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## Systemic inflammatory responses following welding inhalation challenge test



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### ABSTRACT

**Aim:** The aim of this study was to investigate inflammatory and respiratory responses to welding fume exposure in patients with suspected occupational asthma.

**Methods:** Sixteen patients referred to the Finnish Institute of Occupational Health underwent mild steel (MS) and stainless steel (SS) welding challenge tests, due to suspicion of OA. Platelet count, leucocytes and their differential count, hemoglobin, sensitive CRP, lipids, glucose and fibrinogen were analyzed in addition to interleukin (IL)-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , endothelin-1, and E-selectin in plasma samples. Peak expiratory flow (PEF), forced expiratory volume in 1 min (FEV<sub>1</sub>) and exhaled nitric oxide (NO) measurements were performed before and after the challenge test. Personal particle exposure was assessed using IOM and a mini sampler. Particle size distribution was measured by an Electric Low Pressure Impactor (ELPI).

**Results:** The number of leukocytes, neutrophils, and platelets increased significantly, and the hemoglobin level and number of erythrocytes decreased significantly after both the MS and SS exposure tests. Five of the patients were diagnosed with OA, and their maximum fall in FEV<sub>1</sub> values was 0.70 l ( $\pm$ 0.32) 4 h after SS exposure. MS welding generated an average inhalable particle mass concentration of 31.6, and SS welding of 40.2 mg/m<sup>3</sup>. The mean particle concentration measured inside the welding face shields by the mini sampler was 30.2 mg/m<sup>3</sup> and 41.7 mg/m<sup>3</sup>, respectively.

**Conclusions:** Exposure to MS and SS welding fume resulted in a mild systemic inflammatory response. The particle concentration from the breathing zones correlated with the measurements inside the welding face shields.

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### 1. Introduction

Welding is a process that joins material, usually metals or alloys, by using heat and/or compression. Welders are exposed to fumes containing different gases and particles, depending on the composition of the welding electrodes,

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welded material, and the welding method used. Welding particles include both fine (0.1–2.5  $\mu\text{m}$ ) and ultrafine particles (<0.1  $\mu\text{m}$ ) [1].

Welding fume exposure has been associated with several adverse health outcomes such as chronic bronchitis, pneumonia, metal fume fever, lung function changes, and a possible elevated risk of lung cancer and death from ischemic heart disease [2–8]. In addition, population-based studies have shown that welders are at an almost twofold risk of developing asthma [9,10]. Moreover, other epidemiological studies have indicated that exposure to welding fumes may indeed be a direct cause of asthma [11,12]. Some case series have revealed occupational asthma (OA) in workers exposed to stainless steel welding fumes in particular [13–15].

The underlying mechanisms responsible for these cardiorespiratory effects are largely unknown. One line of research has investigated the possible association between welding fume exposure and acute systemic inflammatory responses [16–18]. The hypothesis is that short-term exposure to welding fumes may induce a local as well as systemic inflammatory reaction, which may be responsible for chronic lung and cardiovascular disease if the exposure persists for a long time [18]. In our own recent study [19], welding fumes, as well as dusts and fumes from sheet metal work, caused a slight acute inflammation during a work shift in actual workplace conditions.

The aim of the present study was to further investigate, on the basis of prior studies, whether short exposure to high concentration of welding fumes is capable of inducing acute effects on hematological, systemic inflammatory and respiratory parameters by following welding challenge test in patients with suspected OA.

## 2. Subjects and methods

### 2.1. Study subjects

The study consisted initially of 18 patients who were referred to the Finnish Institute of Occupational Health (FIOH) by pulmonologists of local central hospitals or by physicians of local occupational health units from all over Finland in 2007. Sixteen of the patients had been diagnosed with asthma previously and two patients had asthma like symptoms but not specific diagnosis of asthma; all patients were suspected of having OA caused by welding fumes. One patient was excluded from the study because his exposure tests were not performed on consecutive days, and another was excluded because he was given medication during the study which may have affected the blood results. Therefore, the final study population comprised 16 participants. They were all male, and worked as welders ( $N=7$ ), sheet metal workers ( $N=7$ ), assemblers ( $N=1$ ) and metal workers ( $N=1$ ). All of them were exposed to welding fumes in their work regardless of their occupational title. Asthma medication was discontinued before testing at FIOH.

### 2.2. Study protocol

Welding challenge tests were performed in a special welding chamber (6 m<sup>3</sup>). Fifteen of the participants were exposed to mild steel (MS) (control test) and stainless steel (SS) welding fumes on consecutive days as described earlier for suspicion of OA [15]. One subject was exposed to MS welding fumes only. The manual metal arc welding (MMAW) exposure time was 30 min. During the exposure, five rods were consumed in the MS control test (OK 48.00; ESAB AB, Gothenburg, Sweden) and 11 rods in the ST welding test (OK 63.30; ESAB AB, Gothenburg, Sweden) [14].

OA was diagnosed according to European guidelines [20]. Study participants were monitored for 24 h after each challenge.

Altogether five venous blood samples (one blood sample before each of the challenge tests and one blood sample after each of the challenge tests and the fifth one on the next day after the tests) were taken from each of the subjects (Fig. 1). Baseline measurements of peak expiratory flow (PEF), forced expiratory volume in 1 min (FEV<sub>1</sub>), and exhaled nitric oxide (NO) were performed before MS exposure, and then approximately 22 h after the MS and SS exposure. Each participant gave written informed consent and filled in a questionnaire concerning work and exposure history, smoking habits, lung and cardiovascular disease history, and medication. The study protocol was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa.

