Clin Chem Lab Med 2006;44(4):391–395 © 2006 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2006.084

Increased free malondialdehyde concentrations in smokers normalise with a mixed fruit and vegetable juice concentrate: a pilot study

Fabrizia Bamonti[1,*], Cristina Novembrino[2], Silvia Ippolito[1], Enzo Soresi[3], Alberto Ciani[1], Silvia Lonati[1], Elisabetta Scurati-Manzoni[1] and Giuliana Cighetti[4]

[1] Department of Medical Science,
[2] Department of Surgical Science,
University of Milan, Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Fondazione IRCCS, Milan, Italy
[3] Director of Pneumology, Niguarda Hospital, Milan, Italy
[4] Department of Preclinical Sciences, LITA Vialba, University of Milan, Milan, Italy

Abstract

Background: Cigarette smoking, a cardiovascular risk factor leading to oxygen free radical formation, is involved in the development of serious pathological conditions. On the other hand, a healthy diet and adequate supplementation can help prevent many diseases. The aim of our study was to evaluate in healthy light smokers the effects of supplementation with mixed fruit and vegetable juice powder concentrate on homocysteine metabolism and oxidative status.

Methods: In this pilot study, 32 healthy volunteers, 16 light smokers and 16 non-smokers, on twice daily supplementation were monitored at time zero and after 30 days. Plasma homocysteine, and serum vitamin B_{12} and folate concentrations were measured by immunoenzymatic assays; reactive oxygen species, total antioxidant capacity and thiol groups by spectrophotometric methods; and total and free malondialdehyde concentrations by gas chromatography-mass spectrometry with isotopic dilution.

Results: Baseline free malondialdehyde concentrations were significantly higher in smokers than in non-smokers and normalised after 30-day supplementation. Baseline results for all the other parameters remained unchanged after supplementation, with no significant differences between smokers and non-smokers.

Conclusion: This is the first study showing a significant decrease in free malondialdehyde levels in light smokers after 1-month phytonutrient supplementation.

Keywords: homocysteine; malondialdehyde; oxidative stress; phytonutrients; smoking.

Introduction

Clinical and epidemiological studies have shown that malnutrition, metabolic diseases and a sedentary lifestyle, together with oxidative stress, hyperhomocysteinaemia and tobacco smoking, are risk factors for many serious diseases (1–6). Oxidative stress is a pathological condition caused by the formation of reactive oxygen species (ROS). If produced in excess, they can have a pro-oxidant action (''noxa pathogena''), damaging fundamental molecules (lipids, proteins and DNA) and irreversibly modifying cellular homeostasis (3, 4, 6–8). Cells are protected against ROS by an integrated antioxidant defence system (total antioxidant capacity, TAC), including many defence mechanisms (e.g., thiol groups, -SH) and some interacting elements (9, 10). Active ROS result in lipid peroxidation, generating terminal carbonyl compounds, among which malondialdehyde (MDA) is often used as an index of oxidative damage.

In biological matrices, MDA exists both in free form (f-MDA) and bound to nucleophilic groups (SH or NH_2) of various biomolecules. Only the free form is chemically active and represents a potential damaging agent. Bound MDA (b-MDA) excreted in urine (11) is indicative of an old injury (12). While other commonly used analytical assays detect only total MDA (t-MDA = free + bound forms) as thiobarbituric acid-reactive substances, the use of a ''reference method'' based on gas chromatography-mass spectrometry (GC-MS) with isotope dilution (13) allows the direct measurement of both f-MDA and t-MDA concentrations and the indirect measurement of b-MDA concentrations. Monitoring f-MDA leads to a better understanding of actual oxidative status.

Elevated plasma total homocysteine (tHcy) concentrations can result from inadequate vitamin intake (5) and may indirectly contribute to ROS formation. A reduction in plasma tHcy concentrations due to short-term folic acid supplementation has been observed in chronic smokers (14). Long-term smoking has been associated with an increased risk of developing chronic diseases and several authors recently reported that a smoker's diet is more unbalanced than a non-smoker's (15–17). Generally, healthy lifestyle habits, such as a prudent diet and not smoking, may help to

*Corresponding author: Fabrizia Bamonti, Dipartimento Scienze Mediche, Università degli Studi di Milano, Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Fondazione IRCCS, Padiglione Granelli, Via F. Sforza, 35, 20122 Milano, Italy
Phone: +39-02-55033473, Fax: +39-02-55034736,
E-mail: fbamonti@policlinico.mi.it,
fabrizia.bamonti@unimi.it

prevent pathological conditions (18, 19). Polidori et al. (20) reported that healthy smokers showed a marked increase in plasma antioxidant status after giving up smoking.

