ORIGINAL ARTICLE

Levels of hemoglobin and lipid peroxidation metabolites in blood, catalase activity in erythrocytes and peak expiratory flow rate in subjects with passive exposure to tobacco smoke

Marcin Zawadzki[1], Paweł Gać[2], Rafał Poręba[3], Eugenia Murawska-Ciałowicz[4], Tomasz Wielkoszyński[5], Lidia Januszewska[1], Krystyna Pawlas[1], Ryszard Andrzejak[3]

1 Department of Hygiene, Wrocław Medical University, Wrocław, Poland
2 Student Scientific Association of Environmental Medicine and Toxicology at the Department of Hygiene, Wrocław Medical University, Wrocław, Poland
3 Department of Internal Diseases, Occupational Diseases and Hypertension, Wrocław Medical University, Wrocław, Poland
4 Department of Physiology and Biochemistry, University School of Physical Education, Wrocław, Poland
5 Department of Chemistry, Medical University of Silesia, School of Medicine and Division of Dentistry, Zabrze, Poland

KEY WORDS

antioxidant enzymes, cotinine, lipid peroxidation, passive smoking

ABSTRACT

INTRODUCTION Exposure to tobacco smoke is an extremely important risk factor determining the development of respiratory diseases, cardiovascular diseases and cancer. Passive exposure is common and often not realized by the exposed subjects. Markers of tobacco smoke exposure are nicotine metabolites, i.e. cotinine and trans-3'-hydroxycotinine.

OBJECTIVES The objective of the study was to assess the level of passive exposure to tobacco smoke among students and the exposure impact on the blood hemoglobin level, peak expiratory flow (PEF), lipid peroxidation level and antioxidant enzyme activity.

PATIENTS AND METHODS A total of 104 subjects were enrolled in the study. The subjects were categorized in 3 subgroups depending on nicotine metabolite levels in blood (subgroup I with metabolite level >100 ng/ml (high exposure); subgroup II with the metabolite level of 10–100 ng/ml; subgroup III with metabolite level <10 ng/ml). The blood hemoglobin level, PEF, levels of lipid peroxidation metabolites – malondialdehyde and 4-hydroxynonenal (MDA + 4-HNE) and catalase (CAT) activity were determined in all the subjects.

RESULTS The study showed statistically significant differences in levels of lipid peroxidation metabolites and CAT activity. Levels of MDA + 4-HNE were higher in subgroup I than in subgroup II or III (I: 3.84 ±1.64 mmol/l; II: 2.25 ±0.94 mmol/l; III: 1.90 ±0.82 mmol/l; $p_{I-II} < 0.01$; $p_{I-III} < 0.001$). CAT activity was statistically significantly lower in subgroup I than in subgroup III (I: 0.38 ±0.01 × 10^6 IU/g hemoglobin [Hb]; II: 0.38 ±0.03 × 10^6 IU/g Hb; III: 0.41 ±0.04 × 10^6 IU/g Hb; $p_{I-III} < 0.05$).

CONCLUSIONS Passive exposure to tobacco smoke in the study population of students is common. The observed effects of passive exposure to tobacco smoke are similar to those of active smoking. It is postulated to undertake actions aiming at limiting passive exposure to tobacco smoke.

Correspondence to:
Rafał Poręba, MD, PhD,
Katedra i Klinika Chorób Wewnętrznych, Zawodowych i Nadciśnienia Tętniczego,
Akademia Medyczna, ul. Pasteura 4,
50-367 Wrocław, Poland,
phone: +48-71-784-25-20,
fax: +48-71-784-09-54,
e-mail: sogood@poczta.onet.pl
Received: April 15, 2008.
Revision accepted: June 19, 2008.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2008;
118 (12): 705-712
Translated by Iwona Rywczak, MD, PhD

TABLE 1 Characteristics of study subgroups established based on nicotine metabolite level in blood

	Subgroup I	Subgroup II	Subgroup III	p
Enrolment[b]	38	32	34	NS
Age[a] (years)	21.6 ±1.2	22.2 ±1.6	22.2 ±1.5	NS
Height[a] (cm)	175.5 ±8.1	176.5 ±7.5	178.8 ±4.2	NS
Weight[a] (kg)	75.5 ±9.2	73.7 ±11.2	74.7 ±7.5	NS
BMI[a] (kg/m^2)	24.5 ±2.3	23.6 ±3.0	23.6 ±2.1	NS
Sex[b] (M/F)	38/0	32/0	34/0	NS

a mean values ±standard deviation
b absolute values

Abbreviations: BMI – body mass index, NS – not significant

INTRODUCTION In Poland, smoking is estimated to cause every second death in males aged 30–70.[1] Exposure to tobacco smoke is an extremely important risk factor determining the development of respiratory diseases, cardiovascular diseases and cancer. Cigarette smoking is the main cause of chronic obstructive pulmonary disease (COPD), which trebles the risk of myocardial infarction and a stroke.[2] Smokers are reported to have high blood cholesterol, higher prevalence of atherosclerosis and peripheral vascular diseases.[3] Smoking is one of causes of impotence in males and female fertility disorders, and increases the risk associated with use of contraceptives.[4] Smokers are also more prone to osteoporosis and Crohn's disease, and they more commonly develop resistant hypertension.[1] Respiratory tract, bladder, pancreas, kidney and stomach cancer is also more common in smokers.[5]

