Free Radical Biology & Medicine, Vol. 28, No. 3, pp. 428–436, 2000

0891-5849/00/$–see front matter

PII S0891-5849(99)00260-9

Original Contribution

VITAMIN C PREVENTS THE ACUTE ATHEROGENIC EFFECTS OF PASSIVE SMOKING

MIIA M. VALKONEN and TIMO KUUSI

Department of Medicine, University of Helsinki, Helsinki, Finland

(*Received* 30 *August* 1999; *Revised* 18 *November* 1999; *Accepted* 29 *November* 1999)

Abstract—During passive smoking the body is attacked by an excess of free radicals inducing oxidative stress. In nonsmoking subjects even a short period of passive smoking breaks down serum antioxidant defense (TRAP) and accelerates lipid peroxidation leading to accumulation of their low-density lipoprotein (LDL) cholesterol in cultured human macrophages. We now studied whether these acute proatherogenic effects of secondhand smoke could be prevented by an effective free radical scavenger, vitamin C. Blood samples were collected from nonsmoking subjects ($n = 10$) as they were consecutively exposed to normal air or cigarette smoke during four separate days. During the last 2 d, a single dose of vitamin C (3 g) was given, which doubled its plasma concentration. Vitamin C did not influence the plasma antioxidant defense or the resistance of LDL to oxidation in normal air, but prevented the smoke-induced decrease in plasma TRAP ($p < .001$), the decrease in the resistance of LDL to oxidation ($p < .05$), and the accelerated formation of serum thiobarbituric acid reactive substances (TBARS) ($p < .05$) otherwise observed 1.5 h after the beginning of passive smoking. Vitamin C protected nonsmoking subjects against the harmful effects of free radicals during exposure to secondhand smoke. © 2000 Elsevier Science Inc.

Keywords—Passive smoking, Atherosclerosis, Coronary disease, Vitamin C, Lipid peroxidation, Antioxidant defense, Free radicals

INTRODUCTION

The protective role of vitamin C against cardiovascular diseases (CVD) is still controversial [1,2]. Epidemiological studies have shown that low-plasma vitamin C is a risk factor for coronary heart disease and its acute clinical manifestations [3–5], but the few existing supplementation trials with vitamin C have thus far failed to show an effect on cardiovascular morbidity [2].

The mechanism by which vitamin C could be protective against CVD can be attributed to its effects on vascular cells and lipoprotein profile or to its antioxidative characteristics [1,6,7–9]. Vitamin C is an effective free radical scavenger and among the strongest determinants of plasma total antioxidant defense [10,11]. Furthermore, it effectively reduces LDL oxidation in vitro [10,12]. Oxidized LDL promotes the initiating events of atherosclerosis and has a regulatory role for several aspects of arterial wall metabolism that might render it atherogenic and contributory to manifestation of CV morbidity. Thus, by inhibiting LDL oxidation vitamin C could both reduce atherogenesis and its clinical consequencies [1,13–17]. In nonsmoking subjects, however, trials on the efficacy of vitamin C supplementation in protecting lipids from peroxidation have provided conflicting evidence [18].

Cigarette smoking (CS) increases the risk of CHD, myocardial infarction, and cardiac death [19]. Smoking also increases the rates of in vivo and in vitro lipid peroxidation [20–23] and reduces the levels of plasma ascorbic acid [24,25]. Thus, smokers could be a target group for vitamin C supplementation. Indeed, vitamin C reduces the increased circulating products of lipid peroxidation, F2-isoprostanes, in smokers [26] and in some studies also the susceptibility of LDL to ex vivo oxidation [27–29]. In addition, vitamin C has other beneficial effects in smokers, because it prevents the increased adhesiveness of monocytes to endothelium and the formation of leukocyte-platelet aggregates [30,31]. It also neutralizes reactive oxidants released by hyperactive

Address Correspondence to: Dr. Timo Kuusi, Department of Medicine, Helsinki University Hospital, Haartmaninkatu 4, 00290 Helsinki 29, Finland; Tel: +358 9-4712213; Fax: +358 9-4714012; E-Mail: timo.kuusi@huch.fi.

phagocytes [32] and improves endothelial dysfunction [33]. However, no clinical trials yet exist to clarify the effect of vitamin C on cardiovascular morbidity in smokers.

An analysis of recent epidemiological studies showed that passive smoking increases the risk of CHD by a fourth [34]. Thus, the heart disease risk of a passive smoker is almost half of the risk of an active smoker even if the quantity of inhaled smoke is only one percent of that coming from a daily pack of cigarettes [34]. Furthermore, it has been suggested that in nonsmokers, even a short period (10–30 min) of exposure to CS is enough to induce platelet aggregation, endothelial damage, endothelial dysfunction, and measurable changes in antioxidant defense and lipid peroxidation [22,35,36]. Thus, the smoke induced oxidative stress is probably more pronounced in subjects whose cardiovascular system lacks adaptation to smoke [37].

We have recently demonstrated that an acute exposure of nonsmoking subjects to CS leads to a decrease in plasma TRAP [22]. Furthermore, LDL isolated after passive smoking appeared to be susceptible to further peroxidation by copper leading to accelerated production of lipid peroxidation products, conjugated dienes and TBARS, and finally to accelerated accumulation of LDL cholesterol to human monocyte macrophages [22]. The present study was undertaken to find out whether this pro-oxidative cascade induced by CS could be blocked at its initial stage by vitamin C, an effective free radical scavenger.

METHODS

Blood samples were taken from 10 (5 women and 5 men) nonsmoking, normolipidemic subjects, without any evidence of disease and aging from 23 to 48 years. All subjects had smoke-free homes, worked in a smoke-free environment, and were advised to avoid environmental smoke during their free time and for at least 48 h before entering the study.