Fruit and vegetables contain many phytonutrient compounds, including antioxidants, vitamins, trace elements, and fibre. In fact, fruit and vegetables are considered a source of nutrients. By interacting with biomolecules, they can protect against ROS damage and improve antioxidant status and endothelial functions (21, 22). Many governments and health organisations encourage people, including smokers, to increase their daily intake of fruit (two to four servings) and vegetables (three to five servings) (21). The aim of this pilot study was to evaluate in healthy light smokers and non-smokers the effects of 1-month dietary supplementation with fruit and vegetable juice powder concentrate on several parameters indicative of Hcy metabolism and oxidative status. In particular, lipid peroxidation was evaluated by detecting both free and total MDA levels.

Materials and methods

A total of 32 healthy volunteers, 16 light smokers (10–12 cigarettes/day; 8 male, 8 female, aged 37±10 years) and 16 non-smokers (8 male, 8 female, aged 35.9±11.5 years) participated in the study. The two groups were well matched for sex and age.

Major exclusion criteria were the presence of chronic diseases, cardiovascular episodes within the previous 6 months, pregnancy or lactation, body mass index (BMI) >25 kg/m^2, altered lipidic panel, hyperglycaemia, regular medication other than oral contraceptives, and regular use of any vitamin or dietary supplements.

All subjects were asked to maintain their current lifestyle habits, to refrain from taking vitamins or herbal supplements, drinking coffee, tea and alcoholic beverages for 30 min after taking the study capsules and refrain from smoking for 8 h before blood collection. All the subjects agreed to follow regular diets (30 followed a Mediterranean diet and 2 an Oriental diet) and to take four capsules a day according to instructions. Written informed consent was obtained from all study volunteers.

The phytonutrient product consisted of a blend of fruit (apple, orange, pineapple, cranberry, peach, acerola cherry, papaya) and vegetables (carrot, parsley, beet, kale, broccoli, cabbage, spinach, tomato) juice concentrate with the addition of oat and barley bran (Juice Plus+®, NSA Inc., Memphis, TN, USA) (23, 24).

Peripheral venous blood samples were drawn after an overnight fast at the start of the study (baseline, T0) and after 30 days (T1). Serum aliquots were used for measuring vitamin B_{12}, folate (s-folate), ROS, -SH concentrations and TAC. Whole blood containing EDTA was placed on ice and divided into three aliquots as follows: one for a complete blood count (Coulter counter model STKS; Beckman Coulter, Miami, FL, USA); one for measuring erythrocyte folate (ery-folate) concentrations; and the final aliquot was centrifuged within 30 min for plasma tHcy and MDA measurements. Samples for measuring ery-folate, tHcy and MDA concentrations and serum aliquots were frozen after separation and stored at -20°C until assayed. All samples were analysed in batches at the end of the study.

Plasma tHcy concentrations, along with its metabolically related vitamins (vitamin B_{12}, s-folate and ery-folate), were measured using routine laboratory methods (25). Both serum ROS and -SH concentrations and TAC were measured spectrophotometrically using commercial kits (dROMs test, -SHp test, OXY-Adsorbent test, respectively, from Diacron, Grosseto, Italy) on a F.R.E.E. analyser (Diacron) (3, 4). Plasma free and total (free+bound) MDA levels were detected by GC-MS with dideuterated MDA added as an internal standard (13). The bound MDA concentration was estimated by calculating the difference between total and free MDA.

Data were compared with relevant reference intervals established for healthy volunteers and normally used in our laboratory (8, 13).

Statistical analysis

Results, reported as the median value with interquartile range (IQR) (25–75 percentiles), were analysed using Wilcoxon's test for statistical analysis of non-parametric values. Differences were considered significant at $p<0.05$.

Results

At the start of the study all the subjects were well nourished, with fruit and vegetables included in their diets. The baseline blood count indexes for each individual were within the reference intervals, with no significant change at T1. As regards race, no differences were observed in the smokers' group.

Baseline values of vitamin B_{12}, s-folate and ery-folate were within the reference intervals (164–835 pmol/L, 7–28 nmol/L and 421–1462 nmol/L, respectively). Median values and IQR for vitamin B_{12} and s-folate levels were slightly lower in smokers (Table 1).

