Plasma Levels and Redox Status of Ascorbic Acid and Levels of Lipid Peroxidation Products in Active and Passive Smokers

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Both active and passive smoking are regarded as risk factors for various diseases. To clarify the effects of active and passive smoking on plasma vitamin C levels and lipid peroxidation status, we examined the plasma levels of ascorbic acid (AA), its redox status [ratio of dehydroascorbate (DHAA) to total AA], the levels of thiobarbiturate reactive substance (TBARS), and the levels of lipid peroxides (LPO) in smokers, nonsmokers, and nonsmokers regularly exposed to environmental cigarette smoke (passive smokers). The study population consisted of 149 healthy males: 75 active smokers (consumption of > 15 cigarettes/day for more than 5 years), 36 passive smokers (more than 10 hr/week exposure to environmental cigarette smoke), and 38 nonsmokers (no cigarette smoke exposure). There were no significant differences in plasma TBARS and LPO levels among the three groups. Plasma levels of AA, the reduced form of vitamin C, were significantly lower in active smokers than in the combined nonsmoking groups (7.2 ± 3.5 and 8.4 ± 3.4 µg/mL, respectively; $p < 0.05$). Although no significant differences were found in plasma DHAA levels among the three groups, the ratios of DHAA to total AA were significantly higher in active and passive smokers than nonexposed nonsmokers (11.2, 10.3, and 7.1%, respectively; $p < 0.05$). These results indicate that passive smoking, as well as direct inhalation of cigarette smoke, affects the redox status of plasma AA. In passive smokers, the altered redox status of plasma AA suggests an oxidative stress. *Key words*: ascorbic acid, environmental tobacco smoke, lipid peroxidation, passive smoking, redox status, tobacco smoke. *Environ Health Perspect* 108:105–108 (2000). [Online 5 January 2000]
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Environmental tobacco smoke (ETS) inhalation (passive smoking) as well as direct inhalation of smoke (active smoking) is associated with various diseases. Several investigators have reported that ETS exposure leads to increased risks of atherosclerotic vascular disease (*1,2*) and cancer (*3*). A number of mechanisms may be involved in the atherogenesis induced by passive smoking, such as dysfunction of endothelium (*4*), altered lipoprotein profiles in passive smokers (*5,6*), increase in oxidizability of low-density lipoprotein (LDL), and decrease in plasma ascorbic acid (AA) levels caused by ETS exposure (*7*). ETS may play a role in carcinogenesis because it contains a number of carcinogens and because increased levels of these carcinogens have been found in the blood and urine of smokers (*8*).

In recent years there has been increasing evidence that the intake of dietary antioxidant vitamins, such as vitamin C, vitamin E, and β-carotene, is associated with a decreased risk of atherosclerosis (*9*). Smokers have lower blood levels of these antioxidant vitamins (*10*). Vitamin C has antiatherogenic properties, including inhibition of LDL oxidation (*11,12*) and improvement of decreased endothelial function (*13–15*). The antioxidative effect of vitamin C is exerted by its reduced form, AA, whereas its oxidized form, dehydroascorbic acid (DHAA), is considered a marker of oxidative stress, such as smoking (*16*) and diabetes mellitus (*17*). Although the absolute levels of plasma AA in passive smokers are lower than those in nonsmokers (*18*), it remains unclear whether DHAA levels and the proportion of DHAA to total AA are altered in passive smokers.

Increased lipid peroxidation products have been observed in atherosclerotic plaques (*19*), and it has been suggested that smoking could induce atherosclerosis in part through the formation of oxidatively modified lipids and/or the generation of oxidative stress in the vascular wall. It has not only been reported that active smoking was associated with increased lipid peroxides in plasma (*20*), but also that ETS exposure affected plasma lipid peroxidation (*7*).

To investigate the effects of ETS exposure on oxidative stress in plasma and the major risk factors for atherosclerotic diseases, we determined plasma AA, DHAA, lipid peroxidation products, lipid levels, and other biochemical parameters in smokers and in nonsmokers with or without exposure to ETS.

