by reaction with thiobarbituric acid (TBA), the method commonly used to assess lipid peroxidation [17]. However, the chemically reactive fMDA is an index of recent and potential damage, while bMDA, excreted by the kidney, is an index of older damage [16–18].

Another effect of ROS excess is the oxidation of low-density lipoprotein (LDL) with formation of oxidized-LDL (ox-LDL), a marker of oxidative damage, which triggers the atherosclerotic process. It has been reported that ox-LDL and/or MDA-modified LDL can be taken up by tissue macrophages, leading to the formation of foam cells, which generate atherosclerotic plaque [19].

The aim of this study was to evaluate in healthy heavy smokers the potential beneficial effects of 3 months' supplementation with 2 formulations of encapsulated fruit and vegetable juice powder concentrate or placebo on various oxidative status parameters (ROS, ox-LDL, MDA form concentrations, and TAC) recognized as risk factors for cardiovascular disease.

METHODS

Study Population

This randomized, placebo-controlled, double-blind trial was conducted according to the guidelines of the Declaration of Helsinki for Research on Human Subjects and was approved by the Human Ethics Committee at the San Giuseppe e Sacra Famiglia di Erba Hospital, Erba, Italy (Registration number 27/05/CE/smc on September 23, 2005), and by the Human Ethics Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy (Registration number 2552 on November 20, 2008).

One hundred twenty-four subjects (63 M/61 F, mean age 51.3 ± 10.4 years) were enrolled. Subjects were defined as currently healthy heavy smokers (no respiratory complications assessed by spirometric examination nor other chronic health conditions; 20 cigarettes or more per day for at least 10 years).

Exclusion criteria included a history of endocrine or metabolic disease known to affect plasma lipoproteins; a history of cardiovascular disease (coronary artery disease, peripheral or cerebral vascular disease, congestive heart failure or cardiac arrhythmias, clinically significant electrocardiographic [ECG] abnormalities); hypertension, defined as systolic or diastolic blood pressure higher than 140 or 100 mmHg, respectively; impaired renal function (plasma creatinine > 2.01 mg/dL); fasting plasma glucose levels higher than 6.99 mmol/L or a history of diabetes; acute or chronic infection with human immunodeficiency virus (HIV) or hepatitis B or C virus; poor mental function; unstable psychiatric disorders and alcohol abuse; abnormal body mass index (BMI < 19 or BMI >25 kg/m^2); pregnancy; use of oral contraceptives; and a plan to stop or change smoking and lifestyle habits for the duration of the investigation. Moreover, subjects on long-term prescription drugs or regular vitamin supplementation were excluded. To confirm their eligibility for the study, all subjects were evaluated to determine hematologic and biochemical status

through standard routine examinations, including complete blood count (CBC) and lipid panel.

All participants provided written informed consent and completed a questionnaire on medical history and lifestyle. To determine eligibility, each participant was interviewed about his or her general health, habitual dietary intake, lifestyle, and smoking habits.

Dietary Intake Assessment

The Nutrition Status Assessment Score (NSAS) questionnaire [20] consisted of a series of 13 items pertaining to number of cigarettes smoked, use of alcohol, physical activity, and intake of common Italian foods. Items were scored by a 5-level Likert-type scale, and a total NSAS score was calculated, ranging from 1 to 13.

All subjects were asked to maintain the same lifestyle and diet during the 3-month study. Additionally, they were told that they should not change their daily smoking habits during the study.

Study Design

The study population was randomly divided into 3 groups:

- Placebo group (41 subjects, 22 M/19 F, mean age 48.8 ± 11.6 years)
- Fruit-vegetable (FV) group (43 subjects, 22 M/21 F, mean age 47.4 ± 9.5 years): blend of fruit and vegetable juice concentrate capsules
- Fruit-vegetable-berry (FVB) group (40 subjects, 19 M/21 F, mean age 50.4 ± 10.3 years): FV capsules with additional berry and grape juice concentrate ingredients

FV capsules contained primarily fruit and vegetable juice powder concentrates derived from apple, beet, broccoli, cabbage, carrot, cherry (acerola), cranberry, kale, orange, peach, papaya, parsley, pineapple, spinach, and tomato (Juice Plus+, NSA, Collierville, TN). FV capsules provided about 7.5 mg beta-carotene, 234 mg vitamin C, 32 mg vitamin E, 420 μg folate, and 42 kJ per day [21].