Passive exposure is widespread and often not realized by the exposed subjects.[6-9] The impact of passive smoking on the human body has not been fully understood and seems to require investigation to determine its mechanisms.[10]

In order to have smoking-induced health hazards appropriately estimated, the level of exposure to tobacco smoke should be correctly determined. The assessment could be done by a questionnaire or based on determination of the so-called exposure markers. Admittedly, a questionnaire is the simplest and an inexpensive approach to obtain information on smoking, however, it does not represent real exposure to tobacco smoke, especially when non-smokers with passive exposure to environmental tobacco smoke (ETS) are concerned.[11] Currently, it is thought that the most useful markers of exposure to tobacco smoke are nicotine metabolites, i.e. cotinine and trans-3'-hydroxycotinine. Cotinine is the main product of nicotine transformation with long half-life (17–32 h) in the body. Sensitivity of methods measuring the cotinine level in body fluids ranges from 96% to 98%, and specificity from 97% to 100%.[12]

The objective of the study was to assess the level of passive exposure to tobacco smoke among the students of the Faculty of Medicine of Wrocław Medical University, using nicotine metabolites as specific markers, and to evaluate the exposure impact on the blood hemoglobin level, peak expiratory flow (PEF) rate, lipid peroxidation level and antioxidant enzyme activity expressed as catalase (CAT) activity in erythrocytes.

PATIENTS AND METHODS We studied 104 males, second-year students of the Faculty of Medicine, Wrocław Medical University, who declared only passive exposure to tobacco smoke. Students were enrolled into the study at random. The mean age of the study subjects was 22.0 ±1.4 years, height 176.88 ±6.94 cm, weight 74.70 ±9.32 kg, and body mass index 23.86 ±2.51 kg/m^2. Subjects with a history of chronic systemic or metabolic diseases were ineligible in this study.

Biochemical determination of particular substances in blood was performed in the study group. Each subject had an approximately 5-ml blood sample taken and ethylenediaminetetraacetic acid was used as an anticoagulant. Then the blood was centrifuged and the level of main nicotine metabolites, i.e. cotinine and trans-3'-hydroxycotinine and levels of lipid peroxidation markers, i.e. malondialdehyde and 4-hydroxynonenal (MDA + 4-HNE) were determined in the collected supernatant using the immunoenzymatic assay. Determination of the nicotine metabolites was based on an original method. Tests consisted in immunochemical determination using polyclonal antibodies to cotinine and trans-3'-hydroxycotinine. The upper limit of the measurement range expressed as the ED 90 value was 3 ng/ml. The remaining blood cells were rinsed out 3 times with a normal saline solution, and then CAT activity was evaluated. Moreover, each subject had the PEF rate, hemoglobin level and red blood cells count in peripheral blood determined. The PEF was measured using the hand-held Personal Best peak flow meter, according to the manufacturer's instructions. It was used as a respiratory function indicator because of simplicity of determination, better availability as compared to spirometry and published data indicating changes in the PEF rate as the most immediately detectable disorder of respiratory function in subjects exposed to tobacco smoke.

Based on the nicotine metabolite levels, the study group was divided in the following 3 subgroups:

1 subgroup I with nicotine metabolite levels >100 ng/ml, a group of distinct exposure to tobacco smoke including 38 subjects
2 subgroup II with levels 10–100 ng/ml, 32 subjects
3 subgroup III with metabolite levels <10 ng/ml, a group of low (threshold) exposure to tobacco smoke, 34 subjects.

Cut off scores used to categorize the groups were taken from available literature. Due to the possibility of slight exposure to nicotine from the diet, it was assumed that values <10 ng/ml should be considered as negative.

