Effects of Cigarette Smoking and Smoking Cessation on Plasma Constituents and Enzyme Activities Related to Oxidative Stress

ZHOU JUN-FU, YAN XIAO-FENG, GUO FANG-ZHEN, SUN NING-YA, QIAN ZHI-JUN, AND DING DE-YUN

The 2nd Affiliated Hospital of the Zhejiang University, 68 Jiefang Road, Hangzhou, 310009, Zhejiang Province, China

In order to study effects of cigarette smoking and smoking cessation on plasma constituents and enzyme activities related to oxidative stress, 1255 smokers and 524 healthy non-smokers were investigated in terms of plasma levels of lipoperoxides (LPO), nitric oxide (NO), vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR). Additionally, erythrocytes were examined to deterimine the level of LPO, the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The results showed that, when compared with the average values of the non-smoker group, the average plasma values of LPO, NO and the average erythrocyte value of LPO in the smoker group were significantly increased ($P<0.001$), while the average plasma values of VC, VE, β-CAR, and the average erythrocyte activities of SOD, CAT, GSH-Px were significantly decreased ($P<0.001$). A linear regression and correlation analysis for 65 male smokers who were all 40 years old showed that with longer smoking duration and greater daily smoking quantity, the plasma values of LPO, NO and the erythrocyte value of LPO were elevated, while the plasma values of VC, VE, β-CAR and erythrocyte values of SOD, CAT, GSH-Px were decreased. In a group of 73 smokers who stopped smoking completely for six months, the average plasma values of LPO, NO and the average erythrocyte value of LPO decreased, although they were still significantly higher than those in the matched non-smoker group ($P<0.05$). Additionally, the average plasma values of VC, VE, β-CAR and the average erythrocyte values of SOD, CAT, GSH-Px increased, although they were still significantly lower than those in the matched non-smoker group ($P<0.05$). How-ever, after smoking cessation for one year the above average values were not significantly different from those in the matched non-smoker group ($P>0.05$). This finding indicates that the markedly increased oxidative stress in smokers might gradually return to normal but only after a long period of smoking cessation. In conclusion, in the bodies of smokers a series of free radical chain reactions were gravely aggravated, the dynamic balance between oxidation and antioxidation was seriously disrupted, and oxidative stress was clearly exacerbated, which is closely related to many disorders or diseases in smokers. The present study underscored the need, urgency and importance of complete smoking cessation.

INTRODUCTION

Several studies have indicated that cigarette smoke increases lipoperoxidation and also increases the susceptibility of smokers to LDL oxidation (Steinberg *et al.*, 1998), and also has a peroxidant activity that results in the formation of LPO in smokers (Garcia *et al.*, 1998). The concentration of antioxidant vitamin C in smokers' breast milk was also lower and this might result in peroxidation problems for their newborns (Ortega *et al.*, 1998). It has also been shown that nicotine destroys the expression of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in macrophages (Van Straaten *et al.*,

0895-3988/2000
CN 11-2914

1998), and affects plasma NO_x (Miller *et al*., 1998) and stimulates the synthesis and/or release of nitric oxide (NO) (Mayhan *et al*., 1998). Additionally plasma levels of vitamins C, E and betacarotene in smokers are lower than those in non-smokers (Nadif *et al*., 1998; Ortega *et al*., 1998; Plit *et al*., 1998; Powell *et al*., 1998). Smoking aggravates lipoperoxidation associated with superoxide anion free radical (O_2^-) (Garcia *et al*., 1998; Nishio *et al*., 1998; Ueyama *et al*., 1998), and decreases the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) (Nishio *et al*., 1998; Pryor *et al*., 1998). Some researchers emphasize that tar radicals cause DNA damage (Pryor *et al*., 1998). Up to now, however, there have been no reports on erythrocyte level of LPO in smokers, nor comprehensive analyses on association of oxidative stress with cigarette smoking and smoking cessation. There are no studies that correlate oxidative and antioxidative stress with smoking duration or daily smoking quantity, nor comparisons between each of the linear correlation coefficients among ages and the oxidative, antioxidative indexes in healthy non-smokers and in smokers.

Therefore, in order to evaluate the effects of cigarette smoking and smoking cessation on plasma constituents and enzyme activities related to oxidative stress, plasma levels of LPO, NO, VC, VE, and β-CAR, as well as erythrocyte levels of LPO, and the activities of SOD, CAT and GSH-Px were determined in 1255 smokers and 524 healthy non-smokers using spectrophotometric assays. The differences between average values in the two groups were compared by analysis of variance (ANOV). Meanwhile, the differences between average values of the matched non-smoker group and the matched smoker group were analyzed by paired t test, and the differences between average values of the different smoking duration groups or different daily smoking quantity group were also compared and analyzed with ANOV or rank test. Linear regression and correlation analysis of the values in relation to smoking duration and daily smoking quantity were made in 65 male smokers aged 40 years. Furthermore, the values in 73 smokers who stopped smoking for six months or one year were investigated dynamically, and compared with those in matched healthy non-smokers. The difference between each of the linear correlation coefficients for age and for each of the measured values in the non-smokers and the smokers was compared by u test.

