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Oxidative Stress and Potential Free Radical Damage Associated with Photocopying. A Role for Ozone?

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Objective: To study the relationship between oxidative stress and potential free radical damage associated with photocopying and to explore a role for ozone emitted during the photocopying process. *Methods*: 80 photocopying operators (PO) and 80 healthy volunteers (HV) were enrolled in a random control study design, in which the level of lipoperoxide (LPO, thiobarbituric acid reactive substances, TBARS) in erythrocytes and the levels of vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR) in plasma as well as the activities of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes were determined by spectrophotometric methods. *Results*: Compared with the average values of the above biochemical parameters in the HV group, the average value of LPO (TBARS) in erythrocytes in the PO group was significantly increased ($P < 0.0001$), while the average values of VC, VE and β-CAR in plasma as well as those of SOD and CAT in erythrocytes in the PO group were significantly decreased ($P < 0.0001$). Pearson product-moment correlation analysis showed that with the increase of the ozone level in photocopying sites and the PO duration of exposure to ozone, the level of LPO in erythrocytes in the operators was increased ($P < 0.001$), while the levels of VC, VE and β-CAR in plasma as well as the activities of SOD and CAT in erythrocytes in the operators were decreased ($P < 0.01–0.0001$). *Conclusion*: The findings in this study suggest that ozone causes oxidative damage in copier operatives.

Keywords: Ozone; Oxidation; Lipoperoxidation; Lipoperoxide; Antioxidant; Oxidative stress

INTRODUCTION

Copying of references, documents, information and photos plays important roles in social, economic, business, scientific and technologic affairs, however, photochemical smog emitted during photocopying process may be risk factors inducing potential oxidative damage of copying operators' bodies.[1,2] Some authors have reported that laser printers and photocopiers emit significant amounts of ozone, organic volatiles and formaldehyde during printing or operating process in a badly ventilated office environment, thus leading to oxidative stress and potential oxidative damage of the operators' bodies.[1,2] However, up to now, there are no reports on changes of free radical reactions in the bodies of photocopying operators (PO) who are working in the badly-ventilated office environments, nor reports about the relationship between oxidative stress and potential free radical damage associated with photocopying. To estimate the impact of photocopying on indoor air quality, to study oxidative stress and potential free radical damage to the PO bodies, and to explore a role for ozone emitted during photocopying process, 80 PO and 80 healthy volunteers (HV) were enrolled in a random control study design, in which the level of lipoperoxide (LPO, thiobarbituric acid reactive substances, TBARS) in erythrocytes and the levels of vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR) in plasma as well as the activities of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes were determined by spectrophotometric methods. At the same time, the differences between the average values of the biochemical parameters in the PO group and the HV group were compared.

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TABLE I The demographic data and some other data in the PO group and the HV group

Group	*n*	Age (Years)	Gender		Hemoglobin (g/l)	Albumin (g/l)	Body-mass index
			M	F			
PO	80	28.5 ± 4.8	51	29	139.86 ± 6.96	43.86 ± 4.35	23.40 ± 1.25
HV	80	28.4 ± 4.8	50	30	139.47 ± 7.62	43.75 ± 4.03	23.51 ± 1.28
		0.067*	0.027**		0.337*	0.169*	0.571*
P		0.947	0.870		0.736	0.866	0.569

Note: **t* value of independent samples *t* test, ***Chi-square value of Pearson Chi-square test.

Pearson product-moment correlation between the ozone level in the photocopying sites and the biochemical parameters as well as the correlation between the duration of exposure to ozone and the biochemical parameters were analyzed.

MATERIALS AND METHODS

Study Design

A random control study design was used in the present study. In order to obtain an objective research conclusion, the principles of random samples, control, replication and equilibrium and the management factor, experimental effect and subjects and the inclusion criteria and exclusion criteria of subjects, etc. were taken into full consideration, and were strictly executed in the studying practice.[3–6]

Subjects

Photocopying Sites

Eighty photocopying sites without well-ventilated equipment were used as samples. Volumes of these photocopying sites ranged from 20 to 50 m^3 (32.22 ± 9.01), and there were 1–3 photocopiers in the each photocopying site.

Photocopying Operators (PO)

Eighty PO were randomly sampled from 157 PO in some libraries and copying-businessmen with "Select Cases-Random Sample" of "SPSS 11.0 for Windows". The PO duration of exposure to ozone ranged from 2 to 10 years (5.6 ± 2.4) and their ages, hemoglobin levels, serum albumin levels and body-mass indexes were 21–40 years, 120.61–153.16 g/l, 35.82–50.73 g/l and 19.66–25.68, respectively. They were all volunteers in this study.