### 2.3. Hematological and systemic inflammatory analyses

The concentrations of interleukin (IL)-1 $\beta$ , IL-6, IL-8, tumor necrosis factor alpha (TNF- $\alpha$ ), endothelin-1, and E-Selectin in the plasma samples were determined by enzyme immunoassay (EIA) using commercial reagents: IL-1 $\beta$  (sensitivity 0.063 pg/ml) and TNF- $\alpha$  (sensitivity 0.125 pg/ml), Quantikine HS ELISA, R&D Systems Europe Ltd., Abingdon, UK; IL-6 (sensitivity 0.6 pg/ml), Peli-Pair ELISA, Sanquin, Amsterdam, the Netherlands; IL-8 (sensitivity 1.56 pg/ml), Opt EIA, BD Biosciences, Erembodegem, Belgium; endothelin-1 (sensitivity 0.68 pg/ml), QuantiGlo ELISA, R&D Systems Europe Ltd., Abingdon, UK; E-Selectin (sensitivity 20.5 pg/ml), ELISA, HyCult Biotechnology, Uden, the Netherlands).

Platelet count, leucocytes and their differential count, hemoglobin, haematocrit, sensitive C-reactive protein (CRP), lipids, glucose, and levels of fibrinogen were analyzed using established methods.

All laboratory analyses were performed blind to the exposure status of the studied participants.

### 2.4. Respiratory measurements

A portable, pocketsize spirometer (One Flow, STI MEDICAL, Saint-Romans, France) recorded the lung function measurements (PEF, FEV<sub>1</sub>), and a decrease of 20% in PEF or FEV<sub>1</sub> from the baseline value was regarded as significant [21].

Exhaled NO was measured using a chemiluminescence gas analyzer (NIOX, Aerocrine AB, Solna, Sweden),

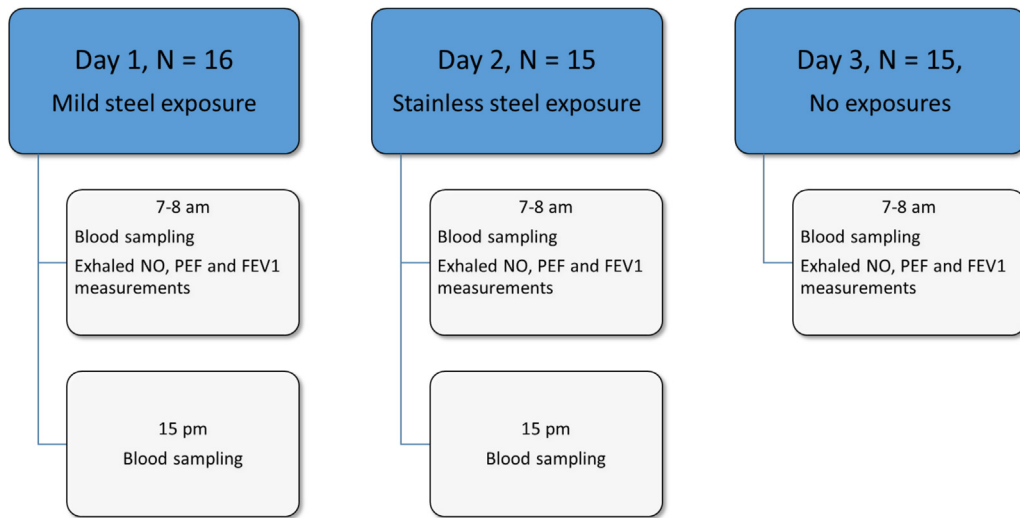


Fig. 1. Description of study protocol.

according to ATS FE<sub>NO</sub> guidelines. Values of >30 ppb were considered to be over normal [22].

### 2.5. Exposure measurements

Particle exposure was assessed by personal sampling from the breathing zone of 14 participants. Inhalable dust samples were collected outside welding face shields using an IOM sampler with a cellulose acetate filter (AAWP, diameter 25 mm; Millipore, Bedford, MA, USA). Sampling time was 30 min and volumetric flow was adjusted to 2.0 l/min. The samples were analyzed gravimetrically [23].

In addition, mini sampler measurements were carried out simultaneously with the IOM sampling [24]. The mini samples were collected from inside the welding face shields of the 15 participants. The sampling volumetric flow was 0.75 l/min and the sampling time 30 min. Dust samples were analyzed gravimetrically.

During the MS and SS welding exposure tests of three participants, particle number size distributions were measured by an Electric Low Pressure Impactor (ELPI) (Dekati Ltd., Finland) in a size range of 30 nm to 10 μm. The ELPI measurement system gives particulate number concentrations in 12 size bins covering the whole measurement size range.

### 2.6. Statistical analyses

Repeated data consisted of the results of inflammatory responses during the exposure in the welding chamber. All response variables were continuous and mostly normally distributed. Therefore, the paired *t*-test was applied when comparing the values before and after a challenge test. If the variable was non-normally distributed, the Wilcoxon one sample test was used. A *P*-value of <0.05 was set to indicate statistical significance. All analyses were performed using the Statistical Analysis System, SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

## 3. Results

### 3.1. Study population characteristics

The mean age of the 16 study participants was 44.6 (range 23–57 years), and they were all men (Table 1). Average work history was 27.5 years of welding. One (6%) was a current smoker, eight (50%) were ex-smokers, and seven (44%) had never smoked regularly. Fourteen of the patients had been diagnosed with asthma previously and two patients had asthma like symptoms but not specific diagnosis of asthma; three patients had hypertension.