The nonsmoking subjects were consecutively exposed to normal air or passive smoking during 4 ordinary working d and thus served as their own controls. The exposure periods were spent in normal office rooms, thus in a smoke-free area, or in a room used specifically by active smokers. On day one, the subjects spent 30 min in normal air; and were, on day 2, exposed to 30 min of passive smoking. On day three, the subjects were exposed to passive smoking, and on day four to normal air. In addition, 3 g of ascorbic acid (C-Vimin, Astra, Sweden) was taken 2 h before the exposure on days three and four. Blood samples were collected before and 1.5 h after the beginning of 30 min exposure. Two and seven normal working days in a smoke-free area separated days two and three, and days three and four, respectively, in order to avoid the possible ongoing effect of CS or vitamin C from the preceding day of the study. The subjects had their normal breakfast before the study and lunch during the study. They were exposed to smoke for 30 min in the 88 m^3 ventilated room (ventilation rate 600 l/s), where 16 cigarettes were consumed during the exposure by active smokers. After passive smoking, the subjects continued working in their smoke-free environment. All participants reported palpitation and symptoms such as headache or nausea. In preliminary experiments no changes were found in plasma HbCO, determined in the routine clinical laboratory, in accordance with previous data [38]. The study was approved by the local ethical committee.

Blood samples were collected at zero time and 1.5 h after the beginning of the exposure into tubes kept on ice. The effect of passive smoking peaks at 1.5 h, which was thus used as study period [22]. Samples for plasma vitamin C determinations were also collected 2 h before time zero, thus before the subjects took vitamin C. Plasma containing 1mg/mL of ethylenediaminetetraacetate (EDTA) was separated by centrifugation at +4°C. Unless used immediately, samples were stored at −80°C and used within 8 weeks. Uric acid was measured in a Cobas Mira-S Centrifugal Analyzer (Roche Inc., Basel, Switzerland) using reagents of Roche (Cat. No: 0736813). Vitamin C was determined according to the method of Denson et al. [39]. Serum lipid soluble antioxidants, α-tocopherol, β-carotene, and retinol (available from seven subjects), were determined by high-performance liquid chromatography [40]. Serum sulphydryl groups were determined by the method described by Ellman et al. [41]. The plasma total antioxidant defense or the combined capacity of all plasma antioxidants to resist artificially induced peroxidation, i.e., the total peroxyl radical trapping potential of plasma (TRAP), was quantitated as described previously [42] in a computerized system. The TRAP can be determined either directly from the serum samples (measured TRAP) or by calculating the sum capacity of all major antioxidants to trap free radicals [42].

LDL was isolated by rate-zonal ultracentrifugation in a density gradient at 40,000 rpm, at 4°C for 2.5 h in a Beckman L8-70 ultracentrifuge using Beckman swing-out rotors, as described in detail in [42] . All gradient solutions contained EDTA. Before oxidation, EDTA was separated from LDL using small dextran-sulfate affinity columns (Liposorber LA-15, Kaneka Co., Osaka, Japan). Lipid peroxidation was initiated by adding freshly prepared $CuSO_4$ solution to a final concentration of 10.4 μmol/l and the formation of conjugated dienes was monitored at a wavelength of 234 nm using standard techniques in a computerized system [43]. The resistance of

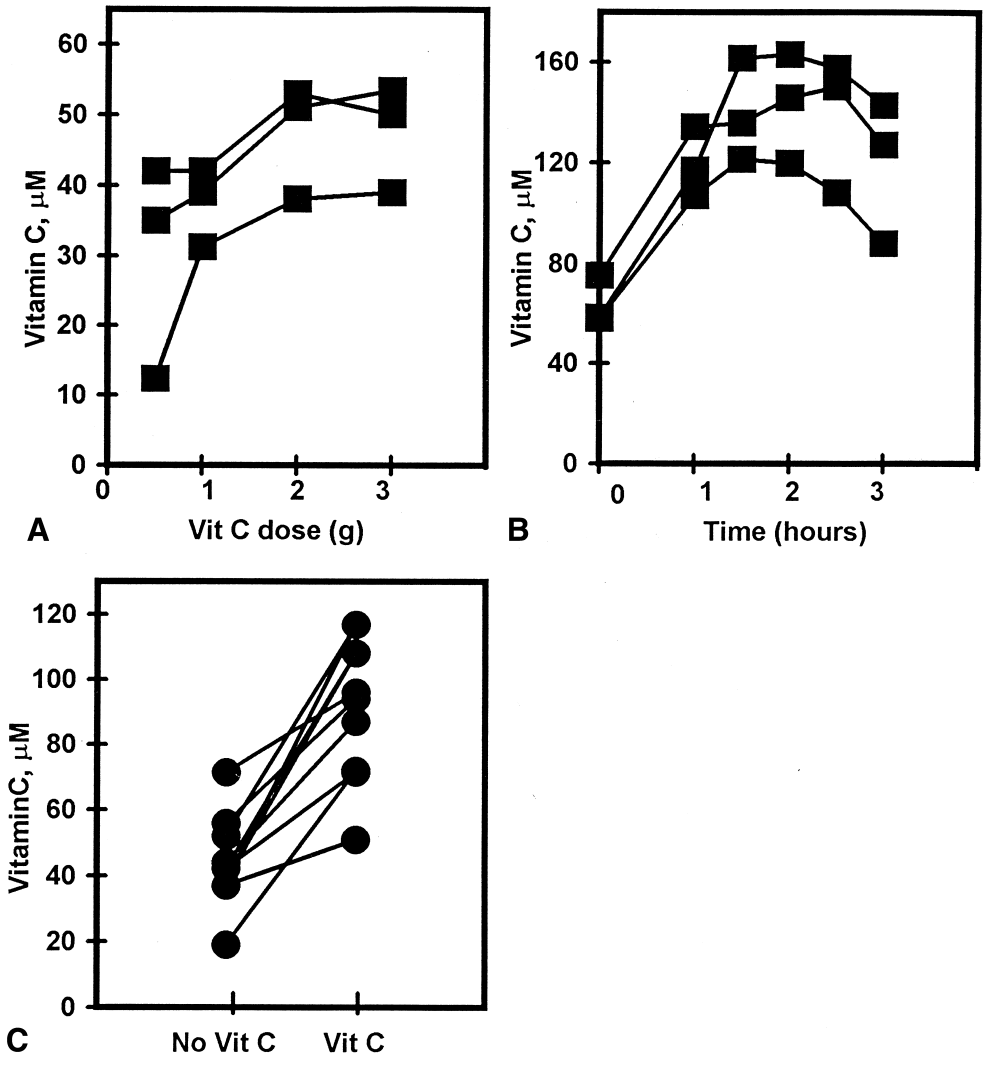

Fig. 1. Plasma vitamin C after ingestion of vitamin C. (A) Plasma vitamin C was determined 2 h after the indicated dose of vitamin C. (B) Plasma vitamin C at the indicated time intervals after ingesting single dose of 3 g. (C) Individual plasma vitamin C concentration ($n = 10$) before entering smoky air without or with vitamin C supplementation 2 h earlier.