SUBJECTS AND METHODS

Subjects

Smokers. A total of 1255 cigarette smokers were randomly sampled through routine physical examinations. The amount of tar in the cigarettes was 14～18 mg/piece, and nicotine in cigarette gas was 1.0～1.5 mg/piece. As for professional distribution, of 1255 smokers there were 326 office workers (26.0%), 734 workers (58.5%), 153 teachers (12.2%) and 41 doctors (3.3%). The subjects were aged between 18 and 76 (45.2±11.5) years. with a smoking duration of 1 to 53 (18.3±10.6) years, and daily smoked between 3 and 60 (17.6±8.7) cigarettes. Of the subjects, 944 were male, aged from 18 to 76 (45.3±11.4) years, with a smoking duration of 1 to 53 (18.4±10.8) years, and smoked 3 to 60 (17.7±9.1) cigarettes per day; 311 were female, aged from 19 to 74 (44.9±10.9) years, with a smoking duration of 1 to 50 (17.9±10.5) years, and smoked 3 to 60 (17.4±8.3) cigarettes per day. There was no significant difference ($P>0.05$) between the average age of the male group and the female group as determined by t test, not a significant difference ($P>0.05$) in the sexual distinction proportion between the two groups as determined with χ^2 test.

Because all of the above values showed a certain degree of linear correlation with the ages of the smokers, with a concentration at 40 years, 65 male 40 year old smokers were randomly selected from 1255 smokers using the random numbers table (RNT). This was done to eliminate analytical errors possibly induced by the differences in the ages and the sexual distinction proportion of the smokers. The above values were used for linear regression and correlation analysis. Their smoking duration was 1 to 21 (9.8 ± 5.8) years, with 18 smokers smoking for $\leqslant$5 years, 16 smokers smoking for 6 to 10 years, 18 smokers smoking for 11 to 15 years, and 13 smokers smoking for $\geqslant$16 years. Their daily smoking quantity was 3 to 60 (23.2 ± 16.4) cigarettes, with 19 smokers consuming $\leqslant$10 cigarettes, 15 smokers consuming 11 to 20 cigarettes, 17 smokers consuming 21 to 40 cigarettes, and 14 smokers consuming $>$40 cigarettes.

In order to study the changes of the above values in smokers who completely abstained from cigarette, 73 smokers (47 male and 26 female) who voluntarily abstained from smoking were randomly sampled from 1255 smokers using the RNT so as to determine and analyze the values in smokers who had completely stopped smoking for six months or one year.

Non-smokers 524 healthy non-smokers were randomly sampled with the RNT through routine physical examinations, and their ages were between 18 and 76 (45.1 ± 11.2) years. Of them, 397 were male, aged from 18 to 76 (45.0 ± 11.3) years, and 127 were female, aged from 20 to 74 (45.4 ± 11.1) years. As for their professional distribution, 136 were office workers (26.0%), 307 were workers (58.5%), 64 were teachers (12.2%) and 17 were doctors 17 (3.3%).

Among the non-smokers, there was no significant difference ($P > 0.05$) in the average age of the male group and the female group as determined by t-test. Meanwhile, with the RNT, 65 male healthy non-smokers, all aged 40 years, were randomly selected from 524 healthy non-smokers as the matched control to compare with 65 male smokers aged 40 years and selected from 1255 smokers. There was no significant difference ($P > 0.05$) in the average age of the smokers and non-smokers by t-test, nor was there a significant difference ($P > 0.05$) in the sexual distinction proportion between the two groups with χ^2 test. For the smokers and non-smokers, there were no significant differences ($P > 0.05$) in the average age of the people in the four groups by ANOV, nor significant differences ($P > 0.05$) in the sexual distinction proportion with χ^2 test.

Furthermore, 73 healthy non-smokers (47 male and 26 female) between the ages of 38~63 (51.8 ± 7.1) years were randomly selected from 524 healthy non-smokers with the RNT so as to make matched analysis with the 73 smokers who voluntarily stopped smoking for six months or one year.

All the subjects were within normal ranges for routine blood, urine and feces tests, ECG and radiographs. Those people with disorders associated with heart, brain, lung, liver, kidney and other organs were excluded, and those with diseases such as hypertension, hyperlipemia, chronic bronchitis, autoimmune disease, diabetes, atherosclerosis, senile cataract were also excluded. Subjects were never exposed to radiation, work related intoxication or pesticides. Within one month before recruitment they took no antioxidants such as vitamin C, vitamin E, ginkgo biloba, theo-polyphenols or other agents.

Methods

Blood samples collection and pretreatment. Fasting venous blood samples were collected in the morning for all the subjects and heparin sodium was added as an-

ticoagulant. The separated plasma and erythrocytes were stored at 4℃ immediately (Zhou *et al*., 1997 a).