As summarised in Table 2, no notable difference between smokers and non-smokers was observed for tHcy and t-MDA concentrations, either before or after supplementation. However, baseline f-MDA levels were significantly higher in smokers than in non-

Table 1 Effect of dietary phytonutrient supplementation on vitamin B_{12}, s-folate and ery-folate in smokers and non-smokers.

Analyte	Time, days	Smokers, n=16	Non-smokers, n=16
Vitamin B_{12}, pmol/L	0	290 (199–544)	431 (275–576)
	30	307 (287–521)	458 (361–546)
s-Folate, nmol/L	0	11.7 (5.2–20.6)	17.9 (11.3–30.8)
	30	25.1 (22–28.5)	33.6 (28.5–34.2)
ery-Folate, nmol/L	0	749 (367–1039)	875 (579–1445)
	30	947 (550–1021)	979 (856–1347)

Results are expressed as median (IQR).

Table 2 Effect of dietary fruit and vegetable juice powder concentrate on total, free and bound (= total–free) MDA and tHcy in smokers and non-smokers.

Analyte	Cut-off	Time, days	Smokers (n = 16)	Non-smokers (n = 16)
t-MDA, μmol/L	<2.5	0	2.3 (1.9–2.5)	2.3 (1.7–2.6)
		30	2.4 (2.1–2.7)	2.4 (1.8–2.6)
f-MDA, μmol/L	<0.43	0	1.23 (0.9–1.6)*	0.44 (0.3–0.6)
		30	0.71 (0.6–0.9)[#]	0.41 (0.4–0.5)
b-MDA, μmol/L	<2.0	0	0.77 (0.57–1.58)**	1.85 (1.25–2.06)
		30	1.62 (1.26–2.07)[§]	1.97 (1.30–2.12)
tHcy, μmol/L	<10	0	9.0 (7.9–11.3)	8.7 (7.1–9.3)
		30	9.1 (7.1–11.2)	8.2 (6.6–8.8)

Results are expressed as median (IQR). *$p < 0.005$ vs. non-smokers; [#]$p < 0.001$ vs. baseline; **$p < 0.001$ vs. non-smokers; [§]$p < 0.002$ vs. baseline.

smokers ($p < 0.005$) and decreased significantly after supplementation ($p < 0.001$, T1 vs. T0).

Baseline b-MDA concentrations were significantly lower in smokers than in non-smokers ($p < 0.001$) and increased significantly at T1 ($p < 0.002$), with no significant difference between the two groups ($p = 0.2$). No change in free and bound MDA levels was observed in non-smokers.

ROS values were below the reference cutoff (<300 UCarr) at both T0 and T1 in non-smokers [268 (222–297) and 298 (259–308) UCarr, respectively]. All ROS concentrations were normal in smokers both before and after supplementation [261 (216–302) and 271 (249–288) UCarr, respectively], except for one smoker, whose ROS levels were high at T0 and normalised after supplementation (352 and 292 UCarr, respectively). TAC for both groups was above the reference cutoff (>350 μmol HClO/mL) at both T0 and T1, without notable difference [smokers: 357 (347–384) μmol HClO/mL at T0 and 372 (341–387) μmol HClO/mL at T1; non-smokers: 377 (337–387) μmol HClO/mL at T0 and 378 (347–394) μmol HClO/mL at T1]. Regarding -SH group concentrations (reference interval 350–650 μmol/L), no statistically significant difference was observed between the two groups at either time [smokers: 371 (311–448) μmol/L at T0 and 349 (336–383) μmol/L at T1; non-smokers: 406 (397–492) μmol/L at T0 and 359 (322–422) μmol/L at T1].

Three non-smokers continued taking daily capsules for 6 months (T2). The oxidative status parameters for all the subjects, monitored monthly, continued to improve, reaching a stable antioxidant capacity; the mean difference (Δ) between T2 and T1 concentrations was 165 μmol HClO/mL for TAC, 229 μmol/L for -SH and 18 UCarr for ROS. No change in either total or free MDA levels was observed.

Discussion

Epidemiological studies have shown how much lifestyle and an adequate intake of antioxidant vitamins can influence the aetiopathogenesis of several pathological conditions (1, 2, 18–21). In this pilot study we focused our attention on possible oxidative status variations in light smokers and non-smokers after short-term dietary supplementation with encapsulated mixed fruit and vegetable juice powder concentrate.