LDL to oxidation was derived from the length of the lag time (min) before the propagation of the reaction. The thiobarbituric acid-reactive substances (TBARS) in plasma were determined as described previously [44]. The samples from different study days from one subject were analyzed simultaneously in parallel cuvettes.

The statistical analysis was performed by using the Systat statistical package. Values obtained before and 1.5 h after the start of spending half an hour in a smoke-free area or in a smoking room were tested by paired two-sample Student's *t*-test for means. Paired Student's *t*-test for means was also used in comparing the baseline values between the days to evaluate the effect of vitamin C in normal air. Analysis of variance (ANOVA) was used to calculate the overall significancies between the 2 d of smoke exposure with or without vitamin C and to compare these to their corresponding control periods. The values are presented as means ± SE.

RESULTS

The dose and timing of vitamin C supplementation were first optimized (Fig. 1). Plasma vitamin C concentration increased dose dependently (Fig. 1A) reaching its maximal level in 2 h after the ingestion of 3 g of vitamin C (Fig. 1B). Accordingly, the subjects took 3 g of vitamin C 2 h before the exposure to CS or normal air. This caused a 2.1-fold increase in the mean plasma vitamin C concentration, within the physiological range (30 to 150 mol/L) [8], from 44.5 to 94 mol/l (Fig. 1C). The concentrations of protein sulfydryl (SH-) groups, retinol, β-carotene, vitamin E, and the values of TRAP, mea-

sured directly from the plasma samples, were similar 2 h after ingestion of vitamin C to those determined without the preceding supplementation (data not shown). However, plasma uric acid was 10.5% lower 2 h after administration of vitamin C than without it ($n = 10$, $p < .05$). Moreover, vitamin C had no effect on the resistance of LDL to oxidation or on the formation of plasma TBARS at baseline.

The nonsmoking subjects were then exposed to secondhand smoke (day two), first without the preceding vitamin C supplementation. In accordance with the previous data [22], a significant decrease in plasma vitamin C 1.5 h after passive smoking was evident (Fig. 2A) and other plasma antioxidant substances, such as urate (Fig. 2B), protein SH-groups (Fig. 2C), or lipid-soluble antioxidants did not change (Figs. 2D–2F) significantly. However, failure in plasma total antioxidant defense occurred after passive smoking, since both measured (Figs. 3A and 4A) and calculated TRAP (Fig. 3B) decreased significantly. Moreover, after passive smoking a uniform decrease in the lag times of LDL oxidation could be demonstrated in all subjects. Thus, the mean lag times were 23% shorter 1.5 h after the beginning of smoke exposure (Figs. 3C and 4C). Similarly, a significant increase in plasma TBARS was evident (Figs. 3D and 4B).

The subjects were then exposed to CS (day three) with a preceding vitamin C supplementation. Their plasma vitamin C was now twice higher than before the first smoke exposure (Fig 1C), followed by a decline after the peak at 2 h after its ingestion (Fig. 1B). The decrease of plasma vitamin C observed 1.5 h after the beginning of the 30 min exposure was more prominent after passive smoking (day three) than after the control period (day four, Fig. 2A). No significant decreases were observed in the plasma concentrations of other individual antioxidants (Figs. 2B–2F). Notably, the CS–induced failure in TRAP, measured directly from the plasma samples, could be prevented by vitamin C (Figs. 3A and 4A). The calculated TRAP decreased also after vitamin C supplementation (Fig. 3B). Furthermore, after vitamin C supplementation the uniform decrease in the lag times of LDL oxidation observed after passive smoking disappeared (Figs. 3C and 4C). Similarly, the formation of plasma TBARS was significantly lower after vitamin C supplementation (Figs. 3D and 4B).

Interindividual variation in the protective effect of vitamin C against LDL oxidation ex vivo was evident. In fact, in 3 out of 10 subjects vitamin C did not prevent the decrease in the lag times. No significant differences after ingestion of vitamin C in these 3 subjects were found in the rise of plasma vitamin C, in other plasma aqueous and lipid soluble antioxidants, in the production of plasma TBARS, or in plasma antioxidant defense, compared to others.

DISCUSSION

We have recently demonstrated that 30 min in CS is sufficient to induce measurable changes in plasma antioxidant defense (TRAP) [22]. Furthermore, a decrease in the resistance of LDL to ex vivo oxidation and accumulation of lipid peroxides (TBARS) in plasma takes place. All these changes could be demostrated also in the present study during the first smoke exposure in the absence of any vitamin C supplementation (Figs. 2–4). Contrary to our previous findings a minor reduction ($p < .05$) in plasma uric acid after passive smoking took place (Fig. 2B). This reduction was, however, not significant when compared with the corresponding change in normal air ($p < .06$). In order to prevent the harmful effects of secondhand smoke the nonsmoking subjects were given vitamin C to prevent the failure in plasma total antioxidant defense.

The 2-fold increase of plasma vitamin C after ingestion of a 3 g dose is in agreement with earlier studies [8]. This dose of vitamin C doubled its mean plasma concentration (Fig. 1C) and caused a significant decrease in plasma uric acid also consistent with an earlier study [45]. The decrease in plasma urate was, however, confirmed in separate experiments where a mean decrease of plasma uric acid from 173 to 126 umol/l occurred in 2 h after the 3 g dose of vitamin C (data not shown, $p < .01$, $n = 3$). The decrease is likely to be due to increased renal excretion of urate induced by dose of vitamin C exceeding 1 g [45]. The concentrations of other antioxidants under study, were not influenced after the single dose of vitamin C (Fig. 2).