Plasma LPO level. Trichloroacetic acid (TCA) solution (20.0 g%, w/v) was used to sedimentate proteins in plasma. The protein sediment having LPO reacted with thiobarbituric acid (TBA) solution (0.67 g%, w/v) and produced red chemical compounds following incubation in a water bath at 100℃. This was detected spectrophotometrically, using tetraethoxypropane (TEP, 5.0 μmol/L) as the standard and a wavelength (WL) of 532 nm. Concentration was expressed as μmol/L (Zhou *et al*., 1991).

Plasma NO level. Colloidal aluminum hydroxide without nitrite was used to adsorb yellow pigments and to sedimentate proteins in the plasma. The nitrite in the supernate, which contained sodium acetate (0.20 mol/L) and sulphanilic acid (3.30 mmol/L), reacted with α-naphthylamine and formed a colored product, which was detected spectrophotometrically using sodium nitrite (2.50 μmol/L) as the standard and a WL of 520 nm. Concentration was expressed as nmol/L (Zhou *et al*., 1997a).

Plasma VC level. TCA (5.0 g%, w/v) was used to sedimentate proteins in plasma, and ferric trichloride was added to the supernate. Vitamin C in supernate reduced Fe^{3+} in ferric trichloride to Fe^{2+}. Fe^{2+}, which reacted with ferocene, produced a coloured end product. This product was detected spectrophotometrically at 563 nm using vitamin C as the stardard. Concentration was expressed as μmol/L (Zhou *et al*., 1997b).

Plasma VE level. Absolute ethyl alcohol was used to sedimentate proteins in plasma and to extract vitamin E from plasma. Vitamin E in the supernate reduced Fe^{3+} in ferric trichloride to Fe^{2+}. Fe^{2+} reacted with ferrocene to form a colored end product that was detected at 563 nm, with its concentration expressed as μmol/L (Zhou *et al*., 1997b).

Plasma β-CAR level. A mixed solution of absolute ethyl alcohol and petroleum ether was used to sedimentate proteins in plasma and to extract β-carotene. The petroleum ether extract containing β-carotene was analyzed colorimetrically, using β-carotene as the standard and a wave length setting of 440 nm. Concentration was expressed as μmol/L (Zhou *et al*., 1997b).

Erythrocyte LPO level. The mixed solution of absolute ethyl alcohol and trichloromethane (5∶3) was used to sedimentate hemoglobin (Hb) from a hemolytic solution (HS) of RBC without WBC and platelets, and to extract LPO in the HS. Hb level was determined in the HS. LPO in the extract solution reacted with TBA-glacial acetic acid solution (1.0 g%, w/v) and produced red chemical compounds in a water bath at 100℃. This was detected using TEP (5.0 μmol/L) as the standard at a WL of 532 nm. Concentration was expressed as μmol/g·Hb (Zhou *et al*., 1998).

Erythrocyte SOD activity. The mixed solution of absolute ethyl alcohol and trichloromethane (5∶3) was used to sedimentate Hb from the HS of RBC without WBC and platelets, and to extract SOD from the HS. Hb level was determined in the HS. Pyrogallol (6.0 mmol/L) auto-oxidized in Tris-HCl buffer (50 mmol/L, pH 8.20), and the SOD extract were added to the buffer in order to inhibit the auto-oxidation of pyrogallol. SOD activity was calculated according to the auto-oxidation rate of pyrogallol and the rate of SOD-inhibited pyrogallol auto-oxidation. A WL of 420 nm was used, and SOD activity indicated as U/g·Hb (Zhou *et al*., 1997b).

Erythrocyte CAT activity. H_2O_2 (0.20 mol/L) was added to phosphate buffer (10 mmol/L, pH 7.0) containing HS of RBC without WBC and platelets. The Hb level was determined in the HS. After a reaction time of 60 s, a solution of potassium bichromate (0.169 mol/L) and glacial acetic acid (1∶3) was added to the reactive solution in order to stop the coloration reaction, and the reactive solution was heated for 10 min at 100℃. Colorimetry was done after cooling of the solution at a WL of 570

nm. CAT activity was expressed as K/g·Hb (Zhou *et al*., 1997a).

Erythrocyte GSH-Px activity. A solution of absolute ethyl alcohol and trichloromethane (5:3) was used to sedimentate Hb from the HS of RBC without WBC and platelets, and to extract GSH-Px in the HS. Hb level was determined in the HS. GSH-Px in the extract catalyzed the reaction of glutathione with 5, 5′-dithiobis-p-nitrobenzoic acid (DTNB) and produced yellow chemical compounds. This was detected at 422 nm, using glutathione as the standard and GSH-Px activity was expressed as U/g·Hb (Zhou *et al*., 1997a).

Major analytical reagents such as VC, VE, CAR, SOD, CAT, α-naphthylamine, 1, 2, 3-trihydroxybenzen, 1, 1, 3, 3-tetraethoxypropane, 2-thiobarbituric acid were all purchased from SIGMA CHEMICAL COMPANY, USA; and the other analytical-grade reagents were all produced in China. The fresh four-distilled-water was prepared with quartz glass distilling apparatus. The main analytical instruments were 721-spectrophotometer, UV-754-spectrophotometer and others.