The average serum cholesterol level and plasma fasting glucose level were high in the total study group. As much as 75% (*N* = 12) had an elevated level of total serum cholesterol, 31% (*N* = 5) of triglycerides and 19% (*N* = 3) of abnormal plasma fasting glucose level. In addition, mean BMI (body mass index) was 27.5, showing mild overweight.

Table 1

Characteristics of participants (*N* = 16). Values for age, BMI, measured laboratory test results, welding years, and exposure given in means with standard deviation.

Age, years	44.6 (10.7)
BMI	27.5 (4.2)
Baseline FEV <sub>1</sub> , l	3.7 (0.47)
Baseline PEF, l/min	552 (92)
Cholesterol, mmol/l	5.5 (1.0)
HDL, mmol/l	1.3 (0.38)
Triglycerides, mg/l	1.6 (0.68)
Glucose, mmol/l	5.7 (0.49)
Diagnosed asthma	14/16
Welding, years	23.8 (13.0)
Smoking habits	
Current smokers, <i>N</i>	1/16
Ex-smokers, <i>N</i>	8/16
Non-smokers, <i>N</i>	7/16

BMI: body mass index; FEV<sub>1</sub>: forced expiratory volume in 1 min; PEF: peak expiratory flow; HDL: high density lipoprotein

### 3.2. Hematological and systemic inflammatory markers

After the MS exposure test, hemoglobin level decreased significantly from 157 g/l to 154 g/l and the number of erythrocytes from 5.0 to 4.9 ( $10^{12}$  cells  $L^{-1}$ ), whereas the number of leukocytes increased significantly from 6.5 to 7.4 ( $10^9$  cells  $L^{-1}$ ), neutrophils from 3.7 to 4.4 ( $10^9$  cells  $L^{-1}$ ), and platelets from 273 to 291 ( $10^9$  cells  $L^{-1}$ ) (Table 2). Similar statistically significant changes were found following the SS exposure test. Of the systemic immune parameters, the concentration of E-selectin decreased significantly, but only in the MS exposure test (from 48.7 to 46.2 ng/ml) (Table 2).

### 3.3. Respiratory function

The base level of exhaled nitric oxide was elevated (>30 ppb) in five participants (31%). There were no statistically significant changes 22 h after the welding exposure tests (Table 3). However, exhaled NO concentration increased by over 30% in five study participants after the MS exposure test, and in two participants after the SS exposure test compared to the base level measured before the first exposure test. There were slight and statistically significant reductions in the FEV<sub>1</sub> (mean FEV<sub>1</sub> 3.74 l in the baseline and 3.65 l after MS ( $P$  0.032) and 3.59 l ( $P$  0.26) after SS welding) and PEF values. Respectively, the mean PEF value was 566 l/min at the baseline, 543 l/min after MS ( $P$  0.017) and 529 l/min ( $P$  0.022) 22 h after SS welding tests.

Five participants had a positive reaction (a decrease of 20% of PEF or FEV<sub>1</sub> from baseline value) in the SS challenge test and were therefore diagnosed with OA. In the

rest of the patients ( $N=11$ ), the final diagnoses after the examinations at the FIOH were asthma ( $N=9$ ), asthma like symptoms ( $N=1$ ) and chronic obstructive pulmonary disease ( $N=1$ ). In the patients with OA ( $N=5$ ), the average FEV<sub>1</sub> value was 4.01 ( $\pm 0.38$ ) and the PEF value 617 l/min ( $\pm 100$ ) before the welding challenge tests. The maximum fall in the FEV<sub>1</sub> and PEF values was 0.701 ( $\pm 0.32$ ) and 140 l/min ( $\pm 62$ ), respectively, 4 h after SS exposure. In participants with no confirmed OA ( $N=11$ ), leukocytes and neutrophils increased significantly, whereas erythrocytes decreased significantly following both the welding challenge tests (Table 4). In participants with confirmed OA, only platelet count increased significantly, from 273  $\pm$  76.5 to 288  $\pm$  83.0 after the MS exposure test ( $P$  0.014), and from 290  $\pm$  81.9 to 304  $\pm$  77.3 after the SS exposure test ( $P$  0.014).

### 3.4. Particle exposure

Particle concentration measured in the breathing zone of 14 participants by the IOM sampler varied from 12.7 to 79.4 mg/m<sup>3</sup> in the MS and from 15.9 to 100 mg/m<sup>3</sup> in the SS exposure tests. The average particle concentrations were 31.6 and 40.2 mg/m<sup>3</sup>, respectively. The mean particle concentration measured inside the welding face shields by the mini sampler was 30.2 mg/m<sup>3</sup> in the MS exposure tests, and 41.7 mg/m<sup>3</sup> in the SS exposure tests. Compared to the IOM results, the particle concentration measured by the mini sampler was 4.4% lower in the MS and 3.7% higher in SS exposure tests.