FVB capsules additionally included juice powder concentrates derived from bilberry, blackberry, black currant, blueberry, cranberry, elderberry, grape (Concord), raspberry, and red currant (Juice Plus+ and Vineyard Blend, NSA, Collierville, TN). FVB capsules provided about 7.5 mg beta-carotene, 200 mg vitamin C, 60 mg vitamin E, 600 μg folate, and 63 kJ per day [22].

Placebo capsules contained primarily microcrystalline cellulose.

Active and placebo capsules were provided in opaque gelatin capsules. Subjects were instructed to take 3 capsules twice a day with meals for the 3-month study period. Compliance with study protocol was determined by review of patient diaries and returned capsule count at the 3-month follow-up appointment. Subjects took 6 capsules a day according to instructions for the duration of the study period, while following their habitual diet and lifestyle. Concentrations of biochemical parameters were measured at baseline (T0) and after 3-month capsule intervention (T1).

Biochemical Analyses

Blood samples were drawn at T0 and T1 the morning after an overnight fast. For the MDA assay, 2 blood specimens from each patient were collected in light protected tubes, either with no additive (for serum ROS, TAC, and ox-LDL assays) or containing EDTA to prevent coagulation. EDTA specimens were immediately centrifuged to obtain plasma samples for MDA determination. Serum and plasma samples were frozen and stored at −80°C until analysis.

Serum ROS concentrations were measured by a spectrophotometric method using a commercial kit (dROMs test, Diacron International, Grosseto, Italy) on F.R.E.E. analyzer (Diacron) as previously described [23]. Serum TAC was measured by a spectrophotometric method using a commercial kit (OXY-Adsorbent test, Diacron International) on F.R.E.E. analyzer (Diacron International) as previously described [23].

A global score for oxidative stress status (Oxidative-INDEX [Oxy-I]), which reflected both oxidant and antioxidant counterparts, was then calculated. To consider parameters with different measurement units and variability, standardized values for the oxidant (ROS) and antioxidant (TAC) indices were calculated as previously reported [24] by using the following formula: $sv_{var} = (v_{var} - m_{var})/ds_{var}$), where sv_{var} represents the standard value of a certain parameter (ROS or TAC), v_{var} its original value, and m_{var} and ds_{var} the mean and standard deviation, respectively, of the parameter obtained from a population of healthy nonsmokers (51 M/45 F, mean age 50.2 ± 10.8 years) selected in our laboratory. The difference between the ROS standardized variable and the TAC standardized variable represented the Oxy-I and was reported as the arbitrary unit (AU).

Serum concentrations of ox-LDL were measured by a commercial enzyme-linked immunoassorbent assay (Competitive ELISA, Mercodia, Uppsala, Sweden) on the EASIA reader (Medgenix Diagnostics, Fleurus, Belgium).

Plasma free and total MDA concentrations were determined using a "reference method" based on gas chromatography-mass spectrometry (GC-MS) with synthetized dideuterated MDA added as an internal standard [25]. Briefly, for fMDA determination, plasma (0.2 mL) was diluted with citrate buffer (0.4 mol/L; pH 4.0), added to dideuterated MDA (0.25 nmol) as an internal standard and to butylated hydroxytoluene (0.5 mmol/L; 5 nmol) to prevent any new possible MDA formation from plasma lipid autoxidation. After derivatization with phenylhydrazine for 30 minutes at room temperature, samples were extracted with hexane and analyzed by GC-MS. For tMDA evaluation, plasma (0.2 mL) was treated as for fMDA