Statistic Analysis

All data were analyzed with SPSS/8.0 and Statistica/5.0 statistic software using a Compaq Pentium Ⅲ/600 computer. Statistical testing methods included the t test, paired t test, analysis of variance (ANOV), rank test, chi square test (χ^2 test), linear regression and correlation analysis, and the u test between two linear correlation coefficients. In the statistic analysis of the study, the level of significance of hypothesis testing (α)$\leqslant$0.05. and the power of test $>$0.75.

RESULTS

The average plasma values of LPO and NO and the erythrocyte values of LPO in each of the smoker groups are significantly higher than those in the corresponding non-smoker groups. The average plasma values of VC, VE and β-CAR, and the erythrocyte values of SOD, CAT, GSH-Px in each of the smoker groups are significantly lower than those in the corresponding non-smoker groups (Table 1).

Compared with the matched non-smoker group, the average plasma values of LPO and NO, and the erythrocyte value of LPO in the matched smoker group are significantly increased, while the average plasma value of VC, VE and β-CAR, and the erythrocyte values of SOD, CAT, GSH-Px are significantly decreased (Table 2).

With prolonged smoking, the average plasma values of LPO and NO, and the erythrocyte value of LPO are significantly raised, while the average plasma values of VC, VE and β-CAR, and the erythrocyte values of SOD, CAT, GSH-Px are significantly lowered (Table 3).

The linear regression and correlation analysis of the above values in comparison with smoking duration in 65 smokers indicates that with prolonged smoking the plasma values of LPO and NO, and the erythrocyte value of LPO are gradually increased, representing a significant linear positive correlation, whereas the plasma values of VC, VE and β-CAR, and

TABLE 1

Comparison of the Above Determined Values ($\bar{x} \pm s$) Between the Smoker Group and the Non-Smoker Group

Group	n	Plasma				
		LPO	NO	VC	VE	β-CAR
Non-smoker (M)	397	10.8±1.5	378±190	53.7±17.6	24.1±6.9	1.68±0.65
(F)	127	10.6±1.3	368±173	54.9±17.7	25.0±7.5	1.69±0.67
(T)	524	10.7±1.4	376±186	54.0±17.7	24.3±7.0	1.68±0.66
Smoker (M)	944	12.4±1.4***	520±229***	41.2±15.8***	19.4±7.5***	1.33±0.57***
(F)	311	12.1±1.5***	515±240***	41.9±16.0***	20.4±7.4***	1.35±0.60***
(T)	1255	12.3±1.4***	519±231***	41.4±15.9***	19.6±7.5***	1.34±0.58***

Group	n	Erythrocyte			
		LPO	SOD	CAT	GSH-Px
Non-smoker (M)	397	28.4±4.3	1921±153	321±91	28.4±5.3
(F)	127	28.1±4.1	1943±155	327±93	28.9±5.7
(T)	524	28.2±4.2	1926±154	322±92	28.5±5.5
Smoker (M)	944	31.8±4.7***	1809±163***	272±81***	23.8±4.8***
(F)	311	31.6±4.5***	1812±164***	276±83***	24.7±5.1***
(T)	1255	31.7±4.6***	1840±163***	273±82***	24.0±4.9***

Note. ANOV, vs corresponding each non-smoker group: *** $P<0.001$; M: male; F: female; T: total.

TABLE 2

Comparison of the Above Determined Values ($\bar{x} \pm s$) in the Matched Smoker Group With the Matched Non-Smoker Group

Group	n	Plasma				
		LPO	NO	VC	VE	β-CAR
Non-smoker	65	10.5±1.2	368±148	55.7±16.9	25.7±7.4	1.71±0.67
Smoker	65	11.9±1.6	509±220	42.3±16.2	20.8±8.3	1.39±0.53
P^a		<0.001	<0.001	<0.001	<0.001	<0.001

Group	n	Erythrocyte			
		LPO	SOD	CAT	GSH-Px
Non-smoker	65	28.7±4.4	1963.2±138.3	317.8±81.4	27.1±4.9
Smoker	65	32.5±4.6	1835.8±161.6	266.7±78.6	23.2±4.7
P^a		<0.001	<0.001	<0.001	<0.001

[a]Paired t test.

the erythrocyte values of SOD, CAT, GSH-Px are gradually decreased, representing a significant linear negative correlation (Table 4).

TABLE 3

Comparison of the Above Determined Values ($\bar{x} \pm s$) in the Different Smoking Duration Groups With the Non-Smoker Group

Group	n	Plasma				
		LPO	NO	VC	VE	β-CAR
Non-smoker	65	10.5±1.2	368±148	55.7±16.9	25.7±7.4	1.71±0.67
≤5 years	18	10.6±1.8	385±242	54.7±5.9	26.4±6.6	1.80±0.20
6 years～	16	11.6±1.4*	429±184**	44.8±10.4*	22.8±5.3**	1.47±0.34*
11 years～	18	12.4±1.0***	576±146***	36.9±14.4***	17.7±7.3***	1.21±0.47**
≥16 years	13	13.2±0.7***	687±172***	29.9±21.3***	14.9±9.8***	0.98±0.70***

Group	n	Erythrocyte			
		LPO	SOD	CAT	GSH-Px
Non-smoker	65	28.7±4.4	1963±138	318±81	27.1±4.9
≤5 years	18	30.5±2.3*	1922±92	304±58	26.4±2.8
6 years～	16	31.9±4.1**	1858±133*	271±65*	24.2±4.1*
11 years～	18	33.3±6.0***	1783±163**	258±78**	22.3±5.8***
≥16 years	13	34.6±6.5***	1764±215***	222±124***	18.7±7.5***

Note. Rank test, vs non-smoker group: * $P<0.05$, ** $P<0.01$, *** $P<0.001$; ≤5 years, 6 year～, 11 years～, ≥16 years are respectively the different smoking duration in the smokers.