Fig. 2 presents the particle number size distributions in the MS and SS exposures of one participant. Particle size

**Table 2**  
Inflammatory and hematological parameters before and after welding exposure tests.

Blood parameters	Day 1, mild steel exposure MS (N = 16)			Day 2, stainless steel exposure SS (N = 15)			Blood sample 5 Mean ( $\pm$ SD)	Comparison 5–1 $P$
	Before	After	$P$	Before	After	$P$		
Hemoglobin, g/l	157 $\pm$ 12.8	154 $\pm$ 11.7	0.016	158 $\pm$ 12.0	155 $\pm$ 12.3	0.019	157 $\pm$ 11.3	n.s.
Leukocytes, $10^9$ cells $L^{-1}$	6.5 $\pm$ 1.2	7.4 $\pm$ 1.5	0.009	6.7 $\pm$ 0.99	7.8 $\pm$ 1.6	0.004	6.7 $\pm$ 0.9	n.s.
Neutrophils, $10^9$ cells $L^{-1}$	3.7 $\pm$ 1.2	4.4 $\pm$ 1.3	0.002	3.8 $\pm$ 0.94	4.8 $\pm$ 1.4	0.004	3.9 $\pm$ 1.0	n.s.
Lymphocytes, $10^9$ cells $L^{-1}$	2.2 $\pm$ 0.66	2.2 $\pm$ 0.45	ns	2.3 $\pm$ 0.56	2.3 $\pm$ 0.46	ns	2.1 $\pm$ 0.64	n.s.
Eosinophils, $10^9$ cells $L^{-1}$	0.20 $\pm$ 0.09	0.16 $\pm$ 0.09	0.012	0.25 $\pm$ 0.09	0.20 $\pm$ 0.09	0.022	0.23 $\pm$ 0.11	0.025
Erythrocytes, $10^{12}$ cells $L^{-1}$	5.0 $\pm$ 0.38	4.9 $\pm$ 0.34	0.013	5.0 $\pm$ 0.34	4.9 $\pm$ 0.31	0.008	4.9 $\pm$ 0.32	n.s.
Monocytes, $10^9$ cells $L^{-1}$	0.40 $\pm$ 0.11	0.51 $\pm$ 0.15	0.012	0.38 $\pm$ 0.08	0.56 $\pm$ 0.20	<0.001	0.37 $\pm$ 0.13	n.s.
Basophils, $10^9$ cells $L^{-1}$	0.03 $\pm$ 0.05	0.06 $\pm$ 0.05	ns	0.04 $\pm$ 0.05	0.06 $\pm$ 0.05	ns	0.04 $\pm$ 0.05	n.s.
Platelet count, $10^9$ cells $L^{-1}$	273 $\pm$ 62.5	291 $\pm$ 61.3	<0.001	282 $\pm$ 57.3	293 $\pm$ 59.9	0.008	278 $\pm$ 52.4	n.s.
Sensitive CRP, mg/ml	1.46 $\pm$ 2.24	1.51 $\pm$ 2.25	ns	1.58 $\pm$ 2.21	1.60 $\pm$ 2.14	ns	1.46 $\pm$ 2.24	n.s.
P-fibrinogen, g/l	3.4 $\pm$ 0.98	3.1 $\pm$ 0.93	ns	3.4 $\pm$ 0.91	3.5 $\pm$ 1.1	ns	3.6 $\pm$ 1.01	n.s.
IL-1 $\beta$ , pg/ml	0.57 $\pm$ 0.43	0.45 $\pm$ 0.30	ns	0.65 $\pm$ 0.86	0.65 $\pm$ 0.76	ns	0.46 $\pm$ 0.30	n.s.
IL-6, pg/ml	2.7 $\pm$ 1.6	2.5 $\pm$ 1.6	ns	2.5 $\pm$ 1.4	2.4 $\pm$ 1.1	ns	2.6 $\pm$ 1.4	n.s.
IL-8, pg/ml	7.7 $\pm$ 2.8	7.2 $\pm$ 3.6	ns	8.3 $\pm$ 4.8	7.4 $\pm$ 2.7	ns	7.4 $\pm$ 3.3	n.s.
TNF- $\alpha$ , pg/ml	2.0 $\pm$ 0.68	2.0 $\pm$ 0.83	ns	2.1 $\pm$ 1.1	1.8 $\pm$ 0.53	ns	1.8 $\pm$ 0.6	n.s.
Endotelin 1, pg/ml	0.92 $\pm$ 0.23	0.86 $\pm$ 0.22	ns	0.87 $\pm$ 0.21	0.88 $\pm$ 0.35	ns	0.90 $\pm$ 0.26	n.s.
E-selectin, ng/ml	48.7 $\pm$ 20.9	46.2 $\pm$ 19.1	0.04	47.2 $\pm$ 19.2	47.3 $\pm$ 20.2	ns	45.9 $\pm$ 19.8	n.s.

Comparison 5–1: comparison of Blood test 5 on Day 3, and of Blood test 1 on Day 1.

**Table 3**Average exhaled nitric oxide (NO), FEV<sub>1</sub> and PEF values with standard deviation (SD) before and after exposure tests.

	Before exposure tests (N = 16)		After mild steel exposure (N = 16)		After stainless steel exposure (N = 15)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Exhaled NO, ppb	25 (22)	4.9–74	25 (24)	4.5–79	28 (27)	6.2–86
FEV <sub>1</sub> , l	3.74 (0.47)		3.65 (0.46)		3.59 (0.48)	
PEF, l/min	566 (92)		543 (79)		529 (87)	

FEV<sub>1</sub>: forced expiratory volume in 1 min; PEF: peak expiratory flow.**Table 4**