The change in free and bound MDA levels observed in smokers after supplementation was the most interesting result of this study. The method used for MDA evaluation, based on GC-MS with the addition of an isotopic internal standard (dideuterated MDA) (13), enhances the accuracy of MDA levels reported. This is the only ''reference method'' accepted for the validation of all other evaluation methods generally used for clinical diagnostic use (26).

No difference in baseline t-MDA levels was observed between the two groups; however, just moderate cigarette consumption was enough to increase the potentially active and dangerous f-MDA, which was significantly higher in smokers than in non-smokers. Daily supplementation with juice powder concentrate capsules for 30 days affected f-MDA levels, which normalised and reached non-smoker values. The decrease in f-MDA was accompanied by an increase in b-MDA, mainly excreted in urine (11). Thus, the decrease in f-MDA could be explained by its transformation into the bound form. This could be because of conjugation with some components of the juice powder concentrate. It has been suggested that the additive and synergistic effects of phytochemicals in fruit and vegetables could be responsible for their antioxidant activity. As reported by Kiefer et al., a supplement such as juice powder concentrate capsules could mimic the antioxidant behaviour of fruit and vegetables (22).

To the best of our knowledge, this is the first study showing changes in f-MDA levels following dietary supplementation. Regarding the effects of fruit and vegetable supplementation on t-MDA levels, some authors observed a decrease in t-MDA (27–29), but others did not find any change at all (30, 31) in agreement with our findings. The different MDA analytical detection techniques adopted could account for this discrepancy, emphasising the importance of f-MDA, the only form directly produced by oxygen radical reaction. Increased oxidative stress in smokers, highlighted only by high f-MDA levels, and its decrease after supplementation are in agreement with the results of others (32–34) who studied the effects of dietary supplementation using F_2-isoprostanes

instead of MDA levels as an index of lipid peroxidation. After smoking cessation, vitamin C therapy (32) or antioxidant supplementation (33), a decrease in high 8-epi-prostaglandin $F_{2\alpha}$ values was observed in smokers. In a pilot study, Dillon et al. (34) observed that 8-iso-prostaglandin $F_{2\alpha}$ concentration was higher in smokers than in non-smokers, but the values significantly decreased after supplementation with a garlic extract.

All the other parameters evaluated in our study did not show a notable pattern of change over 30 days.

Regarding tHcy, in two different crossover intervention trials using the same juice powder concentrate capsules, a significant reduction in tHcy was observed by Panunzio et al. (24) in 26 subjects and by Samman et al. (21) in 13 smokers and 19 non-smokers. The baseline tHcy concentration was below our laboratory reference cutoff ($\leq$10 μmol/L) in all participants, with a slight decrease at T1 only in non-smokers. The lack of a significant reduction in tHcy levels in smokers could be due to the subjects' good health, as shown by baseline vitamin B_{12} and folate concentrations (Table 1). However, a female smoker's plasma tHcy concentration halved after 30-day supplementation and her baseline s- and ery-folate concentrations, below normal (5.2 and 399 nmol/L, respectively), normalised at T1. As reported by other authors (21, 24), in this case, the intake of dietary mixed fruit and vegetable supplements clearly confirmed a negative correlation between tHcy levels and folate status. Moreover, we observed that after 30-day natural phytonutrient supplementation, s- and ery-folate concentrations increased within the physiological reference interval both in smokers and in non-smokers.

As regards antioxidant parameters, TAC and -SH values appeared to be normal and similar in the two groups, with no change after 30-day supplementation. The lack of difference between the two groups could be because the subjects were light smokers, or had stopped smoking for 8 h before blood collection, or possibly followed an adequate daily diet. Our results are in agreement with those of Miller et al. (35), which showed no significant difference in TAC values between healthy smokers and non-smokers on a normal diet. Moreover, the unmodified TAC and -SH values we observed in both smokers and non-smokers at T1 are presumably because supplementation was short-term. In fact, the three non-smokers whose supplementation had been extended by 6 months showed a notable increase in TAC and -SH levels, but no modification in ROS and MDA concentrations. Both light smokers and non-smokers showed similar ROS values, even if f-MDA was higher in smokers than in non-smokers. A recent paper by Cighetti et al. (36) could provide an explanation for this apparent discrepancy: the poor correlation between f-MDA and ROS values is in fact mainly because of possible underestimation of ROS concentrations. Free and total MDA, formed during ''in vivo'' oxidative imbalance, are measured directly by the most specific and sensitive GC-MS method. The ROS formed ''in vivo'' are instead indirectly measured as dROMs after their ''in vitro'' transformation through a reaction probably affected by endogenous antioxidants. However, even if the dROMs assay is reported to discriminate various pathological conditions (3,4,7,8), in this study it did not seem to be sufficiently sensitive to highlight the presence of lipid peroxidation in light smokers.

