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Impact of wildfire emissions exposure on the associations between levels of lung injury, lipid peroxidation, DNA oxidation, and exposure biomarkers

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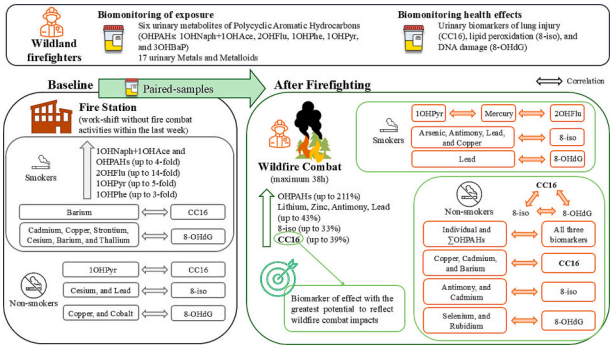
HIGHLIGHTS

- Wildfire combat contributed to lung injury, DNA oxidation, lipid peroxidation.
- Urinary Clara cell 16 arose as a key effect biomarker of wildland firefighting.
- Smokers exposed to wildfires showed increased metal(loid)s-induced oxidative stress.
- Individual and sum of OHPAHs correlated with all three effect biomarkers.
- Wildfire exposure and health impacts were more evident among non-smokers.

GRAPHICAL ABSTRACT



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ABSTRACT

Firefighters face increased risks of developing cardio-respiratory diseases and cancer. This study aimed, for the first time, to simultaneously characterize several biomarkers of effect (lung injury by Clara cell 16 –CC16, lipid

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peroxidation by 8-isoprostane–8-iso, and DNA oxidation by 8-hydroxy-2-deoxyguanosine–8-OHdG) and exposure (polycyclic aromatic hydrocarbons metabolites – 6 OHPAHs and 17 metal(loid)s) in (pre- and post-exposure) paired urine samples of wildland firefighters, while exploring their inter-/intra-associations and accounting for tobacco consumption. Wildfire combat influenced the levels of CC16 (+39 %), 8-iso (+33 %), 8-OHdG (-13 to +19 %), individual and sum of OHPAHs (+75–211 %), and metal(loid)s (up to 43 %, $p > 0.05$: lithium, zinc, antimony, and lead); post-exposure increments were more evident among non-smokers. Post-exposure (individual and sum) OHPAHs and some metal(loid)s (copper, cadmium, barium, antimony, copper, lead, zinc, selenium, and rubidium) were positively associated with CC16, 8-iso and/or 8-OHdG ($0.609 < r < 0.838$; $0.001 < p < 0.047$). Spearman's correlations and principal component analysis highlighted CC16 as the best discriminant effect biomarker of wildland firefighting, correlating positively with individual and sum of OHPAHs, cadmium, barium and copper ($0.647 < r < 0.764$; $0.006 < p < 0.031$). Cumulative exposure to wildfires and tobacco contributed to positive correlations ($0.587 < r < 0.715$; $0.009 < p < 0.045$) between lipid peroxidation and arsenic, antimony, lead, and copper, and between DNA oxidation and lead. Smoking firefighters presented higher OHPAHs baseline concentrations (2- to 14-fold), and lung injury and DNA oxidation induced by cadmium, copper, strontium, cesium, barium and thallium ($0.661 < r < 0.709$; $0.022 < p < 0.038$). Given firefighter's carcinogenic risks, performing similar studies in larger groups is crucial to enhance risk assessment by establishing a well-defined panel of effect and exposure biomarkers.