The calculated TRAP, obtained by calculating the sum capacity of all major antioxidants to trap free radicals, increased significantly after vitamin C supplementation ($p < .05$). The 2-fold increase in plasma vitamin C multiplied by the number of free radicals scavenged by its each molecule, 1.7 (stoichiometric factor) [42] obviously contributes greatly to calculated TRAP (Fig. 3) [42]. At higher concentrations this stoichiometric factor has been shown to overestimate the contribution of vitamin C because the reducing capacity of vitamin C is concentration dependent [46]. Above concentrations exceeding 50 μM a lower stoichiometric factor, 0.5, [46] is recommended and when used in calculations the TRAP no longer significantly differed from that in the absence of vitamin C ($p < .08$). In accordance, the measured TRAP values, determined experimentally from the plasma samples were not influenced by the large dose of vitamin C (Figs. 3A and 4A). This is in agreement with other studies where no significant

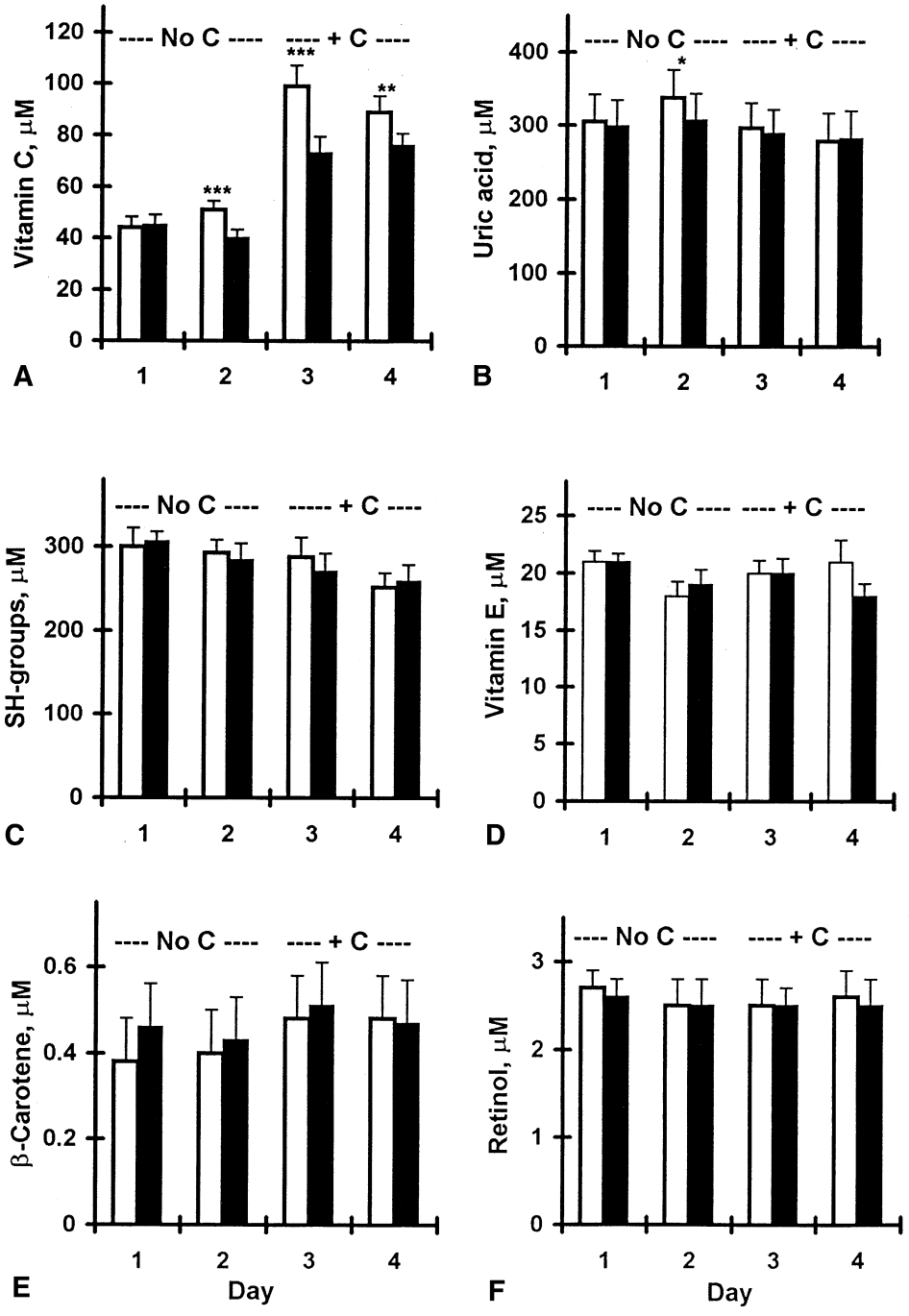

Fig. 2. The effect of passive smoking and vitamin C intake on serum antioxidants. The plasma concentrations of vitamin C (Fig. 2A), uric acid (Fig. 2B), SH-groups (Fig. 2C), vitamin E (Fig. 2D), β-carotene (Fig. 2E), and retinol (Fig. 2F) (mean $\pm$ SE) in healthy subjects (n = 10) before (open bar) and after (closed bar) the exposure periods were determined as described in methods. The same 10 subjects were exposed to normal air (day one), to passive smoking (day two), to passive smoking with vitamin C 2 h earlier (day three), and to normal air with vitamin C 2 h earlier (day four). The significancies were calculated by paired Student's t-test. The supplementation status is indicated by: ----No C---- = no vitamin C, --- + C ----- = vitamin C supplementation. *$p < .050$, **$p < .01$, ***$p < .001$.

changes have been described in plasma TRAP measured by other methods during long-term supplementation with vitamin C [27,47].