Healthy Volunteers (HV)

Eighty HV were randomly sampled from 200 HV confirmed by comprehensive physical examination at the Second Affiliated Hospital, College of Medicine, Zhejiang University, with "Select Cases-Random Sample" of "SPSS 11.0 for Windows". Their ages, hemoglobin levels, serum albumin levels and body-mass indexes were 21–40 years, 125.05–152.02 g/l, 37.25–50.57 g/l and 21.10–25.39, respectively.

The demographic data and some other data of 80 PO and 80 HV are presented in Table I.

The above PO and HV' medical history of disorders associated with brain, heart, lung, liver, kidney and other organs as well as blood system, circulatory system, respiratory system, digestive system and other systems were all excluded by their routine blood, urine and feces examinations as well as radiographs, cardiogram and other necessary examinations. And their medical history of every inflammation, hypertension, hyperlipidemia, acute or chronic bronchitis, autoimmune disease, diabetes, atherosclerosis, tumors and other diseases and subnutrition, malnutrition, supernutrition and other nutritional diseases were also all excluded. In addition, the above subjects all had no smoking history or excessive drinking history.

The above subjects were never exposed to radiation, nor engaged in work exposing them to intoxicating materials or pesticides. Within the prior month in which they volunteered the experimentation in the study, none of the subjects had taken any antioxidant supplements such as VC, VE, β-CAR, ginkgo biloba, tea polyphenols or other similar substances.

METHODS

Measurement of Ozone Level in the Indoor Air of Copying Sites

Ozone-analyzer was used to determine directly ozone level in the indoor air in 80 photocopying sites. They were measured 150 cm, above the ground at four corner places in every photocopying site for 1.0 min at 10a.m. and 3p.m. and the average value of ozone was expressed as ppm.

Collection and Pretreatment of the Blood Samples

Fasting venous blood samples were collected in the morning from all the subjects and heparin sodium was added as anticoagulant and the promptly

separated plasma and erythrocytes were stored at −50°C immediately.[3,4] The blood samples collected did not undergo any hemolysis.

Biochemical Measurements

Erythrocytic LPO (TBARS) Level

Spectrophotometry of TBARS was used to determine erythrocytic LPO (TBARS) level which was expressed as nmol/g Hb.[3,4]

Plasma VC Level

Spectrophotometry of ferrozine coloration was used to determine plasma VC level which was expressed as μmol/l.[3,4]

Plasma VE Level

Absolute ethanol was used to sediment proteins in plasma and to extract VE from plasma. The VE in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}. Fe^{2+} reacted with ferrozine to form a colored end product that was detected at 563 nm, with its level expressed as μmol/l.[3,4]

Plasma β-CAR Level

β-CAR was extracted with a mixture of ethanol and petroleum ether and was assayed with spectrophotometry and its level was expressed as μmol/l.[3,4]

Erythrocytic SOD Activity

Spectrophotometry of inhibiting pyrogallol autooxidation was used to determine erythrocytic SOD activity which was expressed as U/g Hb.[3,4]

Erythrocytic CAT Activity

Spectrophotometry of coloration of hydrogen peroxide and acetic acid-potassium dichromate was used to determine erythrocytic CAT activity which was expressed as K/g Hb.[3,4]

In the determination of the above biochemical substances and enzymes, the main analytical reagents, such as VC, VE, β-CAR, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazinedisulfonic acid disodium salt (ferrozine), Cu/Zn-SOD, CAT, 1,2,3-trihydroxybenzene (pyrogallol), 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid, were purchased from SIGMA® chemical company, USA; and the other analytical reagents were produced in China, the fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. The instrument used to determine ozone level was DCS-1 Ozone-analyzer, Shanghai, China.

In the determination of the above biochemical substances and enzymes, the standardization of experiment, e.g. the same batch number of each reagent, the same quality control, the same lab assistant and the identical analytical apparatus were strictly used for every experiment in order to decrease errors and to ensure the analytical quality of determinations.[3–6]

Medical Statistical Analysis

All data were statistically analyzed with SPSS 11.0 for Windows statistic software using a Compaq Pentium IV/1.6 GHz computer. The biochemical parameters in this study presented normal distributions by Kolmogorov–Smirnov Z test and were expressed as mean plus or minus standard deviation ($\bar{\chi} \pm s$) and 95% confidence interval (95% CI). Hypothesis testing methods included independent-samples t test, Pearson chi-square test (χ^2 test), Pearson product-moment correlation analysis. In the statistical analysis of this study, the level of hypothesis testing (α) was ≤ 0.05 in order to avoid false positives (α-error), and the power of hypothesis testing (*power*) was ≥ 0.80 to avoid false negatives (β-error).[3–6]

RESULTS

Ozone Level in the Indoor Air in the Photocopying Sites

Ozone level in the indoor air in 80 photocopying sites without well-ventilated equipment ranged from 0.054 to 0.204 ppm (0.113 ± 0.042). Pearson product-moment correlation analysis showed that the ozone level in the indoor air was increased with the decrease of volume of photocopying site ($r = -0.578$, $n = 80$, $P < 0.0001$).