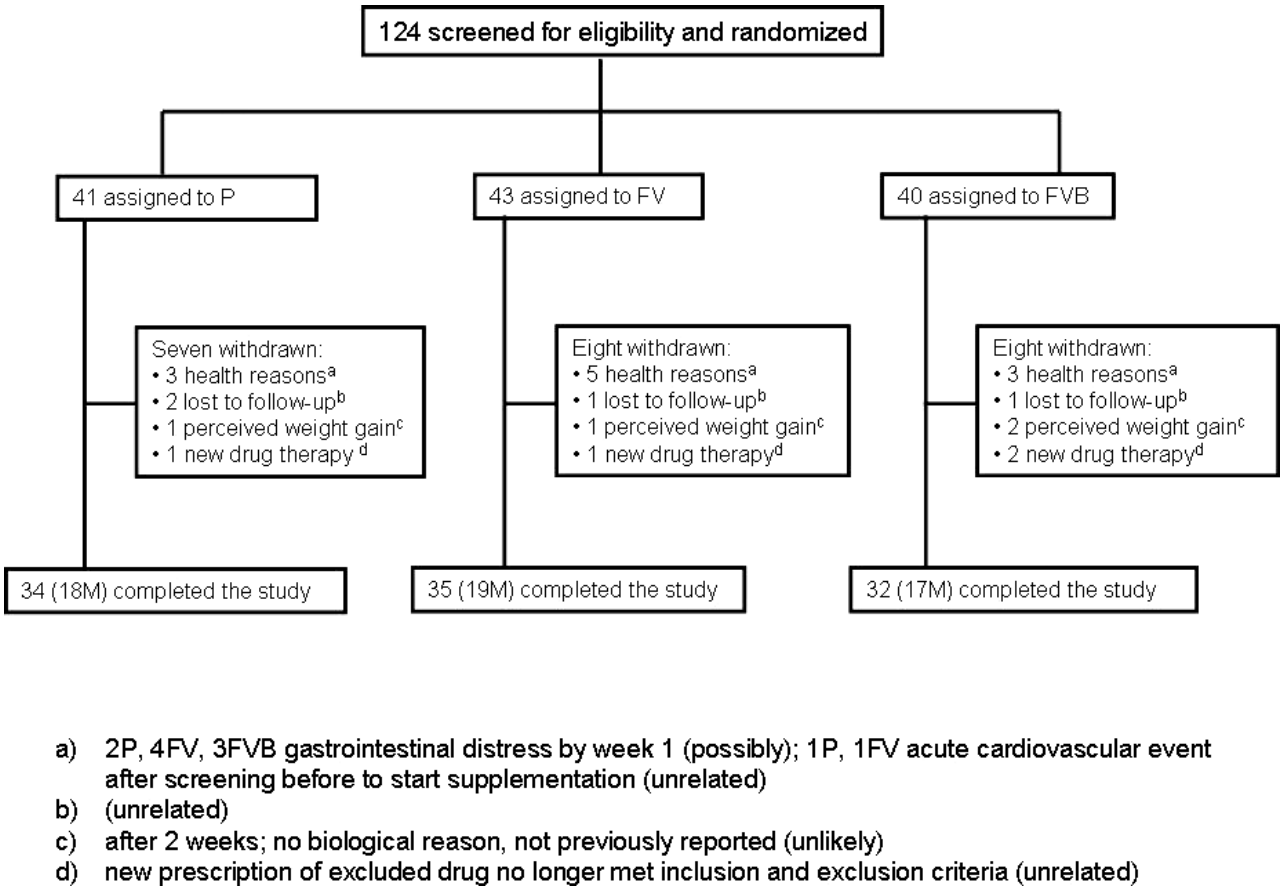

Fig. 1. Trial participant flow. The attribution of adverse event association with subjects is specified within the parentheses.

detection but was subjected, before the derivatization step, to hydrolysis in 1 mol/L NaOH at 60°C for 60 minutes. Bound MDA concentration was calculated by subtracting the fMDA amount from the total.

Statistical Analyses

Continuous variables are reported as means ± standard deviation to properly account for the skewness of most data distributions, and categorical variables are reported as percentage and absolute number of valid observations.