TABLE 4

The Linear Regression and Correlation Between the Above Values and the Smoking Duration of 65 Smokers

Correlative Item	n	Regression Equation	r	P
Plasma LPO with smoking Duration	65	$Y=10.3456+0.1531X$	0.5513	<0.0001
Erythrocyte LPO with Smoking Duration	65	$Y=29.6871+0.2742X$	0.7248	<0.0001
Plasma NO with Smoking Duration	65	$Y=290.243+21.773X$	0.6063	<0.0001
Plasma VC with Smoking Duration	65	$Y=58.3166-1.6241X$	−0.5857	<0.0001
Plasma VE with Smoking Duration	65	$Y=28.7692-0.8116X$	−0.5620	<0.0001
Plasma β-CAR with Smoking Duraton	65	$Y=1.9153-0.0533X$	−0.6274	<0.0001
Erythrocyte SOD with Smoking Duration	65	$Y=1951.86-11.838X$	−0.4257	0.0004
Erythrocyte CAT with Smoking Duration	65	$Y=318.252-5.2104X$	−0.7184	<0.0001
Erythrocyte GSH-Px with Smoking Duration	65	$Y=28.1487-0.5216X$	−0.6987	<0.0001

As the amount of daily smoking increased, the average plasma values of LPO and NO, and the erythrocyte value of LPO in the smokers are significantly increased, while the average plasma values of VC, VE and β-CAR, and the erythrocyte values of SOD, CAT, GSH-Px are significantly decreased (Table 5).

The linear regression and correlation analysis for the above values in relation to the daily smoking quantity in 65 smokers shows that with an increase in the amount of daily smoking, the plasma values of LPO and NO, and the erythrocyte value of LPO are gradually increased, representing a significant linear positive correlation. The plasma values of VC, VE, and β-CAR, and the erythrocyte values of SOD, CAT, GSH-Px are gradually decreased, representing a significant linear negative correlation (Table 6).

TABLE 5

Comparison of the Above Determined Values ($\bar{x} \pm s$) in the Different Daily Smoking Quantity Groups With the Non-Smoker Group

Group	n	Plasma				
		LPO	NO	VC	VE	β-CAR
Non-smoker	65	10.5±1.2	368±148	55.7±16.9	25.7±7.4	1.71±0.67
≤10 pieces	19	10.6±1.8	376±239	54.0±5.9	25.9±6.3	1.78±0.19
11 pieces～	15	11.8±1.5*	443±182**	45.9±10.5*	23.0±5.4	1.51±0.34
21 pieces～	17	12.4±1.0***	569±146***	36.2±14.6***	18.1±7.3**	1.19±0.48***
≥41 pieces	14	13.3±0.7***	689±165***	29.3±20.6***	14.7±9.5***	0.96±0.68***

Group	n	Erythrocyte			
		LPO	SOD	CAT	GSH-Px
Non-smoker	65	28.7±4.4	1963±138	318±81	27.1±4.9
≤10 pieces	19	30.4±2.2*	1927±88	308±59	26.1±2.7
11 pieces～	15	32.1±4.0**	1874±130*	271±64*	24.0±4.2*
21 pieces～	17	33.4±5.9***	1797±162**	257±82**	21.8±5.9***
≥41 pieces	14	34.7±6.9***	1753±210***	217±117***	20.1±6.5***

Note. Rank test, vs non-smoker group: * $P<0.05$, ** $P<0.01$, *** $P<0.001$; ≤10 pieces, 11 pieces～, 21 pieces～, ≥41 pieces are respectively the different daily smoking quantity in the smokers.

TABLE 6

The Linear Regression and Correlation Between the Above Values and the Daily Smoking Quantity of 65 Smokers

Correlative Item	n	Regression Equation	r	P
Plasma LPO with Daily Smoking Quantity	65	$Y=10.7090+0.0490X$	0.4964	<0.0001
Erythrocyte LPO with Daily Smoking Quantity	65	$Y=30.3565+0.0917X$	0.7412	<0.0001
Plasma NO with Daily Smoking Quantity	65	$Y=283.261+7.5684X$	0.6871	<0.0001
Plasma VC with Daily Smoking Quantity	65	$Y=56.5751-0.6201X$	−0.6240	<0.0001
Plasma VE with Daily Smoking Quantity	65	$Y=27.5035-0.2899X$	−0.5751	<0.0001
Plasma β-CAR with Daily Smoking Quantity	65	$Y=1.8585-0.0204X$	−0.6241	<0.0001
Erythrocyte SOD with Daily Smoking Quantity	65	$Y=1945.43-4.7170X$	−0.4778	<0.0001
Erythrocyte CAT with Daily Smoking Quantity	65	$Y=310.458-1.8713X$	−0.7261	<0.0001
Erythrocyte GSH-Px with Daily Smoking Quantity	65	$Y=26.2717-0.1305X$	−0.6847	<0.0001

