ANTIOXIDANT ENZYMES AND PULMONARY FUNCTION IN STEEL MILL WELDERS

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Abstract. It is known that high levels of nitric oxide and ozone lead to disturbances of the balance between oxidants and antioxidants. The purpose of this study was to investigate ventilatory parameters in relation to the antioxidant status measured as total antioxidant status (TAS), superoxide dismutase (SOD) and catalase (CT).

The study group consisted of 94 welders, aged 41.2 ± 10.0 years, employed in the Steel Mill in Kraków, Poland, and exposed to nitric oxides and ozone in concentrations exceeding the threshold limit values. The control group consisted of 115 unexposed healthy workers aged 40.8 ± 10.2 years. All the subjects under study were smokers. Determination of ventilatory efficiency was based on a “flow-volume” curve and spirometry. TAS was measured using reagents from the Randox Laboratories Ltd, SOD according to Fridovich and CT with Aebi’s method.

It was found that in the group of welders, the concentrations of TAS, CT and SOD were lower compared to controls (TAS-1.15/1.33 mmol/ml; CT-18.1/28.4 m/gHb, SOD-767.6/855.6 U/gHb). The incidence of extreme obstructive pulmonary disease and small airway disease in the welder group was more frequent than in controls.

Changes in the concentration (or activity) of antioxidant parameters cannot be used as early markers of ventilatory dysfunction, although the values in the lowest class of TAS, SOD and CT showed a significantly larger number of welders than controls.

Key words: Welders, Total antioxidant status, Superoxide dismutase, Catalase, Chronic obstructive lung disease, Small airway disease

INTRODUCTION

Commonly used welding processes emit fumes, gases, electro-magnetic radiation and noise as by-products. During welding, workers are exposed to all of these agents. The fumes are chemically very complex, arising primarily from filler metal, electrode coating or cores [1]. Oxidant gases such as NO and O₃ are present in welding gases; ozone is generated at a distance from the arc by the action of emitted ultraviolet rays even during gas-shielded arc welding, whereas nitrogen oxides are produced in the welding arc and flames by thermal oxidation of atmospheric nitrogen.

The airways exposure to either of these oxidant gases may result in pulmonary injury characterized by the damage of the epithelium of the upper airways, bronchi, terminal bronchioles, and alveoli. Finally, as a consequence
of inflammation, chronic obstructive pulmonary disease (COPD) and other various diseases of respiratory tract may occur [2].

The biological effects of ozone [3] and nitrogen oxides [4] are attributed to their ability to cause oxidation and peroxidation of biomolecules, directly and/or via free radical reactions [5,6]. A sequence of events after lipid peroxidation is the loss of functional groups of enzymes, alteration of membrane permeability, and cell injury or even release of apoptosis processes. It is also known that collagen exposed to ozone is degraded in a time and dose-dependent manner [7]. Individual lipid ozonation products activate specific lipases, which trigger the release of endogenous mediators of inflammation [8]. Both ozone and nitrogen oxides are capable of depleting antioxidant resources of the organism [3,4,9].

There are a few publications reporting disturbances of the respiratory tract connected with exposure to oxidant gases and fumes generated during welding (10–17). The purpose of this study was to make an attempt to analyze the influence of occupational environment on ventilatory parameters of welders and their antioxidant status.

MATERIALS AND METHODS

The subjects of the study were 94 welders aged 41.2 ± 10.0 years, employed in a big metallurgic enterprise in Southern Poland (Sendzimir Steel Mill, Kraków), and engaged in the manual electric arc welding process and gas welding without an inert gas blanket. The control group, exclusively men, matched by seniority of work to the group of welders, consisted of 115 healthy workers aged 40.8 ± 10.2 years, non-exposed to ozone and nitrogen oxides, performing virtually the same tasks, except welding. Ozone was present at exceeded levels only in the workshops of the welders. The concentration of this gas was monitored using the AID Portable Ozone Meter Model 560. Measured levels varied from 0.1 to 0.4 ppm (at source). They greatly exceeded the threshold limit value-time weighted average, established by the American Conference of Governmental Industrial Hygienists (0.1 ppm for heavy work), which is in accordance with the Polish regulations setting the occupational exposure limit (OEL) value at 0.076 ppm [18,19].

Nitrogen oxides were present in the welders’ workshops at variable concentrations, in some places slightly exceeding the OEL-established level at 5 mg/m³ of air. All the examined subjects (welders and controls) were active smokers.