TABLE 2 Biochemical determinations and peak expiratory flow in the study subgroups established based on nicotine metabolite level in blood

	Subgroup I	Subgroup II	Subgroup III	p
Enrolment[b]	34	32	38	NS
Hb[a] (g/dl)	14.81 ±1.32	14.95 ±0.47	15.27 ±0.78	NS
RBC[a] (x10^6)	5.28 ±0.26	5.33 ±0.36	5.31 ±0.42	NS
PEF[a] (l/min)	592.22 ±145.46	596.15 ±95.94	599.48 ±51.87	NS
MDA + 4-HNE[a] (mmol/l)	3.84 ±1.64	2.25 ±0.94	1.90 ±0.82	I–II: $p < 0.01$ I–III: $p < 0.001$
CAT[a] (×10^6 IU/g Hb)	0.38 ±0.01	0.38 ±0.03	0.41 ±0.04	I–III: $p < 0.05$

a mean values ±standard deviation
b absolute values

Abbreviations: CAT – catalase activity in erythrocytes, HB – hemoglobin level in peripheral blood, MDA + 4-HNE – level of lipid peroxidation markers in blood, PEF – peak expiratory flow, RBC – peripheral blood erythrocyte count, others – see **TABLE 1**

Therefore, subgroup III could be treated as a control subgroup of subjects not exposed to tobacco smoke. Characteristics of subgroups established based on the nicotine metabolite level in blood are presented in **TABLE 1**.

Statictical analysis Statistical analysis was performed based on the STATISTICA 6.0 software (StatSoft Polska, Kraków). The results were presented as mean values and standard deviations. Distribution of variables was checked using the Shapiro-Wilk test. In case of non-normal distribution of variables parameters, the differences between the mean values were tested with the non-parametric equivalent of univariate analysis of variance (ANOVA), i.e. with the Kruskal-Wallis one-way ANOVA. Statistically significant differences were assessed using post-hoc tests. The analysis of correlation and regression was performed to determine the association between the tested variables. A $p < 0.05$ was considered statistically significant.

RESULTS The mean level of main nicotine metabolites, i.e. cotinine and trans-3'-hydroxycotinine in blood supernatant of the subjects was 61.36 ±55.91 ng/ml. The subjects were divided in 3 groups of high, moderate and low exposure to tobacco smoke (36.54%, 30.77% and 32.69%, respectively). Mean hemoglobin levels and erythrocyte counts in peripheral blood in the whole study group were 15.01 ±0.96 g/dl and 5.31 ±0.35 × 10^6, respectively. The mean PEF rate was 595.81 ±106.03 l/min. The mean total level of lipid peroxidation markers, i.e. MDA + 4-HNE, was 2.72 ±1.49 mmol/l, and erythrocyte CAT activity was 0.39 ±0.04.

In the subgroups singled out based on a criterion of nicotine metabolite levels in blood, hemoglobin levels and erythrocyte counts in peripheral blood were similar. The subgroups were not statistically different in the mean PEF rate (**TABLE 2**).

The analysis of oxidative stress parameters in the subgroups showed statistically higher levels of MDA + 4-HNE in subgroup I than in subgroups II or III (I: 3.84 ±1.64 mmol/l; II: 2.25 ±0.94 mmol/l; III: 1.90 ±0.82 mmol/l; $p_{I–II} < 0.01$; $p_{I–III} < 0.001$). CAT activity was statistically significantly lower in subgroup I than in subgroup III (I: 0.38 ±0.01 × 10^6 IU/g Hb; II: 0.38 ±0.03 × 10^6 IU/g Hb; III: 0.41 ±0.04 × 10^6 IU/g Hb; $p_{I–III} < 0.05$) (**TABLE 2**).

The whole study group demonstrated negative linear correlations between blood nicotine metabolite levels and erythrocyte CAT activity ($r = –0.49$; $p < 0.05$) (**FIGURE 1**) and between the nicotine metabolite level and the PEF rate ($r = –0.25$; $p < 0.05$) (**FIGURE 2**). Moreover, blood nicotine metabolite levelsin the whole study group positively correlated with blood MDA + 4-HNE levels ($r = 0.67$; $p < 0.05$) (**FIGURE 3**). Furthermore, subgroup I demonstrated a negative linear correlation between levels of main nicotine metabolites and hemoglobin levels in peripheral blood ($r = –0.73$; $p < 0.05$) (**FIGURE 4**).

The multivariate stepwise reverse regression analysis showed that the independent factor of the oxidative stress increase (expressed by the increased level of lipid peroxidation metabolites) in the study group was the nicotine metabolite level in blood – oxidative stress increases with the increase in cotinine and trans-3'-hydroxycotinine levels, whereas the factor of the oxidative stress decrease (expressed by the decreased level of lipid peroxidation metabolites) in the study group is the CAT – oxidative stress decreases with the increase in the CAT activity level. The association is presented below as a model resulting from regression analysis:

MDA + 4-HNE = 3.35 + 0.02 × cotinine/trans-3'-hydroxycotinine – 4.17 × CAT ±1.12

$SEM_{intercept} = 1.48$
$SEM_{B\ cotinine/trans\text{-}3'\text{-}hydroxycotinine} = 0.002$
$SEM_{B\ CAT} = 3.55$

SEM – standard error of measurement

All parameters of the model are statistically significant at the level of $p < 0.05$. Statistical power of the model is confirmed by the statistical significance level of the model ($p = 0.001$), the model coefficient of determination ($R^2 = 0.6437$) and the proportion of variance explained by the model (61.63%).