Differences among groups at baseline and at follow-up were evaluated using continuous variables, the Kruskal-Wallis test (for not normally distributed data), or analysis of variance (ANOVA) (for normally distributed data). T0-T1 comparisons (within group) were conducted by Student t test or Mann-Whitney-Wilcoxon test. Changes from baseline to follow-up were compared among groups using a robust median regression model, where group comparisons were parameterized as linear contrasts. Statistical significance was assigned as a p value <0.05. Statistical analyses were performed using R statistical software (Revolution Analytics, Palo Alto, CA) [26].

RESULTS

Out of 124 participants entering the study, 101 (54 M/47 F, mean age 49.7 ± 10.3 years) completed the 12-week supplementation period, and 23 dropped out (18.5%). Drop-out subjects were equally distributed into 3 randomized groups (Fig. 1).

At baseline, the 3 randomized intervention groups showed no significant differences in demographic and biochemical parameters (Table 1); all subjects had an adequate hematologic status, based on a standard CBC panel, and normal renal function (data not reported). However, about one third of them showed the polycythemia (hematocrit and red blood cell count above the reference interval; data not shown) typically observed in smokers [3].

Lipid panel parameters in particular were altered in most subjects, with the exception of triglycerides (TGs). Most subjects showed baseline oxidative stress with increased ox-LDL and fMDA concentrations (86% and 82%, respectively); tMDA was elevated in more than 50% of subjects. In particular, ox-LDL levels showed a positive correlation with total cholesterol (tChol; $r=0.67$; $p<0.0001$), LDL cholesterol (LDL-c; $r=0.6$; $p<0.0001$), and TGs ($r=0.4$; $p=0.0001$) but a negative correlation with HDL cholesterol (HDL-c; $r=-0.3$;

Table 1. Baseline Parameters (Mean ± SD) in the Whole Population and in the Randomized Groups (% of Subjects with Pathologic Values Reported in Parentheses)

Parameters (Reference Values)	All Smokers	Placebo	Fruit/Vegetable	Fruit/Vegetable/Berry
Number of subjects	101	34	35	32
Males/females	54/47	18/16	19/16	17/15
Age (years)	49.7 ± 10.3	49.7 ± 11.5	48.5 ± 8.8	51.1 ± 1.6
Cigarette count (daily)	24.7 ± 8.8	24.6 ± 9.3	24.8 ± 7.3	24.9 ± 9.9
Duration of smoking (years)	25.0 ± 8.8	25.5 ± 9.3	23.9 ± 5.0	26 ± 12.3
BMI (19–25 kg/m^2)	23.4 ± 2.3 (2%)	23.5 ± 2.6 (3%)	23.5 ± 1.6 (0%)	23.4 ± 3.8 (3%)
TGs (<170 mg/dL)	116.4 ± 60.1 (15%)	115.4 ± 56.2 (12%)	128.2 ± 77.5 (20%)	117.7 ± 60 (12.5%)
tChol (<200 mg/dL)	226.2 ± 41.2 (72%)	228.6 ± 41.8 (77%)	227.5 ± 46.5 (69%)	222 ± 34.7 (69%)
HDL-c	(70%)	(71%)	(68%)	(73%)
(M >55 mg/dL)	47.9 ± 12.1	48.6 ± 9.7	47.4 ± 12.2	47.9 ± 14.6
(F >65 mg/dL)	61.2 ± 13.3	59.6 ± 11.4	59.6 ± 16.0	64.6 ± 12.4
LDL-c (<130 mg/dL)	135 ± 36.8 (58%)	139.8 ± 36.2 (56%)	132.7 ± 43.0 (54%)	133.2 ± 30.1 (62%)
Oxy-I (0.0004 ± 1.25 AU)	0.45 ± 1.10	0.44 ± 1.10	0.51 ± 1.14	0.42 ± 1.05
ox-LDL (<70 U/L)	91.1 ± 20.5 (86%)	93 ± 20.0 (88%)	95 ± 22.4 (89%)	86 ± 18.1 (81%)
tMDA (<2.5 μmol/L)	2.7 ± 0.80 (58%)	2.6 ± 0.80 (57%)	2.7 ± 0.74 (57%)	2.7 ± 0.76 (60%)
fMDA (<0.43 μmol/L)	0.8 ± 0.4 (82%)	0.7 ± 0.4 (77%)	0.9 ± 0.5 (95%)	0.8 ± 0.5 (75%)
bMDA (<2.0 μmol/L)	1.9 ± 0.8 (42%)	2.0 ± 0.8 (52%)	1.8 ± 0.8 (35%)	1.9 ± 0.7 (40%)