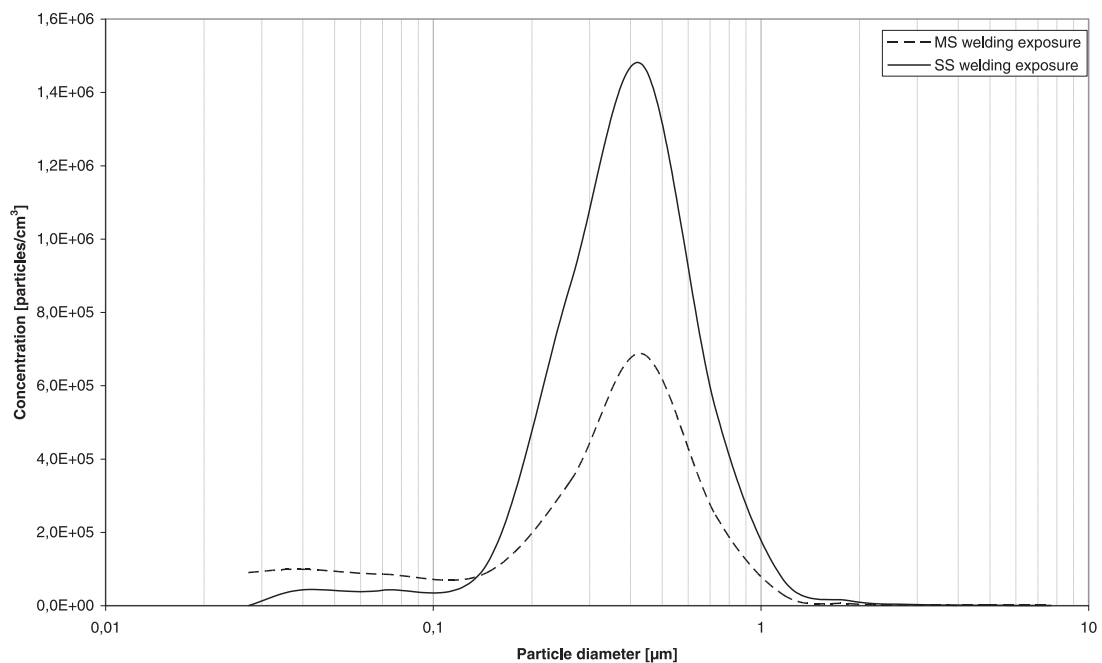
Inflammatory blood parameters before and after welding exposure tests on participants with no confirmed occupational asthma.

	Day 1, mild steel exposure (N = 11)			Day 2, stainless steel exposure (N = 10)		
	Before	After	P	Before	After	P
Hemoglobin, g/l	159 ± 13.3	156 ± 11.6	0.031	159 ± 12.8	156 ± 13.8	ns
Leukocytes, 10 <sup>9</sup> cells L <sup>-1</sup>	6.6 ± 1.4	7.5 ± 1.5	0.030	6.7 ± 0.81	8.2 ± 1.7	0.001
Neutrophils, 10 <sup>9</sup> cells L <sup>-1</sup>	3.4 ± 1.4	4.5 ± 1.4	0.003	3.7 ± 1.02	5.0 ± 1.7	0.002
Lymphocytes, 10 <sup>9</sup> cells L <sup>-1</sup>	2.4 ± 0.68	2.3 ± 0.37	ns	2.4 ± 0.48	2.4 ± 0.39	ns
Eosinophils 10 <sup>9</sup> cells L <sup>-1</sup>	0.21 ± 0.09	0.17 ± 0.10	0.017	0.27 ± 0.10	0.23 ± 0.08	ns
Erythrocytes, 10 <sup>12</sup> cells L <sup>-1</sup>	5.1 ± 0.36	5.0 ± 0.31	0.013	5.1 ± 0.36	5.0 ± 0.33	0.024
Monocytes, 10 <sup>9</sup> cells L <sup>-1</sup>	0.41 ± 0.13	0.48 ± 0.13	0.030	0.37 ± 0.09	0.54 ± 0.19	0.010
Basophils, 10 <sup>9</sup> cells L <sup>-1</sup>	0.05 ± 0.05	0.06 ± 0.05	ns	0.05 ± 0.05	0.08 ± 0.04	ns
Platelet count, 10 <sup>9</sup> cells L <sup>-1</sup>	274 ± 59.4	292 ± 53.6	0.008	278 ± 46.8	288 ± 53.1	ns

distribution was very similar in both exposures. Total particle number concentration in SS welding was  $3.2 \times 10^6 \text{ cm}^{-3}$  and in MS welding  $1.7 \times 10^6 \text{ cm}^{-3}$ . SS welding generated a higher particle number concentration, presumably due to the higher rod number consumption than in MS welding. In both exposure tests, most of the welding fume particles were smaller than 1  $\mu\text{m}$ . The middle point of the mode was around 430 nm in both welding challenge tests.

#### 4. Discussion

Systemic inflammatory, hematological and respiratory responses were studied in 16 middle-aged male workers exposed to welding fumes and of whom 14 had asthma, one chronic obstructive pulmonary disease and one had asthma like symptoms. Previous studies on this subject have been epidemiological [11,12,25,26], carried out on healthy volunteers [16–18] or in workplace settings [19,27]. This is

**Fig. 2.** Particle number size distribution in one participant's mild steel (MS) and stainless steel (SS) exposure tests.



the first study to report the systemic inflammatory and hematological responses in patients with asthma.

Blood leukocytes and neutrophils increased significantly following both the MS and the SS welding exposure tests. This is consistent with other studies dealing with the acute effects of welding exposure [17–19]. Peripheral blood neutrophilia has also been found following short-term inhalation exposure to other substances, such as ozone [28] and compost dust [29].

Blood platelets increased significantly after both the MS and SS welding challenges. This finding contradicts those of Hartmann et al. [18] and those we ourselves have previously reported [19]. The fact that these present observations could be seen as being at odds with these prior findings might also be due to differences in exposure characteristics. In the former study, the participants were exposed to fumes of metal-inert-gas (MIG) welding of aluminum and MIG soldering of zinc-coated steel and, in the latter study, to MS welding fumes and to dusts and fumes generated from grinding MS plates or pieces. In addition, there was a tenfold difference between the average particle concentrations in the earlier studies and those in the present study. Increased platelet count in peripheral blood has previously been reported as also occurring after inhalation of diesel exhaust [30]. Another reason could be reactive thrombocytosis due to increased inflammation or bone marrow stimulation.