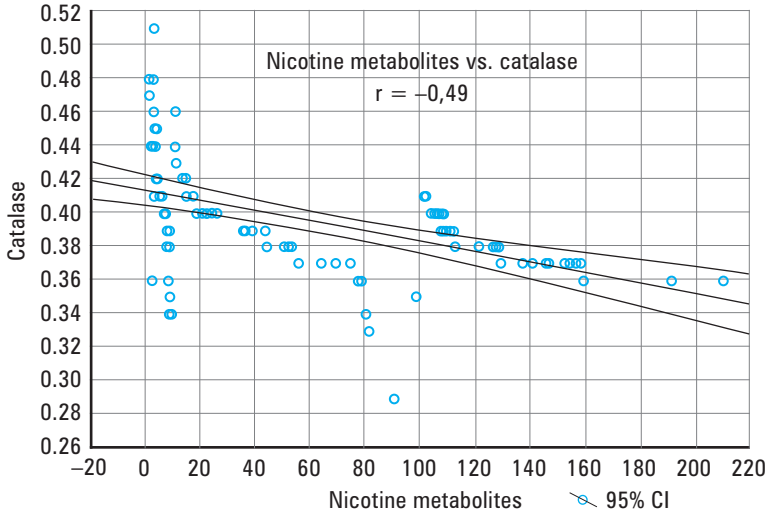
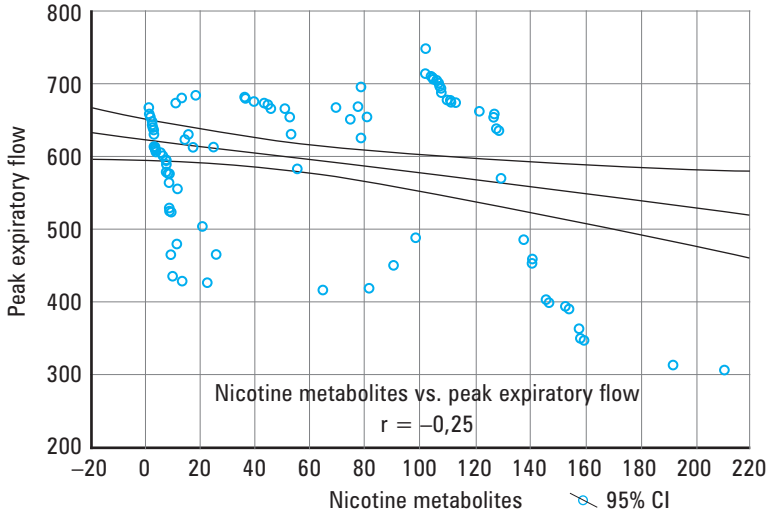


FIGURE 1 Association between catalase activity in erythrocytes and nicotine metabolite level in blood ($p < 0.05$) in the whole study group ($n = 104$)

DISCUSSION According to NHANES III (Third National Health and Nutrition Examination Survey 1988–1991), passive exposure to tobacco smoke assessed based on serum cotinine involves 87.9% of the USA population and is significantly more common than it would appear from the results based on a questionnaire survey (43% of the subjects exposed to ETS).[13] The proportion achieved in this study, i.e. almost 70% of subjects with the cotinine level in blood supernatant exceeding 10 ng/ml, seems to demonstrate that exposure to ETS in Poland is also common. This result exceeds the ones obtained in previous studies made for selected populations in Poland based on questionnaire surveys, both multicenter and conducted by individual researchers.[6,7,9]

One of the most immediately revealed effects of cigarette smoking is airflow impairment, expressed by abnormal spirometry results.[14] Components of tobacco smoke contribute to activation of pulmonary macrophages. Release of several inflammatory mediators and lytic enzymes (metalloproteinases, elastase, cathepsin) by granulocytes, stimulated by cytokines secreted by macrophages, migrating to interstitial pulmonary tissue, favors the remodeling of the bronchial tree and in consequence leads to development of COPD.[15] According to Lubiński et al., the most immediately detectable change in the spirometric test in smokers is a decrease in the PEF rate. In a study conducted in 2000 on a group of 3004 healthy young males aged 18–23, the PEF rate in smokers was statistically significantly lower than in the non-smoker group (96.6% of a normal value in smokers vs. 103% in non-smokers, $p < 0.05$).[16] Although the current study did not demonstrate a statistically significant difference in the PEF rate between the study groups, it should be noted that the lowest PEF rate was characteristic of subjects with the highest exposure to ETS. Furthermore, the study showed a statistically significant adverse linear correlation between the PEF rate and the nicotine metabolite level in blood. This may indicate that passive exposure to tobacco smoke also leads to tobacco smoke-related lesions in the human respiratory tract.