1. Introduction

The occupational activity as a firefighter encompasses many stressors such as nightshifts, paramedic emergency calls, and fire combat. During fire combat, firefighters are simultaneously exposed to physical activity under high temperatures and chemical mixtures of pollutants, with polycyclic aromatic hydrocarbons (PAHs) and metal (loid)s among the most relevant for wildland firefighters (Sousa et al., 2022; Barros et al., 2023a). Toxic pollutants can be absorbed *via* inhalation, ingestion, or dermal contact, and distributed throughout the human body (Barros et al., 2021). Some of these pollutants are classified by the International Agency for Research on Cancer (IARC) as known [Group 1: benzo(*a*)pyrene, nickel compounds, arsenic (and inorganic arsenic compounds), cadmium (and cadmium compounds), lead, beryllium], probable (Group 2A: dibenzo(*a*,*l*)pyrene, dibenz(*a*,*h*) anthracene, cobalt, trivalent antimony) and possible carcinogenic to humans (Group 2B: naphthalene, chrysene, anthracene, benz(*a*) anthracene, benzo(*c*)phenanthrene, dibenzo(*a*,*i*)pyrene, dibenz(*a*,*h*) pyrene, indeno(1,2,3-c,d)pyrene, nickel (metallic), molybdenum trioxide, methylmercury compounds) (IARC, 2024). Given the toxicologic profile of PAHs and metal(loid)s, they are also listed as priority substances by the Agency for Toxic Substances and Disease Registry (ATSDR: 13 PAHs, 16 metal(loid)s), the United States Environmental Protection Agency (USEPA: 16 PAHs, 13 metal(loid)s), and the Human Biomonitoring for Europe Initiative (HBM4EU: 23 PAHs, 5 metals) (USEPA, 2014; HBM4EU, 2021; Ougier et al., 2021; ATSDR, 2022).

Besides carcinogenicity, PAHs are associated with lung function decline, asthma exacerbation, and augmented risk of developing chronic obstructive pulmonary disease (COPD) and cardiovascular illnesses (WHO, 2021). Exposure to metals has been linked to metabolic syndrome, diabetes, increased cardiovascular, respiratory, kidney, and skin toxicity (Kiran et al., 2022; Mitra et al., 2022; Renu et al., 2022). Consequently, the broad exposome firefighters face promotes an increases the risk of developing cardio-respiratory diseases (Cuenca-Lozano and Ramírez-García, 2023).

Therefore, it is of utmost importance to explore biomarkers of effect among firefighters (Oliveira et al., 2017a, 2017b; Engelsman et al., 2019), especially with non-invasive matrices to support and simplify risk assessment. Clara cell 16 (CC16), 8-iso prostaglandin $F_{2\alpha}$ (8-iso), and 8-hydroxy-2-deoxyguanosine (8-OHdG) are key biomarkers of lung injury, lipid peroxidation (oxidative stress), and DNA oxidative damage, respectively, that can be used to characterize inflammatory processes at a local and/or systemic range (Jaishankar et al., 2014; Pinto et al., 2017; Zhou et al., 2023). These biomarkers are detectable in urine, enabling a painless sample collection (Andersson et al., 2007; Zanolin et al., 2015; Zhou et al., 2018; Van der Plas et al., 2019). However, few studies have investigated urinary levels of CC16, 8-iso, and 8-OHdG in firefighters, with the available research limited to structural firefighting [CC16 and 8-iso: (Keir et al., 2017)], prescribed burns [8-iso or 8-OHdG: (Adetona et al., 2013, 2019; Wu et al., 2020)], and wildfire combat activities [8-iso and 8-OHdG: (Gaughan et al., 2014)].

To the best of the author's knowledge, no studies have been conducted on firefighters specifically investigating the health impacts of wildland fire combat activities by simultaneously biomonitoring urinary CC16, 8-iso, and 8-OHdG, as well as characterizing their relationship with biomarkers of exposure of PAHs and metal(loid)s. Thus, in this study, given the importance of urine to assess total body burden, its non-invasive collection, and its relevance for bladder cancer (IARC, 2022), the aforementioned biomarkers of effect (CC16, 8-iso, 8-OHdG) and exposure (six OHPAHs, and 17 metal(loid)s) were characterized in paired (baseline and post-exposure) urine samples of Portuguese wildland firefighters while simultaneously accounting for the contribution of tobacco consumption (as a main confounder), and exploring associations within and between biomarkers.