Hemoglobin and erythrocyte levels decreased significantly following both the MS and SS welding exposure tests, which is consistent with our earlier findings [19]. Although this finding has not been observed in other studies dealing with the acute effects of welding fumes [17,18], a significant association between the concentration of particulate matter (PM<sub>10</sub>) and blood hemoglobin levels has been reported by Seaton et al. [31]. It is unclear how fine particulate matter exposure leads to changes in hemoglobin or erythrocyte levels, but it has been speculated that this could be due to a combination of mechanisms such as volume status or stress, which could affect blood viscosity [30]. As a possible mechanism, Seaton et al. suggested that the inhalation of some component of PM<sub>10</sub> may cause the sequestration of red cells in circulation [31]. In our prior study, the decrease in hemoglobin levels may have been caused by differences in metabolic intensity and fluid shifts during moderate- or low-level physical work [19]. Our present results could also be influenced by these factors, although the welding was not as physically demanding as welding in actual workplace conditions.

Taken together, our peripheral blood findings (the increased level of blood leukocytes, neutrophils, and platelets, and the decreased level of hemoglobin and erythrocytes) are in line with the suggestion that a mild systemic inflammatory response takes place during welding exposure [16,17,19]. This response was a time-limited process, because 22 h after the last exposure test, the response was attenuated.

The present study observed no statistical differences in CRP levels, which is in accordance with our earlier results [19]. In general, the available data on this subject is mixed,

since some of the studies support significant CRP changes related to welding fume exposure [17,29] while others do not [16,19]. This inconclusive situation demands further investigation. No statistical differences in the concentrations of acute-phase mediators such as TNF- $\alpha$ , IL-6 and IL-8 have been reported in humans following welding exposure [16,19]. We also found no statistical differences in the levels of these cytokines, and therefore, our current findings confirm previous observations.

The levels of IL-1 $\beta$ , which is a proinflammatory cytokine, in relation to welding have thus far only been studied once [19]. The study found that IL-1 $\beta$  levels decreased significantly, which was unexpected. In the present study, no significant changes were observed in IL-1 $\beta$  levels. E-selectin levels in relation to welding have also only been studied once to date [18]. In our previous study, the E-selectin level decreased significantly, which was also observed in the present study, but only following exposure to the MS welding fumes. We also studied the levels of endothelin-1, which is a vasoconstrictor peptide, and fibrinogen, which is a soluble plasmaprotein, but found no significant changes in either, which is in accordance with previous reports [16,18,19].

Exposure to welding fumes has been associated with reduced pulmonary function [25–27] and asthma [9,10,11]. It has been suggested that changes in welders' lung functions are transient, occurring at the time of exposure at the workplace and returning to normal during non-exposed periods. Welding fumes of stainless steel can cause OA [13,15]. In this study we found five cases of OA, and these individuals showed marked late phase changes in pulmonary function (FEV<sub>1</sub>, PEF) in response to the SS welding challenge test. Interestingly, significant changes in hematological parameters were found in non-occupational asthma patients but not in patients with confirmed OA. Behndig et al. reported parallel findings in their study, in which both asthma patients and healthy individuals were exposed to diesel exhaust particles or filtered air. They observed a significant increase of submucosal neutrophils and of neutrophil numbers in the bronchial wash of the healthy participants but not among the asthmatics [32].

SS welding generated a higher particle mass and number concentration, presumably due to the higher rod number consumption than that in MS welding. In both exposure tests, most of the welding fume particles were smaller than 1  $\mu$ m, and the particle size distribution was similar in both exposures. The average welding fume exposure in challenge tests was high, but similar high momentary exposures can also occur in workplace conditions. The measured particle mass range was quite wide, depending mostly on the participant's working method. Some participants were bent very close to the object being welded, therefore their breathing zone particle concentrations were higher than those of the participants who were at a longer working distance from the object being welded. The fumes generated during mild steel welding contain mainly iron (80–95%) and also manganese (1–15%), whereas stainless steel welding produces smaller amounts of these but high amounts of chromium (15–30%) and nickel (5–10%) [4]. We did not determine the metal

composition of the fumes generated in the challenge tests in the present series, but we have done this previously [33]. These published results are in line with those published in the literature.

The strength of our study was the possibility to objectively monitor both the welding exposure and the inflammatory, hematological and respiratory responses at the individual level. The limitations of the study were the lack of an unexposed control group and the relatively small number of participants. Since the welding challenge tests in the laboratory environment were the only procedures with the study individuals during the days, there is no other explanation for the slight inflammatory response even in the lack of unexposed control group. The study participants stayed at the FIOH during the test days and thus work, exercise, or other environmental factors cannot be used as an explanation. Also, the results are in line with our previous study on welding in an occupational/workplace setting [19]. Furthermore, a larger study group would probably have given more significant results, rather than diminish the findings. The presence of Cr and Ni in the fumes may cause lung injury and inflammation, lung tumor formation, immune dysfunction, and systemic toxicity [34]. However, welding processes produce fumes consisting of gaseous and aerosol by-products composed of metals, metal oxides and volatilized chemical species from the base metals, welding electrode, or flux material [1]. In the present study, we could not elucidate which component of the welding fumes was responsible for the observed findings.