FIGURE 2 Association between peak expiratory flow rate and nicotine metabolite level in blood ($p < 0.05$) in the whole study group ($n = 104$)



The smoker's body is also subject to quantitative and qualitative changes in blood composition. It is assumed that active smokers demonstrate a significantly higher number of leukocytes in peripheral blood.[17,18] A number of neutrophils is particularly increased.[19] Moreover, the peripheral blood erythrocyte count and the hemoglobin level are increased, and erythropoiesis is enhanced. This provides adaptation to chronic hypoxemia resulting from prolonged exposure to carbon oxide contained in tobacco smoke.[20] Published data concerning altered complete blood counts in subjects with passive exposure to tobacco smoke are limited, apart from being incomplete and contradictory. Lack of statistically significant differences between mean values of the erythrocyte count and the hemoglobin level in blood in the compared subgroups, with the simultaneous strong adverse linear correlation between nicotine metabolite levels and hemoglobin levels in blood in the subgroup with high exposure to ETS, seems to exclude the chance of identical mechanisms underlying the impact of active and passive smoking on hematological parameters. The negative linear correlation between blood levels of hemoglobin and nicotine metabolite in subgroup I may even show no more options in adaptation of the body to changes occurring during clearly enhancedintensified exposure to ETS. For existing discrepancies, the impact of ETS on the erythrocytic system seems to require continuation and expanding research.

Most study findings seem to show that numerous oxidants in tobacco smoke induce oxidative damage and lead to enhanced oxidative modification of macromolecules.[21] Smokers demonstrate increased amounts of generated reactive oxygen species resulting in lipid peroxidation products having documented cytotoxic effect.[22] In a study conducted in 1994, Biagci et al. confirmed that oral administration of aqueous extracts of tobacco to rats, in the amounts corresponding both to active and passive exposure in humans, induced peroxidation of microsomal and mitochondrial lipids expressed by an increase in excretion

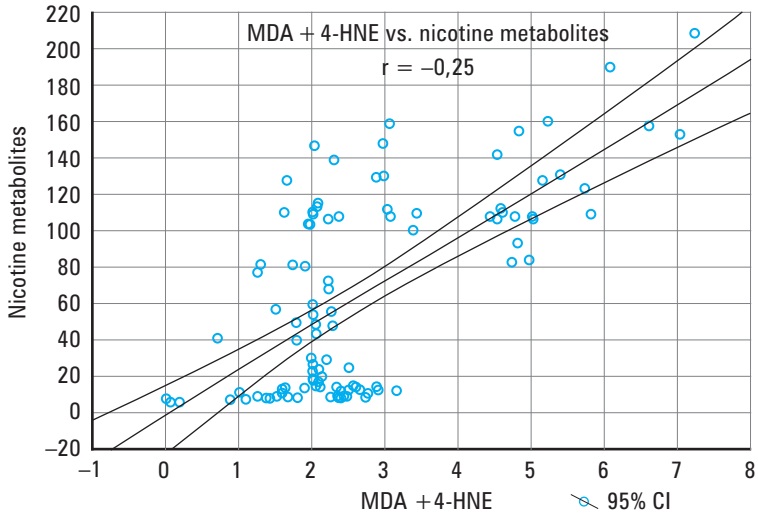
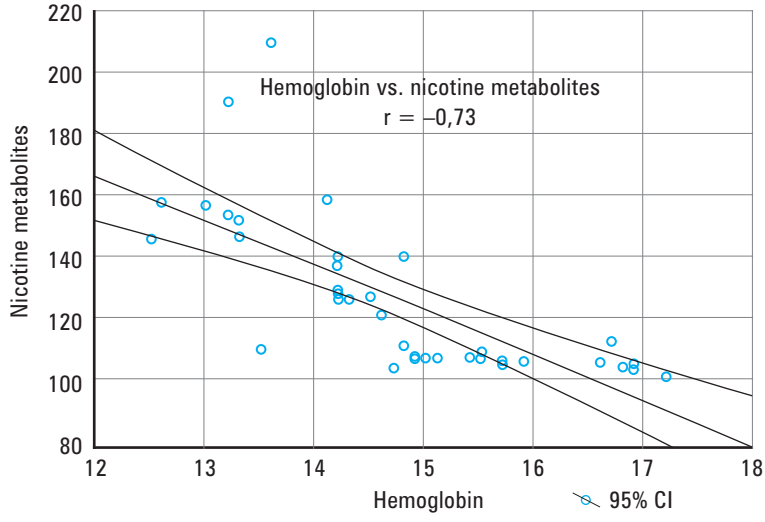