The results from 73 smokers who stopped smoking completely for six months showed that although the average plasma values of LPO, and NO, and the erythrocyte value of LPO decreased, they were significantly higher than those in the matched non-smoker group; and although the average plasma values of VC, VE and β-CAR, and the erythrocyte values of SOD, CAT, GSH-Px increased, they were still significantly lower than those in the matched non-smoker group. However, one year after their complete smoking cessation, there were no significant differences in all of the values between the two groups (Table 7).

TABLE 7

Comparison of the Above Values ($\bar{x} \pm s$) in 73 Smokers Completely Stopped Smoking for Six Months or One Year and 73 Non-Smokers

Group	n	Plasma				
		LPO	NO	VC	VE	β-CAR
Non-smoker	73	10.9 ± 1.5	374 ± 144	53.4 ± 14.7	24.1 ± 6.9	1.64 ± 0.63
Six months	73	11.5 ± 1.7*	438 ± 193*	48.5 ± 13.8*	21.6 ± 6.7*	1.41 ± 0.67*
One year	73	11.1 ± 1.6	399 ± 163	49.8 ± 14.5	22.3 ± 6.2	1.47 ± 0.71
Group	n	Erythrocyte				
		LPO	SOD	CAT	GSH-Px	
Non-smoker	73	29.3 ± 4.7	1928 ± 152	309 ± 99	26.4 ± 5.0	
Six months	73	30.9 ± 5.1*	1870 ± 158*	274 ± 87*	24.6 ± 5.1*	
One year	73	25.5 ± 4.7	1890 ± 163	29.7 ± 4.8	284 ± 92	

Note. ANOV, vs non-smoker group: * $P < 0.05$; six months, one year are respectively the duration abstained from smoking in 73 smokers.

Comparison of each of the linear correlation coefficients between the ages and the above values in 524 healthy non-smokers and 1255 smokers indicates that the oxidative stress experienced by smokers is significantly more severe than that of the non-smokers (Table 8).

TABLE 8

Comparison of the Each of the Linear Correlation Coefficients Between the Ages and the Above Values in the Non-Smokers and the Smokers

Comparative Item	r Value		u Value	P
	Non-Smoker ($n = 524$)	Smoker ($n = 1255$)		
Age with Plasma LPO	0.2347	0.4318	4.276	< 0.001
Age with Erythrocyte LPO	0.2429	0.4593	4.768	< 0.001
Age with Plasma NO	0.1796	0.4186	5.072	< 0.001
Age with Plasma VC	−0.2176	−0.3945	3.759	< 0.001
Age with Plasma VE	−0.1984	−0.3716	3.629	< 0.001
Age with Plasma β-CAR	−0.2031	−0.3687	3.471	< 0.001
Age with Erythrocyte SOD	−0.2218	−0.4034	3.877	< 0.001
Age with Erythrocyte CAT	−0.1875	−0.3651	3.702	< 0.001
Age with Erythrocyte GSH-Px	−0.1932	−0.3704	3.706	< 0.001

DISCUSSION

The findings of the present study suggest that a grave imbalance between oxidation and antioxidation, as well as pathological or physiological aggravation of oxidative stress, may occur in smokers. There may be several interpretations. Smokers inhale a great quantity of free radicals (FRs) contained in the gas-phase (oils and smog) of cigarettes. Through a mouthful of cigarette smoke, a smoker may inhale approxi-