bMDA = bound MDA, BMI = body mass index, fMDA = free MDA, HDL-c = HDL cholesterol, LDL-c = LDL cholesterol, ox-LDL = oxidized LDL, Oxy-I = oxidative index, tChol = total cholesterol, TGs = triglycerides, tMDA = total MDA.

$p = 0.004$). Oxy-I was significantly higher in all smokers than in healthy nonsmokers evaluated for standardization and Oxy-I calculation (0.45 ± 1.07 vs 0.0004 ± 1.25 AU; independent sample t test $p = 0.009$). Oxy-I was significantly higher in female than in male smokers (0.75 ± 1.18 vs 0.24 ± 0.94 AU; independent sample t test $p = 0.02$) and was also correlated with age ($r = 0.39$; $p = 0.0001$) and daily cigarette count ($r = 0.37$; $p = 0.006$). No other gender differences were observed in the other baseline study parameters.

Each subject was interviewed at the end of the study by means of the same NSAS questionnaire used at enrollment; no study participants demonstrated significant changes in lifestyle and/or dietary intake as indicated by the T1 NSAS total score, which was similar to the T0 NSAS total score (data not shown).

After the 3-month intervention period (T1), no differences were observed between the 3 groups (placebo, FV, and FVB) in terms of CBC (data not shown) or changes from baseline values (Δ = T1−T0) in oxidative status parameters; nevertheless, the following significant correlations were found between biochemical parameters: a positive correlation between ox-LDL and TGs was found in the placebo group ($r = 0.64$; $p = 0.001$) but not in the FV and FVB groups; moreover, ox-LDL levels in the 3 groups positively correlated with tChol (placebo: $r = 0.83$, $p < 0.0001$; FV: $r = 0.7$, $p = 0.0002$; FVB: $r = 0.6$; $p = 0.004$) and with LDL-c (placebo: $r = 0.80$, $p < 0.0001$; FV: $r = 0.75$, $p < 0.0001$; FVB: $r = 0.80$; $p < 0.0001$). In addition, Oxy-I correlated with age and cigarette count only in the placebo group ($r = 0.47$, $p = 0.008$ and $r = 0.38$, $p = 0.03$, respectively).

The mean compliance of the whole study population was about 80% (range 45%–100%). Because low compliance can confound study results, a subgroup analysis was performed on subjects >95% compliant with the protocol.

Oxidative status parameters in compliant subjects are reported in Table 2. Baseline (T0) characteristics of these 75 compliant subjects (46 M, aged 49.2 ± 10.6 years) showed the absence of significant differences in demographic and biochemical parameters in the 3 randomized intervention groups. After 3 months' supplementation (T1), tChol and ox-LDL concentrations decreased significantly in both active groups; moreover, a trend toward a decrease in Oxy-I was observed in the FV and FVB groups ($p = 0.08$ and $p = 0.1$, respectively). Changes from baseline to 3-month follow-up (Δ = T1−T0) showed a significant decrease in tChol levels (ANOVA test, $p < 0.05$) in both FV and FVB groups compared with placebo. Moreover, a significant improvement was observed in oxidative status in both active groups (FV and FVB) with a significant decrease in Oxy-I and in ox-LDL and fMDA concentrations compared with the placebo group. In contrast to fMDA values, no significant change in total or bound MDA concentration was observed. It is interesting to note that a negative correlation between change from baseline to T1 in fMDA and bMDA levels was found in both active groups (FV: $r = -0.36$, $p = 0.09$; FVB: $r = -0.55$, $p = 0.01$).