FIGURE 3 Association between blood levels of lipid peroxidation markers (MDA + 4-HNE) and nicotine metabolites ($p < 0.05$) in the whole study group (n = 104)
Abbreviations: MDA + 4-HNE – malondialdehyde and 4-hydroxynonenal

of MDA, formaldehyde, acetaldehyde and acetone in urine.[23] Likewise, Morrow et al. pointed out enhanced lipid peroxidation as a result of smoking in their study in 1995, when they used free and esterified F_2-isoprostanes as a lipid peroxidation marker.[24] The current study might not only confirm the current concept that also passive exposure to tobacco smoke induces lipid peroxidation (statistically significantly higher levels of lipid peroxidation metabolites in the group of subjects of cotinine level >100 ng/ml in comparison with the groups of moderate and low exposure to ETS), but shows a simple positive linear association between the nicotine metabolite level in blood and the level of lipid peroxidation markers. This could lead to the conclusion that the influence of tobacco smoke on the body does not depend on the exposure mode (active or passive), but on its intensity.

On the other hand, the impact of smoking on the body's antioxidative defense, i.e. on the levels of nonenzymatic "scavengers" of free radicals – vitamins E and C, and enzymes (superoxide dismutase, glutathione peroxidase and CAT) is still not thoroughly examined. Published data assessing vitamin E management in subjects exposed to tobacco smoke yielded conflicting results. Most of the studies showed a decrease in the tocoferol level in this group. Scheffer et al., Marangon et al. or Mezetii et al. found lower vitamin E levels in the tested biological materials.[25-27] However, according to Marangon et al., smoking results in an increase in the vitamin E level.[28] Few papers concerning vitamin C administration in subjects exposed to ETS indicated a decrease in ascorbic acid levels in the body as a result of exposure to tobacco smoke. Significantly lower vitamin C levels in plasma of both active and passive smokers were found by Princen et al.[29] and Howard et al.[30] A similar result, but only for active smokers, was achieved by Riemersma et al.[31] According to the current state of knowledge, the impact of tobacco smoke on the activity of antioxidant enzymes is considered to depend on the size of the exposure dose and the duration of exposure. Exposure to small doses of tobacco smoke, corresponding to passive exposure to ETS, causes a transient increase in the activity of those enzymes. On the other hand, exposure to high amounts of tobacco smoke compounds, of values typical of active smoking, results in exhausting the body's adaptability and a decrease in the activity of antioxidant enzymes.[32-34] This view is, however, a generalization, because there are reports on a different impact of smoking on the activity of antioxidative defense enzymes. Serwin and Chodynicka did not found statistically significant differences in activity of glutathione peroxidase between smokers and non-smokers.[35] Tabacova et al. observed increased CAT activity in pregnant smokers, even in those, who had the smoking index (a product of smoking duration in years and a number of cigarettes per day) >300.[36] The adverse linear correlation between the cotinine level and CAT activity in erythrocytes and statistically significantly lower CAT activity in subgroup I in comparison with subgroup III found in the current study also seem to question the above generalization. Probably even low passive exposure to tobacco smoke may result in exhaustion of adaptive antioxidative mechanisms. This in comparison with the study by Tabacova, may indicate the impact of other, except for exposure intensity only, factors on the direction of changes in the antioxidant enzyme activity in a body exposed to tobacco smoke. The presence of individual tendency to faster collapse of mechanisms "removing" free radicals among part of the population exposed to ETS seems probable.

FIGURE 4 Association between blood levels of hemoglobin and nicotine metabolites ($p < 0.05$) in the subgroup of males with high exposure to tobacco smoke (n = 38)



Oxidative stress is multifactorial. Considering numerous factors at the same time while determining its level seems to be important. Available data do not provide consistent opinions concerning the determination of independent risk factors of oxidative stress. Based on the regression analysis, it was found that the high nicotine metabolite level and decreased antioxidant enzyme activity in blood could be used as such risk factors. The resulting model may serve as a prototype for further studies aiming at its confirmation, supplementation or verification. As for today, it seems

to provide some possibility of predictive determination of oxidative stress intensity based on given 2 independent risk factors.