Comparison Between the Average Values ($\bar{\chi} \pm s$) of the Biochemical Parameters in the PO Group and the HV Group

Compared with the HV group, the average value of LPO (TBARS) in erythrocytes in the PO group was significantly increased ($P < 0.0001$), while the average values of VC, VE and β-CAR in plasma as well as those of SOD and CAT in erythrocytes in the PO group were significantly decreased ($P < 0.0001$) (Table II).

95% CI of the Average Values of the Biochemical Parameters in the PO Group and the HV Group

The lower limit of 95% CI of the average value of LPO (TBARS) in erythrocytes in the PO group was greater than the upper limit of 95% CI of same that in

TABLE II Comparison between the average values ($\bar{\chi} \pm s$) of the biochemical parameters in the PO group in the HV group

			Antioxidative constituents				
		Oxidative constituents	Plasma			Erythrocytes	
Group	*n*	Erythrocytes LPO (nmol/g Hb)	VC (μmol/l)	VE (μmol/l)	β-CAR (μmol/l)	SOD (U/g Hb)	CAT (K/g Hb)
PO	80	32.22 ± 4.62 (31.19 ∽ 33.25)	37.29 ± 9.58 (35.16 ∽ 39.42)	16.08 ± 4.09 (15.17 ∽ 16.99)	1.21 ± 0.32 (1.14 ∽ 1.28)	1862.6 ± 144.6 (1830.4 ∽ 1894.7)	252.2 ± 65.7 (237.6 ∽ 266.9)
HV	80	28.04 ± 3.97 (27.16 ∽ 28.93)	52.84 ± 13.83 (49.76 ∽ 55.91)	23.91 ± 6.07 (22.56 ∽ 25.27)	1.67 ± 0.45 (1.57 ∽ 1.78)	2033.2 ± 159.1 (1997.8 ∽ 2068.6)	309.9 ± 81.3 (291.8 ∽ 328.0)
*t**		6.132	8.265	9.572	7.428	7.098	4.932
P		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Note: *Independent-samples *t* test. The figures in parentheses are 95% confidence interval.

the HV group. The upper limits of 95% CI of the average values of VC, VE, β-CAR, SOD and CAT in the PO group were less than the lower limits of 95% CI of same those in the HV group (Table II).

Pearson Product-moment Correlation Analysis between the Ozone Level in the Indoor Air of the Photocopying Sites and the Biochemical Parameters in 80 Photocopying Operators

The findings of Pearson product-moment correlation analysis showed that the LPO (TBARS) value in erythrocytes in the PO was increased with the increase of the ozone level in the indoor air of photocopying sites, while the values of VC, VE and β-CAR in plasma as well as those of SOD and CAT in erythrocytes in the operators were decreased with the increase of the ozone level in the indoor air of the photocopying sites, See Fig. 1(parts **a**–**f**).

Pearson Product-moment Correlation Analysis between the Duration of Exposure to Ozone and the Biochemical Parameters in 80 Photocopying Operators

The findings of Pearson product-moment correlation analysis showed that the LPO (TBARS) value in erythrocytes in the PO was increased with the duration of exposure to ozone, while the values of VC, VE and β-CAR in plasma as well as those of SOD and CAT in erythrocytes in the operators were decreased with the duration of exposure to ozone (Table III).

DISCUSSION

Lipoperoxide (LPO) and its metabolic products, such as malondialdehyde and conjugated diene, etc. play an important role in the healthy status in the human.[3,4,7–22] VC, VE, β-CAR, SOD and CAT are important antioxidants in the human body.[3,4,7–15,20,23–39] Significant increase of LPO level and marked decrease of antioxidant levels in the human body may cause metabolic disorders and pathological aggravation of a series of free radical chain reactions, thus inducing a variety of diseases related to free radicals.[3,4,7–39]

The findings in this study showed that in the indoor air in the photocopying sites without well-ventilated equipment, the ozone pollution was comparatively serious, even-though the lowest ozone level was beyond the recommended maximum exposure limit of 0.051 ppm in the hygienic standard of public places and far exceeded the ozone olfact (olfactory threshold of ozone) of 0.009 ppm.[40] The findings also showed an imbalance between oxidation and antioxidation, and an oxidative stress and potential free radical damage in the PO bodies. There might be several interpretations.