To the best of our knowledge, this is the first observational study carried out to evaluate the possible relationship between oxidative status, homocysteine status, tobacco smoking and juice powder concentrate supplementation. Above all, this is the first study to show a significant decrease in f-MDA levels in light smokers after just 1 month of daily phytonutrient supplementation. These results have prompted us to carry out further studies on a larger population and for a longer period of time to confirm these preliminary findings.

Acknowledgements

This study was partly supported by grants to F. Bamonti and G. Cighetti from the Ministero Università e Ricerca Scientifica Tecnologica, Rome, Italy. The authors are very grateful to Mrs. Mary Coduri for linguistic consultation.

References

1. Bray GA, Ryan DH, Harsha DW. Diet, weight loss, and cardiovascular disease prevention. Curr Treat Options Cardiovasc Med 2003;5:259–69.
2. Morris CD, Carson S. Routine vitamin supplementation to prevent cardiovascular disease: a summary of evidence for the US Preventive Service Task Force. Ann Intern Med 2003;139:56–70.
3. Trotti R, Carratelli M, Barbieri M, Micieli G, Bosone D, Rondanelli M, et al. Oxidative stress and thrombophilic condition in alcoholics without severe liver disease. Haematologica 2001;86:85–91.
4. Carratelli M, Porcaro L, Ruscica M, De Simone E, Bertelli AA, Corsi MM, et al. Reactive oxygen metabolites and prooxidant status in children with Down's syndrome. Int J Clin Pharm Res 2001;21:79–84.
5. Stanger O, Herrmann W, Pietrzik K, Fawler B, Geisel J, Dierkes J, et al. DACH-LIGA Homocystein (German, Austrian and Swiss Homocysteine Society): consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: guidelines and recommendations. Clin Chem Lab Med 2003;41:1392–403.
6. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3:276–85.
7. Fracchiolla NS, Bamonti-Catena F, Novembrino C, Ippolito S, Maisonneuve P, Cortelezzi A. Possible association between reactive oxygen metabolites and karyotypic abnormalities in myelodysplastic syndromes. Haematologica 2003;88:594–7.
8. Campise M, Bamonti F, Novembrino C, Ippolito S, Tarantino A, Cornelli U, et al. Oxidative stress in kidney transplant patients. Transplantation 2003;76:1474–8.
9. Bounous G, Molson JN. The antioxidant system. Anticancer Res 2003;23:1411–5.
10. Evans P, Halliwell B. Micronutrients: oxidant/antioxidant status. Br J Nutr 2001;85(Suppl 2):S67–74.
11. Draper HH, Csallany AS, Hadley M. Urinary aldehydes as indicators of lipid peroxidation in vivo. Free Radical Biol Med 2000;29:1071–7.