2. Materials and methods

2.1. Study population and sampling

Sampling campaigns were performed during June 2021 to October 2022 in fire stations from Bragança (Northern region, Portugal), which is among the Portuguese districts that are most affected by fires (Tyukavina et al., 2022). The study protocol, according to the Declaration of Helsinki, was previously approved by the Ethics Committee of the University of Porto (Report Nr. 92/CEUP/2020) and all enrolled male firefighters ($n = 23$; Table 1) signed an informed consent and filled out a questionnaire about personal and occupational information. Pre-exposure spot urine samples were collected during regular work-shift without previous exposure to fire combat within the last week and used to represent each individual baseline for occupational exposure. This time frame was chosen based on prior evidence suggesting that non-persistent compounds such as PAHs (Gill and Britz-McKibbin, 2020; Li et al., 2012) and related biomarkers of oxidative stress typically return to baseline levels within a few days after exposure cessation (Adetona et al., 2013; Van der Plas et al., 2019). Whenever a wildfire occurred in the district, participating firefighters who were exposed (*i.e.*, conducted combat activities) provided a post-fire spot urine sample and completed a short questionnaire related to their exposure after returning to the fire station. Aliquots of urine samples were prepared (*e.g.*, 0.005 % of butylhydroxytoluol (5 mg/mL ethanol) was added to ensure the stability of 8-iso) for urinalysis and frozen (up to -80 °C) until analysis. Given the contribution of tobacco consumption to urinary biomarkers, the study population was subdivided by self-reported smoking status (non-smoker ($n = 11$) and smoker ($n = 12$)) at the time of the study. Urinary

Table 1
Characterization of the study population.

Variables (median and range)	All firefighters ($n = 23$)	Non-smokers ($n = 11$)	Smokers ($n = 12$)[a]
Age (years old)	36 (21–55)	40.5 (21–55)	36 (21–43)
≤35 (%)	43	40	45
>35 (%)	57	60	55
Body mass index (kg/m^2)	27.0 (19.4–35.9)	27.6 (24.2–35.9)	26.1 (19.4–33.7)
Normal (%)	33	30	36
Overweight (%)	52	50	55
Obese (%)	15	20	9
Service as a firefighter (years)	16.0 (0.3–34.0)	16.0 (3.0–34.0)	16.0 (0.3–28.0)
≤10 (%)	38	40	36
11–24 (%)	38	30	46
≥25 (%)	24	30	18
Exposure to wildfires (hours)	4.0 (1.0–38.0)	3.5 (1.0–38.0)	7.5 (1.0–29.0)
<8 (%)	59	70	50
≥8 (%)	41	30	50

[a] Median: 10 (range: 10–20) smoked cigarettes/day.

creatinine, quantified by the Jaffe colorimetric method (Kanagasabapathy and Kumari, 2000), was used to normalize the concentrations.

2.2. Urinalysis of CC16, 8-iso, and 8-OHdG

Enzyme-linked immunosorbent assays (ELISA) validated for use in urine samples were applied according with manufacturer instructions to quantify urinary CC16 (sandwich ELISA assay, item RD191022200, BioVendor R and D, Brno, Czech Republic), 8-iso (competitive ELISA, item 516351, Cayman, Ann Arbor, MI, USA), and 8-OHdG (competitive ELISA, item ab201734, Abcam, Cambridge, UK). All assayed samples were analyzed in duplicates in a dilution of 1:25 (CC16), 1:10 to 1:55 (8-iso), and 1:20 (8-OHdG). Sensitivity for CC16, 8-iso, and 8-OHdG were 46 pg/mL, 3 pg/mL and 0.59 ng/mL, respectively, and spike recovery ranged from 91.0 and 109.6 %. Median inter-assay relative standard deviations (RSD) of standards were 6 %, 10 %, and 16 %, whereas intra-assay RSD of duplicates was 2 %, 4 %, and 7 %, respectively for CC16, 8-iso, and 8-OHdG (detailed performance characteristics in Section 2.2S of Supplementary material).