In normal air vitamin C supplementation did not increase the resistance of LDL to oxidation (Fig. 3C). This is in line with earlier studies, which have failed to convincingly demonstrate any effect of vitamin C on the extent of lipid peroxidation in healthy nonsmoking sub-

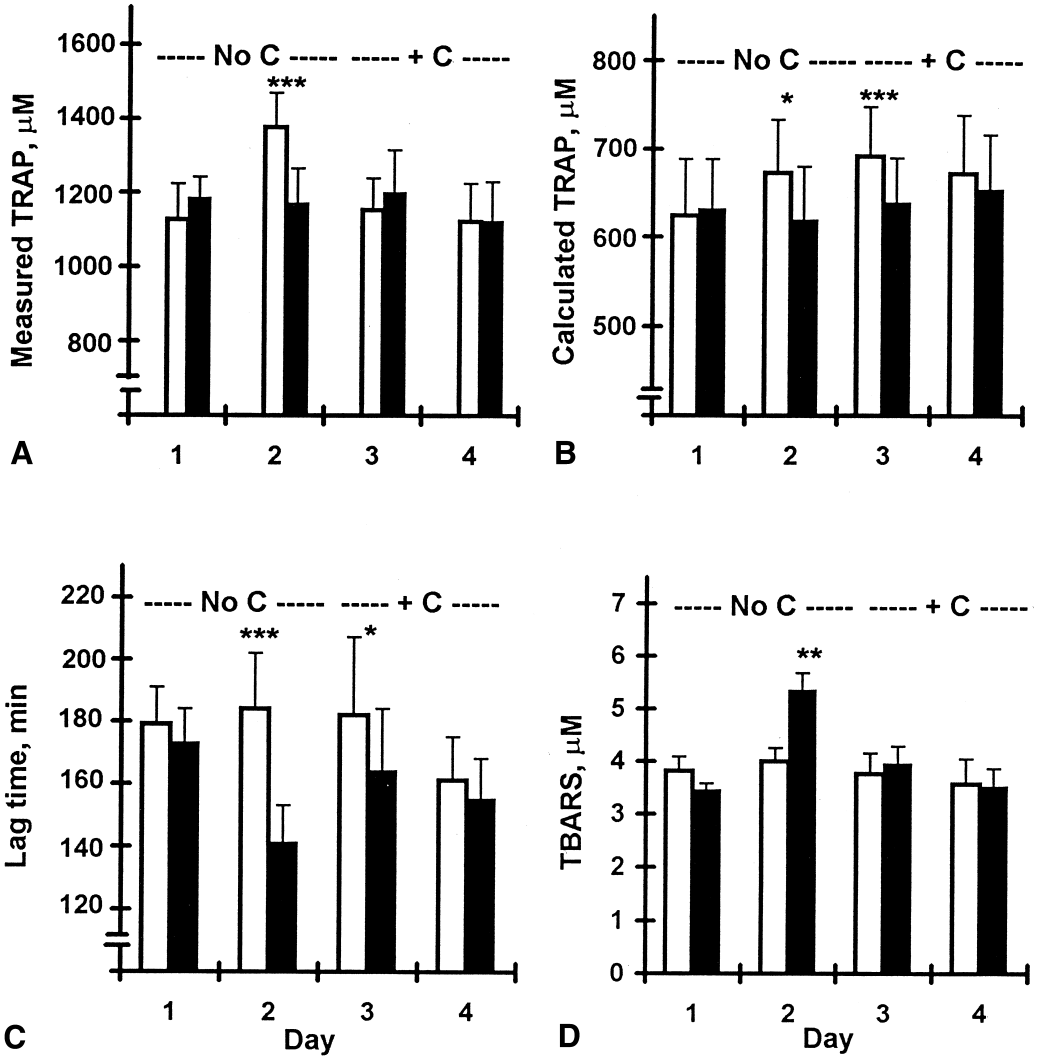

Fig. 3. The effect of passive smoking and vitamin C intake on plasma TRAP and on the indices of lipid peroxidation. The measured TRAP (Fig. 3A), calculated TRAP (Fig. 3B), lag time (Fig. 3C), and TBARS (Fig. 3D) (mean ± SE) in healthy subjects ($n = 10$) before (open bar) and after (closed bar) the exposure periods were determined as described in methods. The same 10 subjects were exposed to normal air (day one), to passive smoking (day two), to passive smoking with vitamin C 2 h earlier (day three), and to normal air with vitamin C 2 h earlier (day four). The significancies were calculated by (Student's) *t*-test. The supplementation status is indicated by: ----No C---- = no vitamin C, --- + C ----- = vitamin C supplementation. Calculated TRAP = 0.44 *[SH] + 2.0 * [E-vit] + 1.3 *[urate] + 1.7 *[ascorbate]. *$p < .050$, **$p < .01$, ***$p < .001$.

jects [18]. The present study thus supports the idea that under normal conditions nonsmoking subjects do not seem to benefit from vitamin C supplementation in order to improve plasma antioxidant defense or the resistance of LDL to ex vivo copper-induced oxidation. The decrease in the reducing capacity of vitamin C at higher concentrations discussed above might explain why no beneficial effects were observed after excess vitamin C under normal conditions. Furthermore, if a pro-oxidant action of vitamin C [48] occurs in vivo it might explain why vitamin C did not operate in normal air or in all subjects of the present study. However, the in vitro experiments carried out among subjects who did not benefit from supplementation during passive smoking, showed that ascorbic acid, in all conditions, acted as an antioxidant in the LDL-oxidation assay (data not shown). This antioxidative action of the suggested pro-oxidant combination of AA and Cu has recently been suggested to be due to the oxidative destruction of Cu binding sites on LDL by ascorbic acid and dehydroascorbic acid (DHA) [49].