Methods

Subjects. Subjects included male soldiers or male employees at the Simofusa Base of the Japan Maritime Self Defense Force (Simofusa, Japan). Subjects were examined by blood and physiologic examinations in periodical medical checks, and those with the following diseases or abnormal findings were excluded from the study population: cardiovascular and cerebrovascular diseases, acute and chronic inflammatory diseases, neoplastic diseases, diabetes mellitus, any endocrine diseases, renal dysfunction, liver dysfunction except for slight elevations of aspartate aminotransferases (up to 60 IU/L), abnormal data in fasting blood glucose (> 120 mg/dL), and significant excretion of urine glucose. Subjects taking supplemental vitamins or routine medication were also excluded.

The remaining subjects consisted of 156 healthy males who read, understood, and answered a questionnaire and signed the informed consent forms. Smoking habits and ETS exposure were evaluated by using a smoking-history questionnaire that allowed an assessment of the hours of ETS exposure in the worksite and the home. According to the results of the questionnaire, the subjects were divided into three groups: 75 active smokers (self-reported smokers), 36 passive smokers [self-reported nonsmokers with > 10 hr/week of ETS exposure for > 6 months; ETS(+)], 38 nonexposed nonsmokers [self-reported nonsmokers with < 2 hr/week ETS exposure for > 6 months; ETS(-)]. Seven subjects that did not fit into these classifications (nonsmokers with between 2 and 10 hr/week ETS exposure) were excluded from the study population. The subjects in the three study groups ranged in age from 35 to 56 years (41.5 ± 7.2, mean ± SD). Vitamin C intake of the subjects was determined by analysis of 3-day food records just before the day of blood sampling.

Blood sampling. After 12 hr fasting and abstinence from smoking, blood samples were obtained from the subjects. Blood was collected into vacutainers containing heparin as an anticoagulant and placed immediately on ice, and plasma was separated by centrifugation. Aliquots of the plasma samples were subjected to biochemical analyses, and the remainder was stored at -80°C for further study.

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Ascorbate and dehydroascorbate determination. Plasma samples were immediately mixed with four volumes of methanol. Precipitates were removed by centrifugation (4,000 × *g* at 4°C for 5 min) and supernatants were kept at -80°C for up to 2 weeks until analysis. AA and DHAA levels were measured by HPLC based on the methods of Barja et al. (*21*). Briefly, AA was separated by reversed-phase HPLC using an NH_2 column (25 × 0.46 cm, 5 µm particle size; Superco, Tokyo, Japan) eluted with methanol/40 mM NaH_2PO_4 (7:3, vol/vol) at 1.0 mL/min as the mobile phase and monitored at 265 nm in an ultraviolet detector. DHAA levels were determined by subtracting AA values from total AA levels after the addition of dithiothreitol (50 mM) as a reducing agent. There was no significant difference in concentrations between the freshly prepared samples and the identical aliquots stored at -80°C.

Biochemical analyses. Total plasma cholesterol, triglycerides, free cholesterol, and phospholipids were determined by enzymatic methods using commercially available enzymatic reagents (Kyowa Medex, Tokyo, Japan). High-density lipoprotein (HDL) cholesterol was measured by precipitating all of the other cholesterol fractions with phosphotungstate-magnesium (*22*). LDL cholesterol was estimated using the formula reported by Friedewald et al. (*23*). Plasma lipid hydroperoxide (LPO) was measured by using a commercially available kit (Determiner LPO; Kyowa Medex) based on a colorimetric method by assessing the reaction of a methylene blue derivative with lipid peroxides in the presence of heme compounds (*24*). Plasma thiobarbiturate reactive substance (TBARS) levels were determined by colorimetric assay reported by Buege and Aust (*25*). Plasma thiocyanate levels were determined by the method of Pettigrew and Fell (*26*).

Statistical analyses. Values were expressed as the mean ± SD. We used unpaired *t*-tests for comparison between groups. Associations between parameters were determined by Pearson's product-moment correlation coefficients. Values with $p < 0.05$ were considered significant.