mately 10^{14-16} FRs, including alkyl, alkoxyl, alkoperoxyl, polyphenoxyl and other FRs such as tar radical (Pryor *et al*., 1998; Zhou *et al*., 1997a, 1997b, 1997c). Tar radical causes DNA damage and produces oxygen free radicals (OFRs) such as O_2^-, hydroxyl radical (·OH), hydrogen peroxy radical ($HO_2^·$) and H_2O_2 (Pryor *et al*., 1998; Zhou et al., 1997a). A great number of FRs stimulate alveolar macrophages and neutrophils to produce and release excess OFRs, which promote lipoperoxidation to form LPO, resulting in lipoperoxidation injury (Pryor *et al*., 1998; Zhou *et al*., 1997a). The gas-phase of cigarettes also contains a large number of nitrogen oxides (NO_x), especially NO and NO_2 (Miller *et al*., 1998; Zhou *et al*., 1997a). Meanwhile, nicotine may destroy the structural integrity of pulmonary tissue and the expression of eNOS and iNOS in macrophages (Van Straaten *et al*., 1998), and stimulate the synthesis and/or release of NO (Mayhan *et al*., 1998; Nadif *et al*., 1998). Cigarette smoke also reduces basal blood flow, increases xanthine oxidase activity, and causes neutrophil aggregation, iNOS activation, and thus increased synthesis of NO (Chow *et al*., 1998; Zhou *et al*., 1997a). No and NO_2 can react with organic molecules such as hydrocarbons and dienes in the smoke smog, generating peroxidation and chain reactions of alkyl FRs (Miller *et al*., 1998; Zhou *et al*., 1997c). Excess NO inactivates antioxidases by combining with hydrosulfide groups (-SH), and NO can combine with O_2^- to produce the superoxide nitroso radical ($ONO-O^-$) that has extra-strong oxidation abilities damaging cell functions and deactivating antioxidases (Zhong *et al*., 1997; Zhou *et al*., 1997a). Again, NO_2 is a very active catalyst in lipoperoxidation, and aggravates the lipoperoxidation of polyunsaturated fatty acids (PUFAs) (Zhou *et al*., 1998). Excess OFRs also can directly attack PUFAs, leading to lipoperoxidation of a large number of PUFAs with subsequent formation of LPO, which damages cell function (Zhou *et al*., 1997a, 1998, 1999). Cigarette smoke also decreases the plasma concentrations of VC, VE and β-CAR as well as the erythrocyte activities of SOD, CAT and GSH-Px, and promotes lipoperoxidation (Plit *et al*., 1998; Nishio *et al*., 1998; Garcia *et al*., 1998; Zhou *et al*., 1997a). In the water soluble product formed by cigarette smog inhaled into lungs, excess OFRs and other FRs all oxidize, decompose and destroy the -SH group of antioxidant constituents, thereby weakening their activities (Ortega *et al*., 1998; Zhou *et al*., 1997a). Heavy smokers appear to have poor appetite, so VC, VE and β-CAR absorbed by their bodies are decreased in quantity, resulting in a significant decrease of the antioxidants (Steinberg *et al*., 1998; Zhou *et al*., 1997a). Smokers have to consume a great quantity of antioxidants and antioxidases so as to catch and clear excess OFRs and other FRs from their bodies, thus their lack of appetite leads to a significant decrease of antioxidants and antioxidases. Oxidative stress in smokers is significantly stronger than that in non-smokers.

In this study we have found that long-term smoking and increased daily smoking cause the values of plasma LPO, NO and erythrocyte LPO to gradually increase, and the values of plasma VC, VE, β-CAR and erythrocyte SOD, CAT, GSH-Px to gradually decrease. Our findings suggest that the longer a person smokes and the more a person smokes daily, the more severe are the pathological or physiological oxidative stress, and the greater the risk of developing lung, cardiovascular, cerebrovascular diseases and cancer (Zhou *et al*., 1997a, 1997c).

In this study, although the average values of plasma LPO, NO and erythrocyte LPO were decreased in 73 smokers who completely stopped smoking for six months, they were significantly higher than in the matched non-smoker group ($P<0.05$). Additionally, although the average values of plasma VC, VE, β-CAR and erythrocyte SOD, CAT, GSH-Px were increased, they were significantly lower than in the matched non-smoker group ($P<0.05$). Only after complete smoking cessation for

one year, the differences in the above average values in comparison with the matched non-smoker group become insignificant ($P > 0.05$). The results show that smokers that stop smoking for less than one year will experience no distinct positive effect until smoking cessation exceeds one year (Zhou *et al.*, 1997a, 1997b, 1997c).

It cannot be denied that a great quantity of carbon monoxide, NOx, nicotine, tar radicals and other FRs inhaled by long-term smokers severely destroy the dynamic balance between oxidation and antioxidation, and enhance oxidative stress in their bodies. The oxidative stress induced by smoking increases the risk of hypertension, hypercholesterolemia, atherosclerosis, cardio-cerebral vascular diseases, lung diseases such as cancer of lung and chronic bronchitis, and other cancers (Britten *et al.*, 1998; Chan *et al.*, 1996; Garcia *et al.*, 1998; Kumagai *et al.*, 1998; Nadif *et al.*, 1998; Nishio *et al.*, 1998; Powell *et al.*, 1998; Pryor *et al.*, 1998; Ueyama *et al.*, 1998). The most healthful choice for every smoker, therefore, is to abstain from smoking completely and with resolve.

REFERENCES

Britten, M. and Schachinger, V. (1998). The role of endothelial function for ischemic manifestations of coronary atherosclerosis. *Herz*. **23**, 97-105.

Chan, P. H. (1996). Role of oxidants in ischemic damage. *Stroke* **27**, 1124-1129.

Chow, J. Y., Ma, L., and Cho, C. H. (1998). Effect of cigarette smoke on ethanol-induced gastric mucosal lesions: the role of nitric oxide and neutrophils. *Eur. J. Pharmacol*. **342**, 253-260.

Garcia Callejo, F. J., Velert Vila, M. M., Esparcia Navarro, M., Martinez Beneito, M. P., and Marco Algarra, J. (1998). Clinical evaluation and application of lipid peroxidation produced by tobacco smoke in the tracheal mucosa. *Acta Otorrinolaringol Esp*. **49**, 264-268.