The serum antioxidant defense of nonsmokers was unaffected by vitamin C in normal air; however, this situation is reversed during an excessive attack by free radicals, such as during passive smoking. The attacking free radicals in CS induce changes, which could be

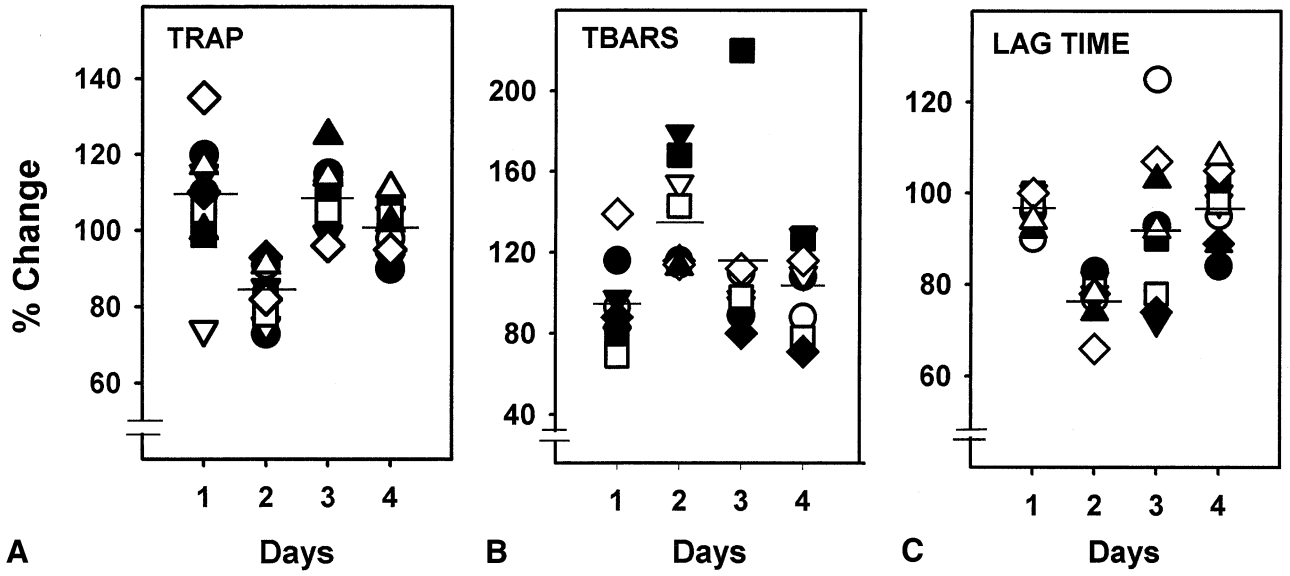

Fig. 4. The effect of passive smoking and vitamin C on plasma TRAP and on the indices of lipid peroxidation. The percentage changes (+ SE) in mean plasma TRAP, TBARS, and lag times of LDL oxidation during the study days.

largely counteracted by the intake of vitamin C (Figs. 2–4). The subsequent doubling of its plasma concentration was evident when the nonsmoking subjects entered smoky air the second time. The oxidative stress induced by secondhand smoke led to more rapid decline of plasma vitamin C than after the control period (Fig. 2A). A large part of this enhanced decline is likely to be attributed to consumption by free radicals in CS or those produced during inflammatory response to inhaled smoke. Some vitamin C might also be consumed in regenerating and protecting other antioxidants such as vitamin E or β-carotene from smoke-induced oxidation [50]. Furthermore, CS exposure might also increase the excretion of vitamin C in urine.

Elevated plasma vitamin C concentration prevented the acute decrease in plasma antioxidant defense (measured TRAP) after passive smoking (Figs. 3A and 4A). This was evident, although the contribution of vitamin C to measured TRAP is only 10%. However, the calculated TRAP decreased, but this can be explained mainly by the close relationship between the changes of vitamin C and calculated TRAP ($r = .74$, $n = 10$, $p < .001$). In addition, the measurement of the concentrations of individual antioxidants does not take into account the cooperations between antioxidants, best known between vitamin E and ascorbate; the contribution of some unknown antioxidants; and the impact of bilirubin and carotenoids. The measurement of plasma TRAP thus seems to quantitate serum antioxidant defense more adequately than calculations of TRAP, since it determines both individual capacity of all antioxidants to resist peroxidation and their cooperation (Figs. 3A and 3B) [42].

In passive smokers the accelerated LDL oxidation and accumulation of TBARS in plasma, otherwise observed 60 min after the smoke exposure (Figs. 3C and 3D) could be greatly prevented by vitamin C (Figs. 3C, 3D, 4B, and 4C). In some studies of active smokers, long-term supplementation with vitamin C reduced urinary F2-isoprostanones, the susceptibility of LDL to ex vivo oxidation, and the concentration of plasma TBARS [18]. The present study is the first to report a protective effect of a single large dose of vitamin C against lipid peroxidation in nonsmoking subjects during acute exposure to secondhand smoke.

Recently, evidence has accumulated to support the increased rate of in vivo and in vitro lipid oxidation in active and passive smokers [18,21,22]. However, among studies comparing smokers and nonsmokers using conventional methods to evaluate lipid peroxidation, ex vivo LDL-oxidation, and TBARS, conflicting evidence exists [51]. Part of this might be explained by inclusion of passive smokers in control populations and by adaptation of antioxidant defenses in active smokers possibly compensating some of the deleterious effects [37]. Furthermore, the in vivo significancies of the TBARS and ex vivo LDL oxidation measurements are hitherto unknown [1]. Although the resistance of LDL to oxidation does not necessarily translate into reduced atherosclerosis [1], one evidence of the atherogenic nature of CS-modified LDL is its interaction with cultured human macrophages, which accumulate cholesterol and develop to foam cells [22].

The free radicals in CS can also cause endothelial cell damage, inactivate nitric oxide, activate platelets and

pulmonary monocytes, and initiate lipid peroxidation leading to vicious circle of these events [33]. Antioxidants could possibly block this vicious circle or neutralize the generated free radicals in situ. This study supports the idea that passive smoking increases circulating free radicals and that these radicals can be scavenged by vitamin C [22,52]. Furthermore, these radicals modify circulating LDL in such a way, that even taken away from the circulating plasma aquaeous antioxidants, like vitamin C, it is oxidized more rapidly. Because the proatherogenic effects of CS might be a sum of its acute effects, studies on nonsmokers provide a good experimental model to study the acute effects of CS and the interactions of free radicals and antioxidants in general. Whatever the effect of antioxidants may be, it is unlikely that they ever could completely delete the harmful effects of CS, which are best prevented by avoiding it.