The simplest reason was that a large amount of ozone emitted by photocopiers could not be adequately exhausted to the open air because of badly-ventilated office environments. Ozone is one of the main components and the most abundant oxidant in photochemical smog.[1,2,40–45] Ozone may cause oxidative decomposition and peroxidative modification of polyunsaturated fatty acids, unsaturated phospholipids, glycolipids and cholesterol in plasma and cell membranes, which may induce aggravation of lipoperoxidation in the human, thus resulting in marked increase of erythrocytic LPO in the copying operators who inhaled a large amount of ozone, and leading to potential free radical damage to the operators.[41,43,46] Such lipoperoxidation and lipoperoxidative damage may lead to the damage of cell functions and to cytostasis.[41,43,46] At the same time, the excessive ozone in the human may generate a large amount of $O_2^{\bullet -}$, •OH, $HO_2^{\bullet}$ and other free radicals,[47] which may also cause a series of oxidative decomposition and peroxidative modification of many organic compounds in the human, thus producing a large amount of LPO, and resulting in significant increase of erythrocytic LPO.[3,4,9–11,26,27,40,41,43,46]

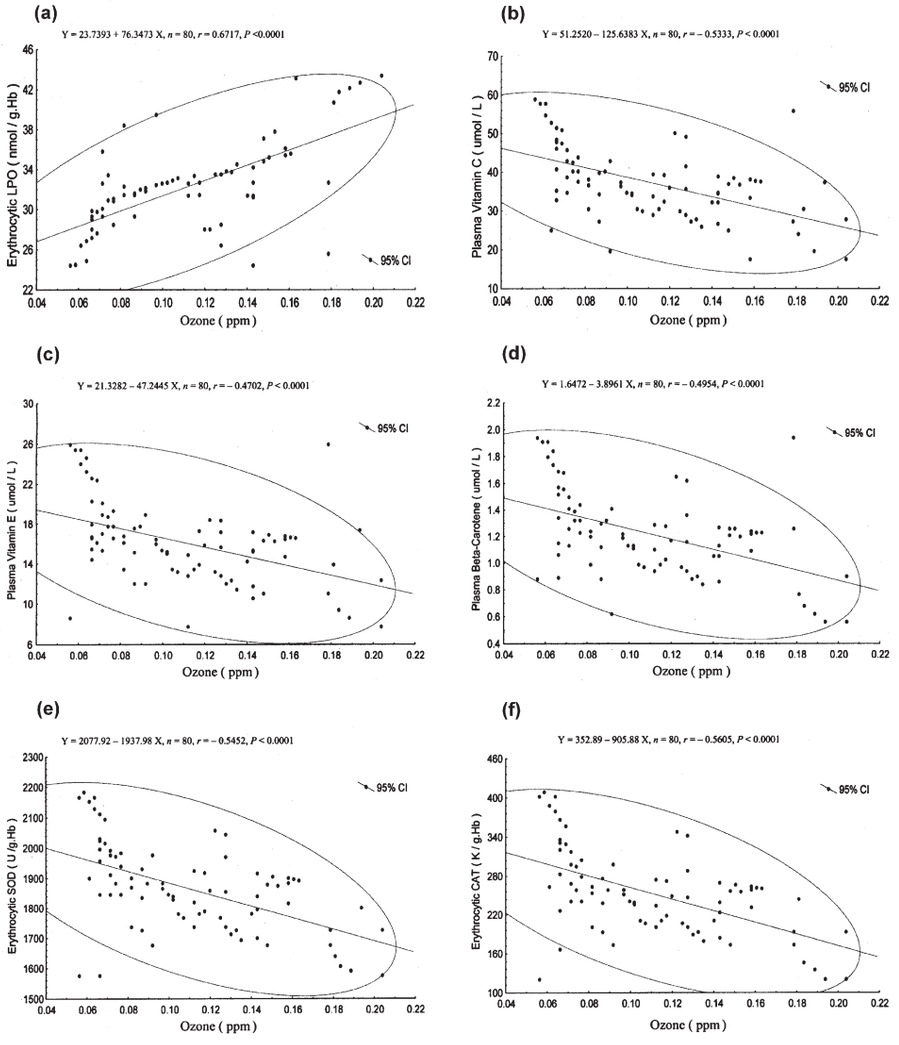

FIGURE 1 Correlations between **a** erythrocytic LPO with ozone; **b** plasma VC with ozone; **c** plasma VE with ozone; **d** plasma β-CAR with ozone; **e** erythrocytic SOD with ozone; **f** erythrocytic CAT with ozone.