DISCUSSION

In the present study, metabolic alterations in oxidative status due to cigarette smoking and consequently the possible

Table 2. Oxidative Status Parameters Evaluated in 75 Compliant (>95%) Subjects Divided into 3 Randomized Groups (Baseline [T0], after Supplementation [T1], and Variations between T1 and T0 [Δ] Reported and Data Expressed as Means [±SD])

	Placebo			Fruit/Vegetable			Fruit/Vegetable/Berry		
M/F	15/10			16/10			15/9		
Age (years)	51.4 ± 12.0			46.6 ± 7.9			49.9 ± 11.4		
	T0	T1	Δ (T1 − T0)	T0	T1	Δ (T1 − T0)	T0	T1	Δ (T1 − T0)
tChol (mg/dL)	234.3 (±41.5)	234.8 (±42.0)	9.3 (±18.4)	218.8 (±45.2)	206.2[a] (±43.3)	−11.6* (±22.9)	226.3 (±38.3)	214.3[a] (±45.3)	−6.3* (±25.5)
Oxy-I (AU)	0.53 (±1.14)	0.74 (±1.22)	0.51 (±0.53)	0.97 (±1.1)	0.73 (±1.3)	−0.24** (±0.60)	0.56 (±0.9)	0.43 (±1.0)	−0.13** (±0.46)
ox-LDL (U/L)	90.0 (±21.6)	85.8 (±19.5)	−2.3 (±11.3)	88.3 (±21.8)	76.4[b] (±19.7)	−13.8* (±12.5)	82.5 (±20.4)	69.5[b] (±17.8)	−11.2* (±12.2)
tMDA (μmol/L)	2.6 (±0.8)	2.9 (±0.9)	0.23 (±0.90)	2.7 (±0.7)	2.8 (±1.1)	0.04 (±0.85)	2.7 (±0.8)	3.0 (±1.1)	0.26 (±0.75)
fMDA (μmol/L)	0.71 (±0.29)	0.88 (±0.39)	0.26 (±0.45)	0.92 (±0.49)	0.78 (±0.32)	−0.14*** (±0.446)	0.77 (±0.51)	0.67 (±0.29)	−0.11*** (±0.36)
bMDA (μmol/L)	2.0 (±0.8)	2.0 (±0.8)	−0.03 (±0.9)	1.8 (±0.8)	2.0 (±1.0)	0.18 (±0.9)	1.9 (±0.7)	2.3 (±1.1)	0.37 (±0.89)

[a] 0.03 vs T0, Student's *t* test for pair data.

[b] 0.0001 vs T0, Student's *t* test for pair data.

* $p < 0.05$ vs placebo, ** $p < 0.001$ vs placebo, *** $p < 0.01$ vs placebo: ANOVA test.

bMDA = bound MDA, fMDA = free MDA, ox-LDL = oxidized LDL, Oxy-I = oxidative INDEX, fChol = total cholesterol, tMDA = total MDA.

effects of 2 formulations of a nutraceutical in attenuating some of these alterations were assessed in heavy smokers.

Oxidative status was evaluated as the balance between oxidant species (ROS concentrations) and the total antioxidant barrier (TAC), calculating a global oxidative stress index (Oxy-I) that was able to reflect both oxidative and antioxidant components. At baseline, our findings showed in these otherwise healthy heavy smokers an imbalanced oxidative status in favor of pro-oxidants, as shown by an Oxy-I significantly higher in smokers than the Oxy-I reference value measured in a nonsmoker population. This observation is consistent with the high ROS content in cigarette smoke [10,11], together with the low consumption of antioxidants described in smoker subjects [12,13]. Moreover, our data are consistent with results reported by Vassalle et al. for a population of current smokers who also showed a positive correlation between Oxy-I and age, partially caused by a longer duration of smoking [27]. It is interesting to note that the influence of smoking on oxidative imbalance was also highlighted in our study by the positive correlation between Oxy-I and the number of cigarettes smoked at baseline.