Results

Table 1 shows the characteristics of the subjects included in this study, including age, body mass index (BMI), blood pressure, number of cigarettes smoked per day, alcohol consumption, and vitamin C intake. Although alcohol consumption was significantly higher in the active smoking (AS) group than in the nonsmoking (NS) group, there were no significant differences in age, BMI, blood pressure, or vitamin C intake between the groups.

Biochemical parameters of blood samples in each group were assessed (Table 2). Plasma triglyceride levels were higher in the AS group than in the NS group. Besides the triglyceride levels, there were no other significant differences in fasting blood glucose, uric acid, or plasma lipid levels. Plasma thiocyanate, a marker of smoking exposure, was significantly higher not only in the AS group, as compared to the NS group, but also in the ETS(+) group, as compared to the ETS(-) group. There was a significant positive correlation between plasma thiocyanate levels and cumulative hours of exposure to ETS in the ETS(+) group (Figure 1), indicating the reliability of the questionnaire used to assess ETS exposure. Table 3 shows the levels of plasma AA and DHAA and the ratio of DHAA to total AA (%DHAA). Total plasma AA levels did not differ significantly between the AS and NS groups or between the ETS(+) and ETS(-) groups. However, the mean level of the reduced form of vitamin C was significantly higher in the NS group than in the AS group. There was no significant difference in AA levels between the ETS(+) and the ETS(-) groups. Although absolute DHAA levels were not significantly different among the groups, %DHAA was significantly higher in the AS and ETS(+) groups than in the NS and ETS(-) groups, respectively. Plasma LPO and TBARS levels showed no significant differences among the groups (Table 4).

Discussion

ETS is related to important medical consequences, including increased risk of cardiovascular disease (*1,2*) and cancer (*3*). Several epidemiologic studies investigating atherosclerotic cardiovascular disease have been reported in recent years, including one estimate of up to 40,000 excess heart disease deaths/year attributed to ETS exposure (*1*). It is likely that the mechanisms of passive smoking-induced atherogenesis are similar to the mechanisms of active smoking-induced disease. Several studies have investigated the effects of ETS and its role in atherogenesis, such as increased thickness of carotid artery walls in humans (*27*), enhanced platelet aggregation (*28*), altered atherogenic lipoprotein profile (*5,6*), and inhibition of endothelium-dependent vasodilatation (*4*).

Decreased levels of antioxidative vitamins such as vitamins C and E are considered one of the mechanisms for the induction of atherosclerosis by smoking. The protective effect of fruit consumption against atherosclerotic disease has been well documented in several epidemiologic studies, suggesting the beneficial contribution of increased vitamin C intake (*29*). Kritchevsky et al. (*30*) reported a significant inverse relationship between vitamin C intake and carotid artery wall thickness as evaluated by B-mode ultrasound.

AA, the reduced form of vitamin C, is an important cofactor in several enzyme reactions and plays a pivotal role in the defense against oxidative stress (*31,32*). Mammalian cells efficiently regenerate AA from its two-electron oxidized form, DHAA. The recycling of DHAA to AA is mediated by glutathione (*33*). Kagan et al. (*34*) reported that AA had the potential to regenerate α-tocopherol from

Table 1. Clinical characteristics of subjects.

Characteristics	AS (*n* = 75)	NS (*n* = 74)	NS ETS(-) (*n* = 38)	NS ETS(+) (*n* = 36)
Age (years)	42.6 ± 5.7	43.0 ± 5.8	44.0 ± 6.5	42.0 ± 4.8
BMI (kg/m^2)	24.0 ± 2.5	23.9 ± 2.3	23.7 ± 1.8	24.1 ± 2.5
Systolic blood pressure (mmHg)	124.6 ± 14.9	125.2 ± 13.6	124.1 ± 13.4	126.4 ± 13.9
Diastolic blood pressure (mmHg)	78.1 ± 11.7	79.3 ± 10.4	78.7 ± 9.6	79.9 ± 11.2
Cigarettes (no./day)	20.9 ± 7.1	–	–	–
Alcohol consumption (g/week)	388 ± 427	166 ± 199*	138 ± 182	191 ± 214
Vitamin C intake (mg/day)	112 ± 65	120 ± 78	119 ± 57	122 ± 63

Abbreviations: AS, active smokers; NS, nonsmokers, BMI, body mass index.
Values represent mean ± SD. Differences between the groups were compared by unpaired Student's *t*-test.
*$p < 0.001$, AS versus NS.