We recognize that TBARS is only an approximate assay of LPO,[3,4,8–10,26,27,53–55] but it is widely used. In this study, the LPO level in erythrocytes and erythrocytic membranes had been determined to substitute for determination of LPO level in serum or plasma. Serum or plasma LPO level may not be able to reflect the lipoperoxidation and lipoperoxidative damage in the human because it can be affected by many factors, such as change of serum or plasma volume, food and others, whereas the determination of erythrocytic LPO level may not be affected by these factors.[3,4,8–10,26,27,53–55]

As a strong oxidant, ozone or its reactive products may interact directly with DNA, thus causing DNA damage and inhibiting or depressing DNA replication.[41,43] Ozone may also linearize

TABLE III Pearson product-moment correlation analysis between the duration of exposure to ozone and the every biochemical parameter

Correlative item	*n*	Correlative coefficient (*r*)	*t*	P
Duration exposed to ozone with erythrocytic LPO	80	0.4416	4.3468	0.0001
Duration exposed to ozone with plasma VC	80	−0.3005	2.7829	0.0068
Duration exposed to ozone with plasma VE	80	−0.3052	2.8306	0.0059
Duration exposed to ozone with plasma β-CAR	80	−0.3342	3.1312	0.0025
Duration exposed to ozone with erythrocytic SOD	80	−0.3959	3.8071	0.0003
Duration exposed to ozone with erythrocytic CAT	80	−0.3714	3.5329	0.0007

circular DNA, [41,43] which leads to a significant decrease in synthesis or regeneration of SOD and CAT and results in marked decrease of their activities. Additionally, excessive $O_2^{\bullet -}$, •OH, and other free radicals as well as $^1O_2$, H_2O_2 and other reactive oxygen species[3,4,9–11,26,27,40,41,43,46] may also cause DNA damage and attack strongly the structures of VC, VE, β-CAR, SOD, CAT and other antioxidants and may inactivate these antioxidants, thus resulting in significant decreases of the levels of VC, VE and β-CAR as well as the activities of SOD and CAT in the operators' bodies.[41,43,46,48]

In the present study, the findings of Pearson product-moment correlation analysis showed that the levels of the above biochemical constituents were closely related to the ozone level in the indoor air in the copying sites and the operators' duration of exposure to ozone.[49] Therefore, the ozone level in the indoor air should be controlled and kept to below the recommended maximum exposure limit by a well-ventilated office environment in every photocopying site.[1,2,40]

The matching of controls for occupational levels is very important in the medical research.[3–5,8–10,25–27] According to the data that we collected in this study, the average values of annual earning of the PO and the controls (HV) were about RMB YUAN 15,000–22,000 (US$ 1800–2650) and 14,000–23,000 (US$ 1700–2770), respectively (in years 2000 and 2001) and the result of independent samples *t* test showed there was no significant difference between the annual earning in the PO group and the HV group. In the education level between the two groups there was no significant difference by Pearson Chi-Square test, too. Therefore, in our study the social-economic status was comparable between the two groups. As for their nutritional status, the albumin levels and the body-mass indexes that reflected, in general, the nutrition levels in the human, we did not find that there was significant difference between the two groups. So, in our study the biological status was also comparable between the two groups. In the study design and study practice we executed strictly the principles of random samples, control, replication and equilibrium, and executed strictly the inclusion criteria and exclusion criteria in the inclusion of the subjects, too. The confounder (confounding bias) would not almost be occurred in our study.

In summary, the findings in the present study suggested that the dynamic balance between oxidation and antioxidation was abnormal in the bodies of PO who were working in the copying sites with badly ventilated office environment, and the oxidative stress in their bodies was aggravated, thereby leading to potential oxidative damage in their bodies. We, therefore, recommend that there should be well-ventilated equipment in every copying site so that the ozone concentration in the indoor air may be decreased to below 0.051 ppm, and that antioxidant vitamins at suitable doses, such as VC and VE, should be given to copying operators daily in order to alleviate potential oxidative damage in their bodies because these antioxidant vitamins are free-radical scavengers and have a protective effect against photo-oxidant exposure damage of ozone.[3,4,9–11,13,14,20,26,27,40,50–58]

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