The welders started smoking when they were 17.0 ± 2.0 years old, whereas subjects in the control group started smoking at the age 16.8 ± 1.8 years (SD). The number of cigarettes smoked a day by welders was 19.7 (Mean) ± 3.5 (SD). The subjects of the control group did not differ in the amount of cigarettes smoked a day (16.8 ± 1.8). As controls were matched to the welder group not only by age, but also by seniority of work, the cumulative amount of cigarettes smoked by the subjects of both groups was virtually the same. Both groups underwent prophylactic, periodic examinations at the Industrial Health Centre. In addition to the physical examination, the workers under study were asked a number of questions by a dietician. Clinical and laboratory tests were performed at the annual, obligatory medical check-up.

ASPAT, ALAT, GGTP, alkaline phosphatase activities in venous, fasting blood were measured by common methods, using Biochemical Analyser Cone-Pro (Finland). A17- parameter morphology was determined by TOA Sysmex K4500, Japan.

Two ml of blood were centrifuged at 1000 • g for 15 min at room temperature.

Approximately 0.8 ml of red blood cells pipetted from the bottom of the centrifuged samples were suspended in 150 mMol/l NaCl, centrifuged at 2000 • g for 10 min, washed four times with 150 mMol/l NaCl and lysed in a total volume of 2 ml double deionized water, by freezing and thawing three times. Hemolysates were used for determining superoxide dismutase (SOD) and catalase (CT). SOD activity was determined by the adrenaline method (according to Misra, Fridovich) based on generating free radicals in the process of adrenaline conversion to adrenochrome [20]. Catalase activity was measured (according to Aebi’s method) by decreasing the absorbance within 15 sec due to H₂O₂ decay by this enzyme (λ = 240 nm at 22°C) [21].
The total antioxidant status (TAS) was measured by using the spectrophotometric Randox reagent kits (Randox Laboratories Ltd., UK). In this method, 2,2’-azino-di-[3-ethyl-benzethiazoline sulphonate] is incubated with a peroxidase (metmyoglobin) and \( \text{H}_2\text{O}_2 \) to produce the radical cation. This has blue-green colour, which is measured at 600 nm.

All the participants completed a questionnaire on smoking history, chronic respiratory diseases (according to MCR) and cardiac disease (according to Rose). They underwent a physical examination, ECG, the chest x-ray and measurement of ventilation efficiency. Flow-volume curves and spirometry were measured by a computer–aided system (Lungtest MES Poland).

In order to eliminate the influence of age, height and body mass on spirometric parameters, all the obtained values were expressed as percent of the predicted values (%N) derived from appropriate equations of regression [22].

The diagnosis of COPD was based on the criteria outlined by the NHLBI/WHO GOLD (Global Initiative for Chronic Obstructive Lung Disease) Workshop Report [23].

1. COPD severity was defined according to the GOLD criteria:
   a) At risk
      - Normal spirometry
      - Chronic symptoms (cough, sputum production)
   b) Mild COPD
      - \( \text{FEV}_1\%\text{FVC} < 70\%\ N \),
      - \( \text{FEV}_1 > 80\%\ N \),
      - With or without chronic symptoms (cough, sputum production)
   c) Moderate COPD
      - \( \text{FEV}_1\%\text{FVC} < 70\%\ N \),
      - \( 30\% < \text{FEV}_1 < 80\%\ N \),
      - With or without chronic symptoms (cough, sputum production)
   d) Severe COPD
      - \( \text{FEV}_1\%\text{FVC} < 70\%\ N \),
      - \( \text{FEV}_1 < 30\%\ N \) or \( \text{FEV}_1 < 50\%\ N \) plus respiratory failure or
      - clinical signs of right heart failure

2. Small bronchi obturation (internal diameter < 2mm) was diagnosed in case of:
   - \( \text{MEF}_{50} < 80\%\ N \),
   - \( \text{MEF}_{25} < 80\%\ N \),
   - \( \text{MEF}_{25/75} < 80\%\ N \)

**Statistical analysis**

The statistical package “Statistics for Windows” (version 5.5) was used for the data analysis. As numeric results obtained in this study could not be normally distributed, the non-parametric Mann-Whitney U test was used to ascertain statistical differences between arithmetic means. Linear correlations were calculated by employing the least squares linear regression.

**RESULTS**

Table 1 presents briefly descriptive statistics of total antioxidant status, superoxide dismutase and catalase in the welder and control groups. Statistical analysis (Mann-Whitney U test) did not reveal any significant differences between means, although the activity of catalase in the group of welders was much lower (approximately 36%) than in controls. This difference however, cannot be regarded as statistically significant (\( p < 0.08 \)).

Frequency distributions of TAS, activity of SOD and CT are presented in Figs. 1–3.