12. Esterbauer H, Schaur RJ, Zoller H. Chemistry and biochemistry of 4-hydroxynonenal and malondialdehyde and related aldehydes. Free Radical Biol Med 1991;11: 81–128.
13. 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 1999; 226:222–9.
14. Mangoni AA, Arya R, Ford E, Asonganyi B, Sherwood RA, Ouldred E, et al. Effects of folic acid supplementation on inflammatory and thrombogenic markers in chronic smokers. A randomised controlled trial. Thromb Res 2003;110:13–7.
15. Carnevali S, Petruzzelli S, Longoni B, Vanacore R, Barala R, Cipollini M, et al. Cigarette smoke extract induces oxidative stress and apoptosis in human lung fibroblasts. Am J Physiol Lung Cell Mol Physiol 2003;284:L955–63.
16. Tsiara S, Elisaf M, Mikhailidis DP. Influence of smoking on predictors of vascular disease. Angiology 2003;54: 507–30.
17. 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 2001;131:1952–8.
18. Clarke R, Armitage J. Antioxidant vitamins and risk of cardiovascular disease. Review of large-scale randomised trials. Cardiovasc Drugs Ther 2002;16:411–5.
19. Mayne ST. Antioxidant nutrients and chronic disease: use of biomarkers exposure and oxidative stress status in epidemiologic research. J Nutr 2003;133(Suppl 3): 933S–40S.
20. Polidori MC, Macocci P, Stahl W, Sies H. Cigarette smoking cessation increases plasma levels of several antioxidant micronutrients and improves resistance towards oxidative challenge. Br J Nutr 2003;90:147–50.
21. 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 2003; 133:2188–93.
22. Kiefer I, Prock P, Lawrence C, Wise J, Bieger W, Bayer P, et al. Supplementation with mixed fruit and vegetable juice concentrates increased serum antioxidants and folate in healthy adults. J Am Coll Nutr 2004;23:205–11.
23. Plotnick GD, Corretti MC, Vogel RA, Hesslink R, Wise J. Effect of supplemental phytonutrients on impairment of the flow-mediated brachial artery vasoactivity after a single high-fat meal. J Am Coll Cardiol 2003;41:1744–9.
24. Panunzio MF, Pisano A, Antoniciello A, Di Martino V, Frisoli L, Cipriani V, et al. Supplementation with fruit and vegetable concentrate decrease plasma homocystine levels in a dietary controlled trial. Nutr Res 2003; 23:1221–8.
25. De Vecchi AF, Patrosso C, Novembrino C, Finazzi S, Colucci P, De Franceschi M, et al. Folate supplementation in peritoneal dialysis patients with normal erythrocyte folate: effect on plasma homocysteine. Nephron 2001;89:297–302.
26. Cighetti G, Allevi P, Anastasia L, Bortone L, Paroni R. Use of methyl malondialdehyde as an internal standard for malondialdehyde detection: validation by isotope-dilution gas chromatography-mass spectrometry. Clin Chem 2002;48:2266–9.
27. Young JF, Nielsen SE, Haraldsdottir J, Daneshvar B, Lauridsen ST, Knuthsen P, et al. Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status. Am J Clin Nutr 1999;69:87–94.
28. Bub A, Watzl B, Abrahamse L, Delincee H, Adams S, Wever J, et al. Moderate intervention with carotenoid-rich vegetable products reduces lipid peroxidation in man. J Nutr 2000;130:2200–6.
29. Leeds AR, Ferris EAE, Staley J, Ayesh R, Ross F. Availability of micronutrients from dried, encapsulated fruit and vegetable preparations: a study in healthy volunteers. J Hum Nutr Diet 2000;13:21–7.
30. Van den Berg R, van Vliet T, Broekmans WM, Cnubben NH, Vaes WH, Roza L, et al. A vegetable/fruit concentrate with high antioxidant capacity has no effect on biomarkers of antioxidant status in male smokers. J Nutr 2001; 131:1714–22.
31. Freese R, Alfthan G, Jauhiainen M, Basu S, Erlund I, Salminen I, et al. High intakes of vegetables, berries, and apples combined with a high intake of linoleic or oleic acid only slightly affect markers of lipid peroxidation and lipoprotein metabolism in healthy subjects. Am J Clin Nutr 2002;76:950–60.
32. Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of oxidant stress in vivo in chronic cigarette smokers. Circulation 1996;94:19–25.
33. Dietrich M, Block G, Hudes M, Morrow JD, Norkus EP, Traber MG, et al. Antioxidant supplementation decreases lipid peroxidation biomarkers F_2-isoprostanes in plasma of smokers. Cancer Epidemiol Biomarkers Prev 2002;11:7–13.
34. Dillon SA, Lowe GM, Billington D, Rahman K. Dietary supplementation with aged garlic extract reduces plasma and urine concentration of 8-iso-prostaglandin $F_{2\alpha}$ in smoking and nonsmoking men and women. J Nutr 2002;132:168–71.
35. Miller ER III, Appel LJ, Jiang L, Risby TH. Association between cigarette smoking and lipid peroxidation in a controlled feeding study. Circulation 1997;96:1097–101.
36. Cighetti G, Paroni R, Marzorati S, Borotto E, Giudici R, Magnanimi G, et al. Evaluation of oxidative stress in serum of critically ill patients by a commercial assay and gas chromatography-mass spectrometry. Clin Chem 2005;51:1515–7.

Received July 28, 2005, accepted January 18, 2006