Table 2. Biochemical parameters of plasma.

Biochemical parmeters	AS (*n* = 75)	NS (*n* = 74)	NS ETS(-) (*n* = 38)	NS ETS(+) (*n* = 36)
Fasting blood glucose (mg/dL)	97.4 ± 10.0	101.4 ± 15.0	101.1 ± 18.7	101.8 ± 9.5
Uric acid (mg/dL)	6.1 ± 1.1	5.7 ± 1.3	5.8 ± 1.4	5.7 ± 1.3
Total cholesterol (mg/dL)	202.4 ± 32.5	198.8 ± 29.3	196.7 ± 28.9	201.2 ± 30.3
HDL cholesterol (mg/dL)	55.6 ± 15.6	57.0 ± 14.1	58.5 ± 16.0	55.4 ± 11.8
LDL cholesterol (mg/dL)	116.6 ± 30.8	121.1 ± 26.8	116.6 ± 22.6	126.0 ± 30.3
Triglycerides (mg/dL)	150.9 ± 113.2	103.4 ± 56.9**	107.6 ± 65.6	98.9 ± 46.1
Phospholipids (mg/dL)	218.9 ± 84.2	213.0 ± 74.1	205.1 ± 75.3	221.1 ± 73.0
Thiocyanate (µmol/L)	78.2 ± 33.9	23.0 ± 20.3#	18.1 ± 10.6	28.0 ± 26.2##

Values represent mean ± SD. Differences between the groups were compared by unpaired Student's *t*-test.
**$p < 0.01$ and #$p < 0.001$, AS versus NS. ##$p < 0.05$, ETS(-) versus ETS(+).

the α-tocopheryl radical with the subsequent change of AA to DHAA. Thus AA, either directly or indirectly, contributes to an antioxidative effect. DHAA is believed to be a marker of oxidative stress caused by diabetes mellitus (*17*) or smoking (*16*). Although cigarette smoke affects the redox status of plasma AA, forming DHAA (*35*), several investigators have also reported unfavorable effects of DHAA itself. Patterson (*36*) reported that DHAA had cytotoxic and diabetogenic effects, and Stait and Leake (*11*) reported that whereas AA inhibited LDL oxidation by copper, DHAA accelerated this process.

We found decreased absolute levels of AA and increased %DHAA in the AS group, which is consistent with previous studies (*16*). Because there were no differences in vitamin C intake among the groups, the decrease of plasma AA caused by cigarette smoking appears to occur predominantly via mechanisms independent of dietary vitamin C intake levels (*37*). Although cigarette smoke directly induces the depletion of vitamin C (*35*), the kinetic studies of Kallner et al. (*38*) showed an increased turnover of vitamin C in smokers. Therefore, the low levels of plasma vitamin C in smokers may be due to increased consumption by oxidants contained in cigarette smoke. Moreover, %DHAA levels were higher in the AS group, implying that smoking could induce oxidative stress. Of further interest is the finding that the %DHAA in passive smoking subjects, although it is lower than that observed in the AS group, was still significantly higher than that in nonsmokers without ETS exposure. Several investigators have suggested that plasma thiocyanate is a good marker of passive smoking (*39*). We also observed significantly higher plasma thiocyanate levels in passive smokers and a positive correlation between plasma thiocyanate and cumulative hours of ETS exposure, indicating that sidestream smoke inhaled indirectly could induce biologic reactions. Davis et al. (*28*) reported that toxic compounds were present in sidestream smoke, which contributes more to ETS than mainstream smoke. Although the actual levels of oxidants contained in environmental polluted air were unclear, it was suggested that inhalation of sidestream smoke could increase the oxidation of AA at least as well as mainstream smoke (because of the radical properties of AA). We found no significant relationship between %DHAA and any of the examined passive or active smoking parameters such as cumulative hours of ETS exposure, plasma thiocyanate, or the number of cigarettes smoked per day (data not shown). However, it has been suggested that the concentrations and the species of oxidants contained in mainstream smoke and environmental air are heterogeneous (*40*).