Acknowledgements — The authors are grateful to Mrs. Satu Laurema for excellent technical assistance. This study was supported by Finnish Heart Research Foundation, Ida Montini Foundation, Yrjö Jansson Foundation, and Helsinki University.

REFERENCES

[1] Diaz, M. N.; Balz, F.; Vita, J. A.; Keaney, J. F. Mechanisms of disease: antioxidants and atherosclerotic heart disease. *N. Engl. J. Med.* **337:**408–416; 1997.

[2] Lonn, E. M.; Yusuf, S. Is there a role for antioxidant vitamins in the prevention of cardiovascular diseases? An update on epidemiological and clinical trials. *Can. J. Cardiol.* **13:**957–965; 1997.

[3] Nyyssönen, K.; Parvianen, M. T.; Salonen, R.; Tuomilehto, J.; Salonen, J. T. Vitamin C deficiency and risk of myocardial infarction: prospective population study of men from eastern Finland. *Br. Med. J.* **314:**634–638; 1997.

[4] Ramirez, J.; Flowers, N. C. Leukocyte ascorbic acid and its relationship to coronary heart disease in man. *Am. J. Clin. Nutr.* **33:**2079–2087; 1980.

[5] Simon, J. A.; Hudes, E. S.; Browner, W. S. Serum ascorbic acid and cardiovascular disease prevalence in U.S. adults. *Epidemiology* **9:**316–321; 1998.

[6] Lynch, S. M.; Gaziano, J. M.; Frei, B. Ascorbic acid and atherosclerotic cardiovascular disease. *Sub-Cell Biochem.* **25:**331–367; 1996.

[7] Woodhouse, P. R.; Meade, T. W.; Khaw, K. T. Plasminogen activator inhibitor-1, the acute phase response and vitamin C. *Atherosclerosis* **133:**71–76; 1997.

[8] Levine, G. N.; Frei, B.; Koulouris, S. N.; Gerhard, M. D.; Keaney, J. F., Jr; Vita, J. A. Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation* **93:**1107–1113; 1996.

[9] Hornig, B.; Arakawa, N.; Kohler, C.; Drexler, H. Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. *Circulation* **97:**363–368; 1998.

[10] Frei, B. Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage. *Am. J. Clin. Nutr.* **54:**1113S–1118S; 1991.

[11] Nyyssönen, K.; Porkkalasarataho, E.; Kaikkonen, J.; Salonen, J.T. Ascorbate and urate are the strongest determinants of plasma antioxidative capacity and serum lipid resistance to oxidation in Finnish men. *Atherosclerosis* **130:**223–233; 1997.

[12] Martin, A.; Frei, B. Both intracellular and extracellular vitamin C inhibit atherogenic modification of LDL by human vascular endothelial cells. *Arterioscler. Thromb.* **17:**1583–1590; 1997.

[13] Lehr, H. A.; Frei, B.; Olofsson, A. M.; Carew, T. E.; Arfors, K. E. Protection from oxidized LDL-induced leukocyte adhesion to microvascular and macrovascular endothelium in vivo by vitamin C but not by vitamin E. *Circulation* **91:**1525–1532; 1995.

[14] Kugiyama, K.; Motoyama, T.; Hirashima, O.; Ohgushi, M.; Soejima, H.; Misumi, K.; Kawano, H.; Miyao, Y.; Yoshimura, M.; Okawa, H.; Matsumura, T.; Sugiyama, S.; Yasue, H. Vitamin C attenuates abnormal vasomotor reactivity in spasm coronary arteries in patients with coronary spastic angina. *J. Am. Coll. Cardiol.* **32:**103–109; 1998.

[15] Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **320:**915–924; 1989.

[16] Fuster, V.; Badimon, L.; Badimon, J. J.; Chesebro, J. H. The pathogenesis of coronary artery disease and the acute coronary syndromes. *N. Engl. J. Med.* **326:**310–318; 1992.

[17] Gokce, N.; Frei, B. Basic research in antioxidant inhibition of steps in atherogenesis. *J. Cardiovasc. Risk* **3:**352–357; 1996.

[18] McCall, M. R.; Balz, F. Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Radic. Biol. Med.* **26:**1034–1059; 1999.

[19] Milei, J.; Grana, D. R. Mortality and morbidity from smoking-induced cardiovascular diseases: the necessity of the cardiologist's involvement and commitment. *Int. J. Cardiol.* **67:**95–109; 1998.

[20] Morrow, J. D.; Frei, B.; Longmire, A. W.; Gaziano, J. M.; Lynch, S. M.; Shyr, Y.; Strauss, W. E.; Oates, J. A.; Roberts, L. J., II. 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] Miller, E. R., III; Appel, L. J.; Jiang, L.; Risby, T. H. Association between cigarette smoking and lipid peroxidation in a controlled feeding study. *Circulation* **96:**1097–1101; 1997.

[22] Valkonen, M.; Kuusi, T. Passive smoking induces atherogenic changes in LDL. *Circulation* **97:**2012–2016; 1998.

[23] Fickl, H.; Van Antwerpen, V. L.; Richards, G. A.; Van der Westhuyzen, D. R.; Davies, N.; Van der Walt, R.; Van der Merwe, C. A.; Anderson, R. Increased levels of autoantibodies to cardiolipin and oxidized low-density lipoprotein are inversely associated with plasma vitamin C status in cigarette smokers. *Atherosclerosis* **124:**75–81; 1996.

[24] Tribble, D. L.; Giuliano, L. J.; Fortmann, S. P. Reduced plasma ascorbic acid concentrations in nonsmokers regularly exposed to environmental tobacco smoke. *Am. J. Clin. Nutr.* **58:**886–890; 1993.

[25] Schectman, G.; Byrd, J. C.; Hoffman, R. Ascorbic acid requirements for smokers: analysis of a population survey. *Am. J. Clin. Nutr.* **53:**1466–1470; 1991.

[26] Reilly, M.; Delanty, N.; Lawson, J. A.; Fitzgerald, G. A. Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation* **94:**19–25; 1996.