Kumagai, Y., Pi, J. B., Lee, S., Sun, G. F., Yamanushi, T., Sagai, M., and Shimojo, N. (1998). Serum antioxidant vitamins and risk of lung and stomach cancers in Shenyang, *Chin. Cancer Lett*. **129**, 145-149.

Mayhan, W. G. and Sharpe, G. M. (1998), Nicotine impairs histamine-induced increases in macromolecular efflux: role of oxygen radicals. *J. Appl. Physiol*. **84**, 1589-1595.

Miller, V. M., Lewis, D. A., Rud, K. S., Offord, K. P., Croghan, I. T., and Hurt, R. D. (1998). Plasma nitric oxide before and after smoking cessation with nicotine nasal spray. *J. Clin. Pharmacol*. **38**, 22-27.

Nadif, R., Diallo, L., Mayer, L., Dusch, M., Porcher, J. M., Schneider, P., Urschel, M., Mur, J. M., and Auburtin, G. (1998). Relatioship between blood antioxidants and occupational exposure to polycyclic aromatic hydrocarbons in coke oven workers. *Am. J. Ind. Med*. **34**, 272-279.

Nishio, E. and Watanabe, Y. (1998). Cigarette smoke extract is a modulator of mitogenic action in vascular smooth muscle cells. *Life Sci*. **62**, 1339-1347.

Ortega, R. M., Lopez-Sobaler, A. M., Quintas, M. E., Martinez, R. M., and Andres, P. (1998). The influence of smoking on vitamin C status during the third trimester of pregnancy and on vitamin C levels in maternal milk. *J. Am. Coll. Nutr*. **17**(4), 379-384

Plit, M. L., Theron, A. J., Fickl, H., van Rensburg, C. E., Pendel, S., and Anderson, R. (1998). Influence of antimicrobial chemotherapy and smoking status on the plasma concentrations of vitamin C, vitamin E, beta-carotene, acute phase reactants, iron and lipid peroxides in patients with pulmonary tuberculosis. *Int. J. Tuberc. Lung Dis*. **2**, 590-596.

Powell, J. T. (1998). Vascular damage from smoking: disease mechanisms at the arterial wall. *Vasc. Med*. **3**, 21-28.

Pryor, W. A., Stone, K., Zang, L. Y., and Bermudez, E. (1998). Fractionation of aqueous cigarette tar extracts: fractions that contain the tar radical cause DNA damage. *Chem. Res. Toxicol*. **11**, 441-448.

Steinberg, F. M. and Chait, A. (1998). Antioxidant vitamin supplementation and lipid peroxidation in smokers. *Am. J. Clin. Nutr*. **68**, 319-327.

Ueyama, K., Yokode, M., Arai, H., Nagano, Y., Li, Z. X., Cho, M., and Kita, T. (1998). Cholesterol efflux effect of high density lipoprotein is impaired by whole cigarette smoke extracts through lipid peroxidation. *Free Radic. Biol. Med*. **24**, 182-190.

Van Straaten, J. F., Postma, D. S., Coers, W., Noordhoek, J. A., Kauffman, H. F., and Timens, W. (1998). Macrophages in lung tissue from patients with pulmonary emphysema express both inducible and endothelial nitric oxide synthase. *Mod. Pathol*. **11**, 648-655.

Zhong, C. S. and Sun, A. Y. (1997). *Biomedicine of Nitric Oxide*, First ed. pp. 238-249. Press of Shanghai Medical University, Shanghai. (in Chinese)

Zhou, J. F., Guo, F. Z., Sun, N. Y., Qian, Z. J., and Ding, D. Y. (1997a). A study on the relationship between smoking and plasma nitric oxide, vitamin C, vitamin E, β-carotene concentration in elderly. *Chin. J. Geriatr.* **16**, 87-89.

Zhou, J. F., Guo, F. Z., Qian, Z. J., Sun, N. Y., and Ding, D. Y. (1997b). Study the effects of injury induced by nitric oxide and other free radicals to smokers. *China Public Health*, **13**, 90-92.

Zhou, J. F., Guo, F. Z., Qian, Z. J., and Ding, D. Y. (1997c). Effects of Cigarette Smoking on Antioxidant Vitamins and Activities of Antioxidases. *Chin. J. Prev. Med.* **31**, 67-70.

Zhou, J. F., Du, Y. H., Wang, Y. L., and Ding F. Y. (1999). The Correlation Between Abusing Alcohol And Antioxidases. *The American Journal of Comprehensive Medicine*, **1**, 287-288.

Zhou, J. F., Zhang, Y., and Guo, F. Z. (1998). Effects of copying operation on oxidation and peroxidation of the operators. *Chin. J. Ind. Hyg. Occup. Dis.* **16**, 143-146.

Zhou, J. F., Zhang, X. G., and Zhong, X. J. (1991). Studies on the relationship between CSF-LPO, P-LPO and acute craniocerebral injury. *Chin. J. Traumal.* **7**, 1-4.

(*Recieved May* 10, 1999 *Accepted November* 12, 1999)