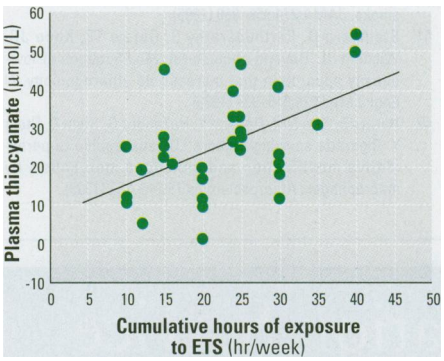

Figure 1. Correlation between plasma thiocyanate levels and cumulative hours of ETS exposure in the ETS(+) group. $r = 0.481$, $p = 0.003$.

We observed that the plasma triglyceride levels in the smokers were higher than those in nonsmokers, whereas HDL cholesterol levels were not different between the two groups. These results might be due to a greater consumption of alcohol in the smokers than in the nonsmokers. According to our questionnaire, there was a high prevalence of alcohol drinkers in the smoking group. The levels of plasma triglyceride were significantly correlated with alcohol consumption in our subjects. In addition, %DHAA had no association with plasma triglyceride levels or alcohol consumption when a multiple regression analysis was performed for all subjects (data not shown). Therefore, plasma triglyceride levels and alcohol consumption were not likely to be the main causes of the difference in %DHAA between the groups.

Oxidative modification of LDL has been implicated in the pathogenesis of atherosclerosis (*41*). Lipid peroxidation products are reportedly found in human atherosclerotic lesions (*19*). Plasma from smokers contains more lipid peroxides (*20*), and LDL from smokers is more susceptible to oxidative modification than that from nonsmokers (*42*). Additionally, Valkonen and Kuusi (*7*) recently reported increased LDL oxidizability in passive smokers. In the present study we found no significant differences in plasma TBARS and lipid peroxide levels among the subject groups. Frei et al. (*32,35*) reported that lipid peroxidation in plasma occurred after complete depletion of vitamin C. Additionally, Harats et al. (*42*) showed that there was no difference in TBARS levels in LDL isolated from smokers and nonsmokers; however, the LDL from smokers was more readily oxidized than the LDL from nonsmokers. Therefore, the decreased plasma vitamin C, increased %DHAA, and unaffected plasma lipid peroxidation products found in the present data are consistent with the results of these previous studies.

We observed that passive smoking, as well as direct inhalation of cigarette smoke, affected the redox status of plasma vitamin C. This indicates that both active and passive smoking could induce the generation of oxidative stress. Unfavorable changes in the redox status of plasma vitamin C may be a mechanism of the induction of atherogenesis induced by passive smoking.

Table 3. Plasma total AA, reduced AA, DHAA, and proportion of DHAA relative to total AA (%DHAA).

Plasma	AS ($n = 75$)	NS ($n = 74$)	NS ETS(-) ($n = 38$)	NS ETS(+) ($n = 36$)
Total AA (µg/mL)	8.12 ± 3.87	9.13 ± 3.49	9.44 ± 3.92	8.80 ± 2.98
Reduced AA (µg/mL)	7.22 ± 3.48	8.38 ± 3.36*	8.80 ± 3.77	7.92 ± 2.84
DHAA (µg/mL)	0.90 ± 0.70	0.76 ± 0.64	0.64 ± 0.65	0.88 ± 0.62
%DHAA (%)	11.2 ± 6.96	8.62 ± 6.77*	7.07 ± 6.24	10.3 ± 7.00**

Values represent mean ± SD. Differences between the groups were compared by unpaired Student's *t*-test.
*$p < 0.05$, AS versus NS. **$p < 0.05$, ETS(-) versus ETS(+).