It is known that oxidative stress promotes lipid peroxidation, evaluated in the present study by assessing ox-LDL levels and MDA concentrations. Again, consistent with other studies [28–31], our baseline findings confirmed an increase in ox-LDL concentration in most of the study population, which indicated that the oxidation of LDL is significantly correlated with the dyslipidemia that characterizes these heavy smokers.

With regard to MDA concentrations, all 3 assessed forms showed higher values than the relevant reference cut-off used in our laboratory. In particular, comparison of the present findings with those reported in our previous study on light smokers (up to 10 cigarettes/d) after 1 month's FV supplementation [32] showed that both total and bound MDA amounts were higher, whereas fMDA was lower, among heavy smokers. It is known that active fMDA can react with different biological compounds containing thiolic and/or aminic groups [16] and with the lysine residues in LDL, forming MDA-modified LDL [19]. This complex reactivity of MDA might explain the reduced although still elevated values compared with normal values for fMDA amounts in heavy smokers compared with light smokers [32]. Unfortunately, the difference in ox-LDL concentrations between heavy and light smokers could not be evaluated because in the present study we assessed ox-LDL levels (in our previous study on light smokers, ox-LDL concentrations were not evaluated). To the best of our knowledge, this is the first study to report elevated levels of fMDA in heavy smokers; generally only tMDA levels have been evaluated by other investigators [31,33,34].

After 3 months of capsule use, both FV and FVB groups demonstrated loss of the positive correlation of Oxy-I with both cigarette count and age observed at baseline, probably caused by the beneficial effects of antioxidant supplementation. This

hypothesis is confirmed by the persistence of the correlation in the placebo group.

When changes in the 75 compliant subjects were evaluated, the improvement in smokers' oxidative status was significant in both active groups compared with the placebo group.

With regard to LDL oxidation, we found that both active formulas were significantly more effective than placebo in decreasing ox-LDL concentrations. Contrasting data are available on this point. In fact, as reported in Kelly's review, these findings agree with others showing positive effects of vitamin supplementation (in particular ascorbic acid) on LDL oxidative susceptibility; however, other investigators have reported no effect [34].

Total cholesterol concentrations were significantly decreased in both active groups at T1. The mechanism by which both supplementations can have an impact on lipid metabolism has not been determined. Similar to the findings of Plotnick et al. on the effects of daily use of FV and FVB supplementation for 1 month, our results are suggestive of a beneficial impact of phyto-antioxidant compounds on the lipid panel [22].

In the present study, the positive effects of both nutraceutical supplementations were evident in the 2 active groups. The antioxidant property of a wide range of bioactive phytonutrients, such as vitamins, flavonoids (free radical scavenger), and polyphenols (chain-breaking action) present in vegetables (spinach, broccoli) and fruit (berry, grape, apple), is known. Their effects are dependent on the hydrogen-donating capacity of the various hydroxyl groups present in these molecules.

A significant decrease in fMDA was noted with a trend towards an increase in both tMDA and bMDA and a significant negative correlation between changes in fMDA and bMDA after 3 months, as observed in both FV and FVB groups but not in the placebo group. It is important to note that the increase in bMDA reduces active fMDA in circulation. This relation between free and bound MDA was also observed in our previous pilot study on light smokers [32]. To explain observed reduced fMDA amounts in supplemented subjects, we might hypothesize some chemical reactions among fMDA and various functional groups present in vitamins, flavonoids, and polyphenols.

Finally, our findings are totally in line with those of other authors who used the same supplementation with healthy volunteers, smokers, and nonsmokers and evaluated antioxidant status [35,36].

In conclusion, administration of both formulations of encapsulated fruit and vegetable juice powder concentrates, with or without the added berry ingredients, provided a good source of bioavailable phytonutrients, resulting in positive metabolic modifications compared with baseline values and with the placebo group. The synergistic antioxidant effects of the multiple nutrients might even be more beneficial for individuals who do not eat sufficient fruits and vegetables (e.g., smokers).

Smoking cessation is important for normalizing Oxy-I. Although the supplements could offer benefits, smokers concerned about their health should consider smoking cessation. These supplements theoretically might hasten biochemical recovery during smoking cessation. Additional research with regard to this topic should be pursued.

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