The present study is not free from limitations and imperfections. The low enrolment of the study group, and in particular the subgroups established based on a criterion of nicotine metabolite levels in blood, could be recognized as one of its main limitations. Although the requirements of statistical reliability of the study were met, i.e. the results were obtained in possibly homogenous groups consisting of more than 30 subjects, the enrolment was too low to provide direct generalizations about the population. The equally important limitation is lack of the comparison between subjects exposed to tobacco smoke and active smokers coming from the same population. Studies with the same group selection are planned soon. In the present paper, the authors tried to concentrate first of all on the interesting, in their opinion, comparison of the determined parameters between non-smokers (subgroup III could be recognized as such) and non-smokers with passive exposure. Another limitation of the present study could be the assessment of respiratory function only based on PEF, instead of spirometry. The reasons for selection of such methodology were already mentioned above.

Despite the described imperfections, the current study seems, in the authors' opinion, to be the basis for further more accurate studies defining the impact of passive smoking on the body's condition. As mentioned above, the impact of passive smoking on the human body has not been sufficiently explained and seems to require studies aiming at the determination of its mechanisms. Furthermore, the study can increase the level of social awareness with regard to toxicity of passive exposure to tobacco smoke. Despite the fact that such exposure is common, the exposed persons often do not realize it. In the future, the results of the studies may help assess changes in the exposure to tobacco smoke in the following years, and the obtained regression model, after its previous confirmation, supplementation or positive verification, may be used for the prediction of oxidative stress intensity in the body, based on given 2 independent risk factors.

In conclusion, passive exposure to tobacco smoke in the study population of students was found to be common. The observed effects of passive exposure to tobacco smoke are similar to the effects of active smoking. The same mechanisms as those indicated for active exposure are probably the basis of lesions developing in the body during passive exposure to tobacco smoke. The level of pulmonary macrophage activation, oxidative stress intensification and dysfunction of antioxidative defense mechanisms of the body during passive exposure to ETS does not differ significantly from the intensity of analogous lesions developing in the body of an active smoker. Therefore, the authors suggest that apart from the continuation of integrated modern educational interventions that focus on restricting the widespread smoking habit and having been conducted in Poland for several years, actions aiming at prevention of passive exposure to tobacco smoke, as a factor equally harmful to health, should be initiated. This suggestion is based on the fact that experience in the field of active smoking has unquestionably showed that in comparison with future costs of potential treatment, modern prophylaxis is far more effective and cost-effective.

REFERENCES

1 Milanowski J. [Smoking. Impact on health and habit control program]. Wydawnictwo Biforium. Lublin 2001. Polish.

2 Tatarczyka A, Wesół D, Kuźnar-Kamińska B. [Assessment of dependence between intensity of smoking habit and efficiency of respiratory system using spirometric test]. Nowiny Lekarskie 2005; 74:623-627. Polish.

3 Wielkoszyński T, Bodzek D. [Smoking and atherosclerosis. Current Views]. Czyn Ryzyka. 2001; 1-2: 39-50. Polish.

4 Mazurkiewicz M. [Tobacco-related cancer as social issue]. Lublin, Wydawnictwo Biforium, 2001. Polish.

5 Błaszczyk J, Pudełko M, Cisarż K, et al. [Malignancy prevalence in Dolnośląskie Province in Poland]. Dolnośląski Rejestr Nowotworów. Wrocław, 2003–2004. Polish.

6 Polakowska M, Piotrowski W, Tykarski A, et al. [Smoking habit in the Polish population. Results of WOBASZ Program]. Kardiol Pol. 2005; 63 (Suppl 4): S626-S631. Polish.

7 Szuba A, Poręba R, Młynek V, et al. [Prevalence, risk factors and treatment of arterial hypertension in dwellers of the village of Boguszyce]. Nadciśnienie Tętnicze. 2006; 2: 121-127. Polish.

8 Nižankowska-Mogilnicka E, Mejza F, Buist AS, et al. Prevalence of COPD and tobacco smoking in Malopolska region – results from the BOLD study in Poland. Pol Arch Med Wewn. 2007; 117: 402-409.

9 Poręba R, Gać P, Zawadzki M. Life style and cardiovascular risk factors among students of Wroclaw postgraduate schools. Pol Arch Med Wewn. 2008; 118: 102-109.

10 Serafin A. [Risk factors, or what everyone should know. Smoking]. Gab Pryw. 2006; 148: 55-56. Polish.

11 Sobczak A, Wardas W, Szołtysek-Bołdys I, et al. [Self-reported cigarette smoking *vs* urinary cotinine among non-smokers, passive and active smokers]. Med Środ. 2005; 8: 39-45. Polish.

12 Sobczak A, Wardas W, Zielińska-Danch W, et al. [Tobacco smoke exposure biomarkers]. Przegl Lek. 2005; 62: 1192-1199. Polish.