Table 4. Plasma LPO and TBARS.

Plasma	AS ($n = 75$)	NS ($n = 74$)	NS ETS(-) ($n = 38$)	NS ETS(+) ($n = 36$)
LPO (nmol/L)	16.6 ± 8.0	15.6 ± 6.4	15.6 ± 6.6	15.6 ± 6.4
LPO/total lipids (nmol/g)	51.3 ± 27.3	56.6 ± 28.3	58.1 ± 29.9	54.9 ± 27.3
TBARS (nmol/L)	5.42 ± 2.32	4.94 ± 0.95	4.89 ± 0.97	4.99 ± 0.94

Total lipids indicate the value of sum of total cholesterol, triglyceride, and phopholipids. Values represent mean ± SD. Differences between the groups were compared by unpaired Student's *t*-test.

References and Notes

1. Glantz SA, Parmley WW. Passive smoking and heart disease. Epidemiology, physiology, and biochemistry. Circulation 83:1–12 (1991).
2. Steenland K. Passive smoking and the risk of heart disease. JAMA 267:94–99 (1992).
3. Wald NJ. Does breathing other people's tobacco smoke cause lung cancer? Br Med J 293:1503–1504 (1986).
4. Celermajer DS, Adams MR, Clarkson P, Robinson J, McCredie R, Donald A, Deanfield JE. Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. N Engl J Med 334:150–154 (1996).
5. Moffatt RJ, Stamford BA, Biggerstaff KD. Influence of worksite environmental tobacco smoke on serum lipoprotein profiles of female nonsmokers. Metabolism 44:1536–1539 (1995).