In the lowest range of activity of both enzymes studied, and in TAS, the frequency in welders was significantly higher than in controls in the same range of values.

![Fig. 1. Total antioxidant status (TAS) in the welder and control groups. Levels of significance by the Mann-Whitney U test: * \( p \leq 0.001 \).](image-url)
In Table 2, the age and spirometry parameters, expressed as percent of predicted values found in both groups, were compared. Differences in the arithmetic means of FEV$_1$, FVC, MEF$_{50}$, MEF$_{75}$, and MEF$_{25/75}$ were statistically significant. (Details are given in the legend to Table 2).

Figure 4 presents a histogram of FEV$_1$%FVC, being one of four parameters indicative of air flow in central bronchi listed in Table 2. It was the only parameter, which showed a significant difference between welders and controls. As depicted in Fig. 4, low, pathological values of this parameter were encountered more frequently in welders than in healthy controls.

### Table 1. Descriptive statistics of total antioxidant status (TAS), superoxide dismutase (SOD) and catalase (CT)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>The welder group (n = 94)</th>
<th>The control group (n = 115)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TAS (mmol/L)</td>
<td>SOD (U/g Hb)</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>1.15</td>
<td>767.62</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.815</td>
<td>733.33</td>
</tr>
<tr>
<td>Median</td>
<td>1.06</td>
<td>1160.77</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.169</td>
<td>237.5</td>
</tr>
<tr>
<td>Maximum</td>
<td>4.025</td>
<td>702.7</td>
</tr>
<tr>
<td>Lower quartile</td>
<td>0.66</td>
<td>542.86</td>
</tr>
<tr>
<td>Upper quartile</td>
<td>1.27</td>
<td>1207.69</td>
</tr>
<tr>
<td>Skewness</td>
<td>1.71</td>
<td>4.58</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>3.69</td>
<td>23.91</td>
</tr>
</tbody>
</table>

Fig. 2. Superoxide dismutase (SOD) in the welder and control groups.

Fig. 3. Catalase (CT) in the welder and control groups. Levels of significance by the Mann-Whitney U test: * p ≤ 0.001.

In Table 2, the age and spirometry parameters, expressed as percent of predicted values found in both groups, were compared. Differences in the arithmetic means of FEV$_1$, FVC, MEF$_{50}$, MEF$_{75}$, and MEF$_{25/75}$ were statistically significant. (Details are given in the legend to Table 2).

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### Table 2. Age and spirometry parameters in the welder and control groups

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<th>The welder group (n = 94)</th>
<th>The control group (n = 115)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>41.20±10</td>
<td>40.8±10.2</td>
</tr>
<tr>
<td>FVC ( % N )</td>
<td>98.67±1.08</td>
<td>97.46±1.17</td>
</tr>
<tr>
<td>FEV$_1$ ( % N )</td>
<td>95.67±1.41</td>
<td>98.80±1.23</td>
</tr>
<tr>
<td>MEF$_{50}$ ( % N )</td>
<td>95.96±2.23</td>
<td>99.88±2.11</td>
</tr>
<tr>
<td>PEF ( % N )</td>
<td>100.96±2.46</td>
<td>99.24±1.97</td>
</tr>
<tr>
<td>FEV$_{1}$%FVC</td>
<td>80.29±0.91*</td>
<td>84.08±0.75*</td>
</tr>
<tr>
<td>MEF$_{50}$ ( % N )</td>
<td>88.72±2.59***</td>
<td>110.44±3.57***</td>
</tr>
<tr>
<td>MEF$_{25}$ ( % N )</td>
<td>94.35±2.70**</td>
<td>105.18±2.90**</td>
</tr>
<tr>
<td>MEF$_{75}$ ( % N )</td>
<td>91.23±2.45*</td>
<td>105.96±2.83*</td>
</tr>
</tbody>
</table>

Levels of significance (Mann-Whitney U Test): * p ≤ 0.01; ** p ≤ 0.02; *** p ≤ 0.001.

FVC = forced vital capacity; FEV$_1$ = forced expiratory volume in 1 sec; MEF$_{50}$ = middle expiratory flow 50% FVC; MEF$_{25}$ = middle expiratory flow 25% FVC; MEF$_{75}$ = middle expiratory flow 75% FVC; PEF = peak expiratory flow; MEF$_{25/75}$ = MEF$_{25}$ - MEF$_{75}$. FVC = forced expiratory volume in 1 sec; MEF$_{50}$ = middle expiratory flow 50% FVC; MEF$_{25}$ = middle expiratory flow 25% FVC; MEF$_{75}$ = middle expiratory flow 75% FVC.
In Table 2, the last three parameters listed are indicative of small bronchi airflow. It was found that the arithmetical means of all the three parameters were statistically significantly different between the two groups. We decided to present only the results of MEF<sub>50%N</sub> as a parameter less influenced by a patient’s cooperation (Fig. 5). Low values (less than 80%N) of that parameter were more frequent in welders than in controls.