[27] Steinberg, F. M.; Chait, A. Antioxidant vitamin supplementation and lipid peroxidation in smokers. *Am. J. Clin. Nutr.* **68:**319–327; 1998.

[28] Fuller, C. J.; Grundy, S. M.; Norkus, E. P.; Jialal, I. Effect of ascorbate supplementation on low-density lipoprotein oxidation in smokers. *Atherosclerosis* **119:**139–150; 1996.

[29] Harats, D.; Chevion, S. ; Nahir, M.; Norman, Y.; Sagee, O.; Berry, E. M. Citrus fruit supplementation reduces lipoprotein oxidation in young men ingesting a diet high in saturated fat: presumptive evidence for an interaction between vitamins C and E in vivo. *Am. J. Clin. Nutr.* **67:**240–245; 1998.

[30] Weber, C.; Erl, W.; Weber, K.; Weber, P. C. Increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin C intake in smokers. *Circulation* **93:**1488–1492; 1996.

[31] Lehr, H. A.; Weyrich, A. S.; Saetzler, R. K.; Jurek, A.; Arfors, K. E.; Zimmerman, G. A.; Prescott, S. M.; McIntyre, T. M. Vitamin C blocks inflammatory platelet-activating factor mimetics created by cigarette smoking. *J. Clin. Invest.* **99:**2358–2364; 1997.

[32] Anderson, R.; Theron, A. J.; Ras, G. J. Ascorbic acid neutralizes

reactive oxidants released by hyperactive phagocytes from cigarette smokers. *Lung* **166:**149–159; 1988.
[33] Heitzer, T.; Just, H.; Munzel, T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation* **94:**6–9; 1996.
[34] Law, M. R.; Morris, J. K.; Wald, N. J. Environmental tobacco smoke exposure and ischemic heart disease: an evaluation of the evidence. *Br. Med. J.* **315:**973–980; 1997.
[35] Davis, J. W.; Shelton, L.; Watanabe, I.; Arnold, J. Passive smoking affects endothelium and platelets. *Arch. Int. Med.* **149:**386–389; 1989.
[36] Lekakis, J.; Papamichael, C.; Vemmos, C.; Stamatelopoulos, K.; Voutsas, A.; Stamatelopoulos, S. Effect of acute cigarette smoking on endothelium-dependent arterial dilatation in normal subjects. *J. Am. Cardiol.* **81:**1225–1228; 1998.
[37] Glantz, S. A.; Parmley, W. W. Passive smoking and heart disease. Mechanisms and risk. *JAMA* **273:**1047–1053; 1995.
[38] Husgafvel-Pursiainen, K.; Sorsa, M.; Engstrom, K.; Einisto, P. Passive smoking at work: biochemical and biological measures of exposure to environmental tobacco smoke. *Int. Arch. Occup. Environ. Health* **59:**337–345; 1987.
[39] Denson, K. W.; Bowers, E. F. The determination of ascorbic acid in white blood cells. *Clin. Sci.* **21:**157–162; 1961.
[40] Schafer Elinder, L.; Walldius, G. Simultaneous measurement of serum probucol and lipid-soluble antioxidants. *J. Lipid Res.* **33:** 131–137; 1992.
[41] Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82:**70–77.; 1959.
[42] Valkonen, M. M.; Kuusi, T. Spectrophotometric assay for total peroxyl radical-trapping potential in human serum. *J. Lipid Res.* **38:**823–833.; 1997.
[43] Esterbauer, H.; Striegl, G.; Puhl, H.; Rotheneder, M. Continuous monitoring of in vitro oxidation of human low-density lipoprotein. *Free Radic. Res. Commun.* **6:**67–75; 1989.
[44] Slater, T. F.; Sawyer, B. C. The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. *Biochem. J.* **123:**805–814; 1972.
[45] Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R. W.; Washko, P. W.; Dhariwal, K. R.; Park, J. B.; Lazarev, A.; Graumlich, J. F.; King, J.; Cantilena, L. R. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA* **93:**3704–3709; 1996.
[46] Wayner, D. D.; Burton, G. W.; Ingold, K. U. The antioxidant efficiency of vitamin C is concentration dependent. *Biochim. Biophys. Acta* **884:**119–123; 1986.
[47] Mulholland, C. W.; Strain, J. J.; Trinick, T. R. Serum antioxidant potential and lipoprotein oxidation in female smokers following vitamin C supplementation. *Int. J. Food Sci. Nutr.* **47:**227–231; 1996.
[48] Stait, S.E.; Leake, D. S. Ascorbic acid can either increase or decrease low-density lipoprotein modification. *FEBS* **341:**263–267; 1994.
[49] Retsky, K. L.; Chen, K.; Zeind, J.; Frei, B. Inhibition of copper-induced LDL oxidation by vitamin C is associated with decreased copper-binding to LDL and 2-oxo-histidine formation. *Free Radic. Biol. Med.* **26:**90–98; 1999.
[50] Handelman, G. J.; Packer, L.; Cross, C. E. Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. *Am. J. Clin. Nutr.* **63:**559–565; 1996.
[51] Marangon, K.; Herbeth, B.; Artur, Y.; Esterbauer, H.; Siest, G. Low- and very low–density lipoprotein composition and resistance to copper-induced oxidation are not notably modified in smokers. *Clin. Chim. Acta* **265:**1–12; 1997.
[52] Schwarzacher, S. P.; Hutchison, S.; Chou, T. M.; Sun, Y. P.; Zhu, B. Q.; Chatterjee, K.; Glantz, S. A.; Deedwania, P. C.; Parmley, W. W; Sudhir, K. Antioxidant diet preserves endothelium-dependent vasodilatation in resistance arteries of hypercholesterolemic rabbits exposed to environmental tobacco smoke. *J. Cardiovasc. Pharmacol.* **31:**649–653; 1998.

ABBREVIATIONS

TRAP—total peroxyl radical trapping potential of serum
CS—cigarette smoke
LDL—low-density lipoprotein
CHD—coronary heart disease
TBARS—thiobarbituric acid reactive substances