6. Neufeld EJ, Mietus SM, Beiser AS, Baker AL, Newburger JW. Passive cigarette smoking and reduced HDL cholesterol levels in children with high-risk lipid profiles. Circulation 96:1403–1407 (1997).
7. Valkonen M, Kuusi T. Passive smoking induces atherogenic changes in low-density lipoprotein. Circulation 97:2012–2016 (1998).
8. Hecht SS, Carmella SG, Murphy SE, Akerkar S, Brunnemann KD, Hoffmann D. A tobacco-specific lung carcinogen in the urine of men exposed to cigarette smoke. N Engl J Med 329:1543–1546 (1993).
9. Jha P, Flather M, Lonn E, Farkouh M, Yusuf S. The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. Ann Intern Med 123:860–872 (1995).
10. Mezzetti A, Lapenna D, Pierdomenico SD, Calafiore AM, Costantini F, Riario SG, Imbastaro T, Neri M, Cuccurullo F. Vitamins E, C and lipid peroxidation in plasma and arterial tissue of smokers and non-smokers. Atherosclerosis 112:91–99 (1995).
11. Stait SE, Leake DS. The effects of ascorbate and dehydroascorbate on the oxidation of low-density lipoprotein. Biochem J 320:373–381 (1996).
12. Fuller CJ, Grundy SM, Norkus EP, Jialal I. Effect of ascorbate supplementation on low density lipoprotein oxidation in smokers. Atherosclerosis 119:139–150 (1996).
13. Heitzer T, Just H, Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. Circulation 94:6–9 (1996).
14. Ting HH, Timimi FK, Boles KS, Creager SJ, Ganz P, Creager MA. Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. J Clin Invest 97:22–28 (1996).
15. Ting HH, Timimi FK, Haley EA, Roddy MA, Ganz P, Creager MA. Vitamin C improves endothelium-dependent vasodilation in forearm resistance vessels of humans with hypercholesterolemia. Circulation 95:2617–2622 (1997).
16. Lykkesfeldt J, Loft S, Nielsen JB, Poulsen HE. Ascorbic acid and dehydroascorbic acid as biomarkers of oxidative stress caused by smoking. Am J Clin Nutr 65:959–963 (1997).
17. Som S, Basu S, Mukherjee D, Deb S, Choudhury PR, Mukherjee S, Chatterjee SN, Chatterjee IB. Ascorbic acid metabolism in diabetes mellitus. Metabolism 30:572–577 (1981).
18. Tribble DL, Giuliano LJ, Fortmann SP. Reduced plasma ascorbic acid concentrations in nonsmokers regularly exposed to environmental tobacco smoke. Am J Clin Nutr 58:886–890 (1993).
19. Yla-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest 84:1086–1095 (1989).
20. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LN. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med 332:1198–1203 (1995).
21. Barja G, Hernanz A. Vitamin C, dehydroascorbate, and uric acid in tissues and serum: high-performance liquid chromatography. Methods Enzymol 234:331–337 (1994).
22. Lipid Research Clinics Program. Manual of Laboratory Operations, Vol 1. Lipid and Lipoprotein Analysis. DHEW Publ No. (NIH) 95-628. Bethesda, MD:National Institutes of Health, 1975.
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502 (1972).
24. Ohishi N, Ohkawa H, Miike A, Tatano T, Yagi K. A new assay method for lipid peroxides using a methylene blue derivative. Biochem Int 10:205–211 (1985).
25. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 52:302–310 (1978).
26. Pettigrew AR, Fell GS. Simplified colorimetric determination of thiocyanate in biological fluids, and its application to investigation of the toxic amblyopias. Clin Chem 18:996–1000 (1972).
27. Howard G, Burke GL, Szklo M, Tell GS, Eckfeldt J, Evans G, Heiss G. Active and passive smoking are associated with increased carotid wall thickness. The Atherosclerosis Risk in Communities Study. Arch Intern Med 154:1277–1282 (1994).
28. Davis JW, Shelton L, Watanabe IS, Arnold J. Passive smoking affects endothelium and platelets. Arch Intern Med 149:386–389 (1989).
29. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 342:1007–1011 (1993).
30. Kritchevsky SB, Shimakawa T, Tell GS, Dennis B, Carpenter M, Eckfeldt JH, Peacher RH, Heiss G. Dietary antioxidants and carotid artery wall thickness. The ARIC Study. Atherosclerosis Risk in Communities Study. Circulation 92:2142–2150 (1995).
31. Levine M. New concepts in the biology and biochemistry of ascorbic acid. N Engl J Med 314:892–902 (1986).
32. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA 86:6377–6381 (1989).
33. Rose RC, Bode AM. Tissue-mediated regeneration of ascorbic acid: is the process enzymatic? Enzyme 46:196–203 (1992).
34. Kagan VE, Serbinova EA, Forte T, Scita G, Packer L. Recycling of vitamin E in human low density lipoproteins. J Lipid Res 33:385–397 (1992).
35. Frei B, Forte TM, Ames BN, Cross CE. Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. Biochem J 277:133–138 (1991).
36. Patterson JW. The diabetogenic effects of dehydroascorbic and dehydroisoascorbic acid. J Biol Chem 183:81–88 (1950).
37. Lykkesfeldt J, Prieme H, Loft S, Poulsen HE. Effect of smoking cessation on plasma ascorbic acid concentration. BMJ 313:91 (1996).
38. Kallner AB, Hartmann D, Hornig DH. On the requirements of ascorbic acid in man: steady-state turnover and body pool in smokers. Am J Clin Nutr 34:1347–1355 (1981).
39. Chen Y, Pederson LL, Lefcoe NM. Exposure to environmental tobacco smoke (ETS) and serum thiocyanate level in infants. Arch Environ Health 45:163–167 (1990).
40. Hammond SK, Sorensen G, Youngstrom R, Ockene JK. Occupational exposure to environmental tobacco smoke. JAMA 274:956–960 (1995).
41. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 320:915–924 (1989).
42. Harats D, Ben NM, Dabach Y, Hollander G, Stein O, Stein Y. Cigarette smoking renders LDL susceptible to peroxidative modification and enhanced metabolism by macrophages. Atherosclerosis 79:245–252 (1989).

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