We cannot exclude that performing the exposure tests on consecutive days could have affected the results. Unfortunately, there is no worldwide consensus how welding exposure tests should be performed. However, the situation could be better in the future, because suggestions have been published how experimental welding fume exposure studies under controlled and standardized conditions should be carried out ([35]). The used welding challenge tests in our series have been standardized [15]. The challenge tests are the “golden standard” for diagnosing OA. The procedure includes first the placebo test and the active test second. It is important that the time between the two tests should be minimized since the patients cease their asthma medication, and the longer the period between the two tests is the less comparable the test results are. Furthermore, keeping an asthma patient without regular asthma medication for several days would provoke unspecific asthma symptoms and false positive test results.

#### 4.1. Conclusions

In conclusion, we observed a mild systemic inflammatory response following welding fume challenge tests. An increase was observed in peripheral blood leukocytes, neutrophils, and platelets, and a decrease in hemoglobin level and in erythrocyte count. The particle size distribution of welding fumes was similar regardless of the exposure; most of the welding fume particles were smaller than 1  $\mu\text{m}$ , and the IOM measurements correlated well with the mini sampler measurements.

#### Conflict of interest

Dr. Hannu and Kauppi report grants from The Finnish Work Environment Fund, during the conduct of the study. The other authors have nothing to disclose.

#### Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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#### References

- [1] A.T. Zimmer, P. Biswas, Characterization of the aerosols resulting from arc welding processes, *J. Aerosol Sci.* 32 (2001) 993–1008.
- [2] A.F. Behndig, I.S. Mudway, J.L. Brown, N. Stenfors, R. Helleday, S.T. Duggan, S.J. Wilson, C. Boman, F.R. Cassee, A.J. Frew, F.J. Kelly, T. Sandström, A. Blomberg, Airway antioxidant and inflammatory responses to diesel exhaust in healthy humans, *Eur. Respir. J.* 27 (2006) 359–365.
- [3] N.L. Mills, H. Törnqvist, S.D. Robinson, M. Gonzalez, K. Darnley, W. MacNee, N.A. Boon, K. Donaldson, A. Blomberg, T. Sandstrom, D.E. Newby, Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis, *Circulation* 112 (2005) 3930–3936.
- [4] J.M. Antonini, Health effects of welding, *Crit. Rev. Toxicol.* 33 (2003) 61–103.
- [5] K. Steenland, Ten-year update on mortality among mild-steel welders, *Scand. J. Work Environ. Health* 28 (2002) 163–167.
- [6] B. Sjogren, T. Fossum, T. Lindh, J. Weiner, Welding and ischemic heart disease, *Int. J. Occup. Environ. Health* 8 (2002) 309–311.
- [7] J.J. Moulin, P. Wild, J.M. Haguenoer, D. Faucon, R. De Gaudemaris, J.M. Mur, M. Mereau, Y. Gary, J.P. Toamain, Y. Birembaut, M. Blanc, M.P. Debiolles, D. Jegaden, B. Laterriere, M. Leonard, F. Marini, C. Massardier, M. Moulin, M. Reure, L. Rigal, G. Robert, M. Viossat, A mortality study among mild steel and stainless steel welders, *Br. J. Ind. Med.* 50 (1993) 234–243.
- [8] M.L. Newhouse, D. Oakes, A.J. Woolley, Mortality of welders and other craftsmen at a shipyard in NE England, *Br. J. Ind. Med.* 42 (1985) 406–410.
- [9] A. Karjalainen, K. Kurppa, R. Martikainen, J. Karjalainen, T. Klaukka, Exploration of asthma risk by occupation – extended analysis of an incidence study of the Finnish population, *Scand. J. Work. Environ. Health* 28 (2002) 49–57.
- [10] M. Kogevinas, J.M. Anto, J. Sunyer, A. Tobias, H. Kromhout, P. Burney, Occupational asthma in Europe and other industrialised areas: a population-based study, *European Community Respiratory Health Survey Study Group, Lancet* 353 (1999) 1750–1754.
- [11] Z.P. Wang, K. Larsson, P. Malmberg, B. Sjogren, B.O. Hallberg, K. Wrangskog, Asthma, lung function, and bronchial responsiveness in welders, *Am. J. Ind. Med.* 26 (1994) 741–754.
- [12] J.R. Beach, J.H. Dennis, A.J. Avery, C.L. Bromly, R.J. Ward, E.H. Walters, S.C. Stenton, D.J. Hendrick, An epidemiologic investigation of asthma in welders, *Am. J. Respir. Crit. Care Med.* 154 (1996) 1394–1400.
- [13] K. Toren, B. Jarvholm, J. Brisman, S. Hagberg, B.A. Hermansson, L. Lilienberg, Adult-onset asthma and occupational exposures, *Scand. J. Work Environ. Health* 25 (1999) 430–435.
- [14] H. Keskinen, P.L. Kalliomaki, K. Alanko, Occupational asthma due to stainless steel welding fumes, *Clin. Allergy* 10 (1980) 151–159.
- [15] T. Hannu, R. Piipari, M. Tuppurainen, H. Nordman, T. Tuomi, Occupational asthma caused by stainless steel welding fumes: a clinical study, *Eur. Respir. J.* 29 (2007) 85–90.
- [16] E. Scharrer, H. Hessel, A. Kronseder, W. Guth, B. Rolinski, R.A. Jörres, K. Radon, R. Schierl, P. Angerer, D. Nowak, Heart rate variability, hemostatic and acute inflammatory blood parameters in healthy adults after short-term exposure to welding fume, *Int. Arch. Occup. Environ. Health* 80 (2007) 265–272.