13 National Center for Environmental Heath, Division of Laboratory Sciences. Second national report on human exposure to environmental chemicals. Atlanta, Georgia, 2003 NCEH. Pub No 02-0716.

14 Xu X, Dockery DW, Ware JH, et al. Effects of cigarette smoking on rate of loss of pulmonary function in adults: a longitudinal assessment. Am Rev Respir Dis. 1992; 146: 1345-1348.

15 Zieliński J, Górecka D, Śliwiński P. [Chronic obstructive pulmonary disease (COPD)]. PZWL, Warszawa 1998. Polish.

16 Lubiński W, Targowski T, Frank-Piskorska A. [Evaluation of the influence of tobacco smoking on pulmonary function in young men]. Pneumonol i Alergol Pol. 2000; 5-6: 226-231. Polish.

17 Benowitz NL, Fitzgerald GA, Wilson M et al. Nicotine effects on eicosanoid formation and hemostatic function: comparison of transdermal nicotine and cigarette smoking. J Am Coll Cardiol. 1993; 22: 1159-1167.

18 Lehr HA, Weyrich AS, Saetzler RK, et al. Vitamin C blocks inflammatory platelet-activating factor mimetics created by cigarette smoking. J Clin Invest. 1997; 99: 2358-2364.

19 Petitti DB, Kipp H. The leukocyte count: associations with intensity of smoking and persistence of effect after quitting. Am J Epidemiol. 1986; 123: 89-95.

20 Benowitz NL, Gourlay SG. Cardiovascular toxicity of nicotine: implications for nicotine replacement therapy. J Am Coll Cardiol. 1997; 29: 1422-1431.

21 Frei B, Forte TM, Ames BN, et al. Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. Biochem J. 1991; 277: 133-138.

22 Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991; 11: 81-128.

23 Bagchi M, Bagchi D, Hassoun EA, et al. Smokeless tobacco induced increases in hepatic lipid peroxidation, DNA damage and excretion of urinary lipid metabolites. Int J Exp Pathol. 1994; 75: 197-202.

24 Morrow JD, Frei B, Longmire AW, et al. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med. 1995; 332: 1198-1203.

25 Scheffler E, Wiest E, Woehrle J, et al. Smoking influences the atherogenic potential of low-density lipoprotein. Clin Investig. 1992; 70: 263-268.

26 Marangon K, Herbeth B, Lecomte E, et al. Diet, antioxidant status, and smoking habits in French men. Am J Clin Nutr. 1998; 67: 231-239.

27 Mezzetti A, Lapenna D, Pierdomenico SD, et al. Vitamins E, C and lipid peroxidation in plasma and arterial tissue of smokers and non-smokers. Atherosclerosis. 1995; 112: 91-99.

28 Marangon K, Herbeth B, Artur Y, et al. Low and very low density lipoprotein composition and resistance to copper-induced oxidation are not notably modified in smokers. Clin Chim Acta. 1997; 265: 1-12.

29 Princen HM, van Poppel G, Vogelezang C, et al. Supplementation with vitamin E but not beta-carotene in vivo protects low density lipoprotein from lipid peroxidation in vitro. Effect of cigarette smoking. Arterioscler Thromb. 1992; 12: 554-562.

30 Howard DJ, Ota RB, Briggs LA, et al. Oxidative stress induced by environmental tobacco smoke in the workplace is mitigated by antioxidant supplementation. Cancer Epidemiol Biomarkers Prev. 1998; 7: 981-988.

31 Riemersma RA, Wood DA, Macintyre CC, et al. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. Lancet. 1991; 337: 1-5.

32 Bolzán AD, Bianchi MS, Bianchi NO. Superoxide dismutase, catalase and glutathione peroxidase activities in human blood: influence of sex, age and cigarette smoking. Clin Biochem. 1997; 30: 449-454.

33 Brown KM, Morrice PC, Arthur JR, et al. Effects of vitamin E supplementation on erythrocyte antioxidant defence mechanisms of smoking and non-smoking men. Clin Sci. 1996; 91: 107-111.

34 Kocyigit A, Erel O, Gur S. Effects of tobacco smoking on plasma selenium, zinc, copper and iron concentrations and related antioxidative enzyme activities. Clin Biochem. 2001; 34: 629-633.

35 Serwin AB, Chodynicka B. [Smoking and glutathione peroxidase activity in psoriasis patients]. Żyw Czł Metabol. 1999; 26: 57-63. Polish.

36 Tabacova S, Little RE, Balabaeva L, et al. Complications of pregnancy in relation to maternal lipid peroxides, glutathione, and exposure to metals. Reprod Toxicol. 1994; 8: 217-224.