The comparison of COPD frequency in the welder and control groups is presented in Fig. 6

Nearly twice as many welders as controls were at risk of COPD (p < 0.001). Mild symptoms of COPD were found also in the same proportion, but statistical analysis showed no difference. Moderate symptoms of COPD were found in 8 welders (8.5%), but only in one subject (0.9%) in the control group (p < 0.02). Neither in the welders nor in the controls the symptoms of severe COPD fully clinically developed were found.

The values presented in Fig. 7 show the frequency of pathological air flow through the small bronchi (below 80% of the predictable values) in the welder and control groups. Levels of significance by the Mann-Whitney U test: * p ≤ 0.01; ** p ≤ 0.02; *** p ≤ 0.001.

Low, albeit significant correlations were found between CT and FEV<sub>1</sub> (r = -0.250; p < 0.05), and also between TAS and FVC (r = -0.40; p < 0.05), TAS and FEV<sub>1%FVC</sub> (r = -0.36; p < 0.05), TAS and MEF<sub>25</sub>.DISCUSSION

According to the results of spirometric examinations reported by several authors, the influence of welding...
fumes and gases on the ventilatory efficiency of welders is ambiguous. Nakadate [14] found that welding processes caused obstructive changes. Wolf et al. [15] observed the small airway disease more frequently in welders than in the control group. Welders had a decreased mean respiratory flow at the 25% and 50% of vital capacity (MEF<sub>25</sub>, MEF<sub>50</sub>) compared with controls, while FVC, and FEV<sub>1</sub> were unchanged.

In another study it was shown that FVC, FEV<sub>1</sub> and PEF were also significantly lower in welders compared with controls. There was no significant difference in the pulmonary function tests between non-smoking welders and non-smoking controls, whereas ventilatory parameters were significantly lower in smoking welders than in smoking controls [16].

There was also no influence of the specific welding processes on the spirographic parameters [17,24]. The pulmonary function analysis revealed no significant difference between FEV<sub>1</sub>, MEF<sub>25/75</sub> and FEF<sub>50</sub> obtained from stainless steel welders and controls. On the other hand, a decrease in the spiographic values after 25 years of welding activity was evident. The higher prevalence of bronchial irritative symptoms, such as cough or sputum production, in welders than in controls was significantly linked to tobacco consumption. [17].

The sub-population of welders, the subject of the present investigation, was a specific one. This group of workers is under constant survey of occupational medicine specialists. Mandatory measurements of biochemical and spirometrical parameters are performed once a year. Persons with noticeable deviations from normal laboratory findings, or claiming substantial worsening of their state of health, are temporarily, or permanently moved to jobs free from occupational exposure to noxious gases and fumes. This probably explains the absence of severe COPD. For the same reasons in the study group there were no persons with a pure form of restrictive ventilatory disorders, and obturation of central and small bronchi was encountered in some workers only in a mild form. Nevertheless, in comparison with the control group, more welders had spirometric parameters below the normal age-adjusted values. The incidence of mild and moderate COPD and the percentage of welders with the first stage of COPD was higher than expected for the healthy, control subpopulation. There was no correlation between pulmonary changes, biological age and seniority of work.

In our search for probable reasons for small, but noticeable abnormalities seen in the group of welders, we resorted to a wide array of biochemical markers of oxidative/antioxidative balance. Indeed, it is known from the literature that occupational exposure to nitrogen oxides and ozone leads to the impairment of oxidant/antioxidant balance [4].

In the lowest class of TAS values, there was a significantly (p < 0.001) larger number of welders than subjects from the control group. Similarly, statistically significant (p < 0.01) results were noted for CT and SOD. It should be pointed out that the lowest class of values for TAS, CT and SOD was below the range of 5–95 percentile values, defined as laboratory reference normal.

The search for a correlation between spirometric parameters and the wide array of blood enzymes and compounds, known to take part in establishing an oxidative/antioxidative balance, produced unsatisfactory results. It is evident that the changes in the concentration (or activity) of these parameters cannot be used as early markers of ventilatory dysfunction.

**CONCLUSIONS**

1. Compared to the controls, the antioxidant system was affected in workers chronically exposed to welding fumes and gases, which are thought to be oxidant pollutants.
2. Chronic obstructive pulmonary and small airway diseases in the welder group was more frequent than in the control group.

**REFERENCES**