- [17] J.Y. Kim, J.C. Chen, P.D. Boyce, D.C. Christiani, Exposure to welding fumes is associated with acute systemic inflammatory responses, *Occup. Environ. Med.* 62 (2005) 157–163.
- [18] L. Hartmann, M. Bauer, J. Bertram, M. Gube, K. Lenz, U. Reisgen, T. Schettgen, T. Kraus, P. Brand, Assessment of the biological effects of welding fumes emitted from metal inert gas welding processes of aluminium and zinc-plated materials in humans, *Int. J. Hyg. Environ. Health* 217 (2013) 160–168.
- [19] M. Järvelä, P. Kauppi, T. Tuomi, R. Luukkonen, H. Lindholm, R. Nieminen, E. Moilanen, T. Hannu, Inflammatory response to acute exposure to welding fumes during the working day, *Int. J. Occup. Med. Environ. Health* 26 (2013) 220–229.
- [20] X. Baur, T. Sigsgaard, T.B. Aasen, P.S. Burge, D. Heederik, P. Henneberger, P. Maestrelli, J. Rooyackers, V. Schlünssen, O. Vandenplas, D. Wilken, ERS Task Force on the Management of Work-related Asthma, Guidelines for the management of work-related asthma, *Eur. Respir. J.* 39 (2012) 529–545.
- [21] A.A. Viljanen, Reference values for spirometric, pulmonary diffusing capacity and body plethysmographic studies, *Scand. J. Clin. Invest.* 42 (1982) 1–50.
- [22] R.A. Dweik, P.B. Boggs, S.C. Erzurum, C.G. Irvin, M.W. Leigh, J.O. Lundberg, A.C. Olin, A.L. Plummer, D.R. Taylor, American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications, An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications, *Am. J. Respir. Crit. Care Med.* 184 (2011) 602–615.
- [23] SFS3860, Measurement of dust concentration in workplace air with filter method, 1988, pp. 1–8.
- [24] G. Liden, J. Surakka, A headset-mounted mini sampler for measuring exposure to welding aerosol in the breathing zone, *Ann. Occup. Hyg.* 53 (2009) 99–116.
- [25] J.M. Mur, D. Teculescu, Q.T. Pham, M. Gaertner, N. Massin, C. Meyer-Bisch, J.J. Moulin, F. Diebold, F. Pierre, B. Meurou-Poncelet, J. Muller, J.C. Henquel, V. Baudin, M. Betz, J.P. Toamain, Lung function and clinical findings in a cross-sectional study of arc welders. An epidemiological study, *Int. Arch. Occup. Environ. Health* 57 (1985) 1–17.
- [26] A.M. Donoghue, W.I. Glass, G.P. Herbison, Transient changes in the pulmonary function of welders: a cross sectional study of Monday peak expiratory flow, *Occup. Environ. Med.* 51 (1994) 553–556.
- [27] F. Akbar-Khanzadeh, Short-term respiratory function changes in relation to workshift welding fume exposures, *Int. Arch. Occup. Environ. Health* 64 (1993) 393–397.
- [28] J.A. Bosson, A. Blomberg, N. Stenfors, R. Helleday, F.J. Kelly, A.F. Behndig, I.S. Mudway, Peripheral blood neutrophilia as a biomarker of ozone-induced pulmonary inflammation, *PLOS ONE* 8 (2013) e81816.
- [29] T. Müller, R.A. Jörres, E.M. Scharrer, H. Hessel, D. Nowak, K. Radon, Acute blood neutrophilia induced by short-term compost dust exposure in previously unexposed healthy individuals, *Int. Arch. Occup. Environ. Health* 79 (2006) 477–482.
- [30] R.M. Krishnan, J.H. Sullivan, C. Carlsten, H.W. Wilkerson, R.P. Beyer, T. Bammler, F. Farin, A. Peretz, J.D. Kaufman, A randomized cross-over study of inhalation of diesel exhaust, hematological indices, and endothelial markers in humans, *Part. Fibre Toxicol.* 10 (2013) 7.
- [31] A. Seaton, A. Soutar, V. Crawford, R. Elton, S. McNerlan, J. Cherrie, M. Watt, R. Agius, R. Stout, Particulate air pollution and the blood, *Thorax* 54 (1999) 1027–1032.
- [32] A.F. Behndig, N. Larsson, J.L. Brown, N. Stenfors, R. Helleday, S.T. Duggan, R.E. Dove, S.J. Wilson, T. Sandstrom, F.J. Kelly, I.S. Mudway, A. Blomberg, Proinflammatory doses of diesel exhaust in healthy subjects fail to elicit equivalent or augmented airway inflammation in subjects with asthma, *Thorax* 66 (2011) 12–19.
- [33] T. Hannu, R. Piipari, H. Kasurinen, H. Keskinen, M. Tuppurainen, T. Tuomi, Occupational asthma due to manual metal-arc welding of special stainless steels, *Eur. Respir. J.* 26 (2005) 736–739.
- [34] J.M. Antonini, M.A. Badding, T.G. Meighan, M. Keane, S.S. Leonard, J.R. Roberts, Evaluation of the pulmonary toxicity of a fume generated from a nickel-, copper-based electrode to be used as a substitute in stainless steel welding, *Environ. Health Insights* 8 (Suppl. 1) (2014) 11–20.
- [35] P. Brand, P. Havlicek, M. Steiners, K. Holzinger, U. Reisgen, T. Kraus, M. Gube, Exposure of healthy subjects with emissions from a gas metal arc welding process. Part 1: Exposure technique and external exposure, *Int. Arch. Occup. Environ. Health* 86 (2013) 25–30.