2.3. Urinalysis of PAH metabolites

OHPAHs were analyzed by high performance liquid chromatography with a fluorescence detector according to a previously validated extraction (enzymatic hydrolysis with β-glucuronidase/arylsulfatase during 120 min at 37 °C) and quantification method (Oliveira et al., 2020). The detection (LOD) and quantification limit (LOQ) were calculated based on 3- and 10-times the standard deviation of the blank value, respectively (Miller and Miller, 2010). LODs and LOQ varied, respectively, 7.34–9.59 and 24.5–32.0 μg/L for 1-hydroxynaphthalene+1-hydroxyacenaphtene (1OHNaph + 1OHAce), 0.03–0.05 and 0.10–0.24 μg/L for 2-hydroxyfluorene (2OHFlu), 0.02–0.06 and 0.06–0.21 μg/L for 1-hydroxyphenathrene (1OHPhe), 0.02–0.05 and 0.06–0.16 μg/L for 1-hydroxypyrene (1OHPyr), 0.02–0.03 and 0.07–0.11 μg/L for 3-hydroxybenzo(*a*)pyrene (3OHBaP). Quality control included triplicate daily analysis of blanks, standards, and spiked pooled urine samples. Recoveries ranged from 70.0 to 117.0 % and intra-/inter-day variations were <10 % (further details in Section 2.3S of Supplementary material).

2.4. Urinalysis of metal(loid)s

The elemental isotopes ^{7}lithium, ^{59}cobalt, ^{60}nickel, ^{65}copper, ^{66}zinc, ^{75}arsenic, ^{82}selenium, ^{87}rubidium, ^{88}strotium, ^{98}molybdenum, ^{111}cadium, ^{121}antimony, ^{133}cesium, ^{137}barium, ^{202}mercury, ^{205}thalium, ^{206}lead, ^{207}lead and ^{208}lead were quantified in urine by inductively coupled plasma spectrometry (ICP-MS) in an iCAP™ Q (Thermo Fisher Scientific, Germany) according to the protocol described by Azevedo et al. (2023). Samples were diluted in a ratio of 1:10 (v/v) with a solution of 2 % HNO_3 (500 μg/L), 1.5 % ethanol, and 10 μg/L ^{71}gallium, ^{103}rhodium, and ^{193}iridium, which were monitored as internal standards. For analytical quality assurance, Seronorm™ Trace Elements Urine L-1 and L-2 (SERO AS, Billingstad, Norway) were repeatedly analyzed at the beginning, middle, and end of each analytical run. LODs varied from 0.0099 (antimony) to 35 (rubidium) μg/L while LOQs varied 0.033–117 μg/L for the same meta(loid)s. Spike recovery tests, internal standards to correct for matrix effects and repeated quality control checks throughout each run ensured analytical accuracy (recoveries ranged 87–120 %). The precision of analytical quality controls varied <10 % (additional information in Section 2.4S of Supplementary material).

2.5. Statistical analysis

Concentrations of exposure biomarkers below the LOD were replaced by the LOD divided by the square root of 2 for statistical purposes (Miller and Miller, 2010). IBM SPSS statistics version 29 and excel (v. 16.0.4266, Microsoft Corporation, USA) including the XLSTAT extension (v. 2023.3.1) were used to conduct statistical analysis. Given the limited sample number and lack of normal distribution, non-parametric Independent-Samples Mann-Whitney *U* Test was used to test for differences in biomarker levels according to smoking status (no adjustments for tobacco use intensity were made in the analysis) and exposure variables. The Wilcoxon Signed Ranks Test was used to test biomarker concentration differences in paired samples. Spearman's correlations were used to explore inter- and intra-associations between biomarker types and personal data (age, body mass index (BMI), and service years as a firefighter). Principal components analysis (PCA) was performed using creatinine-adjusted levels/relative differences (post-exposure to baseline) of biomarkers of effect and exposure. The best fit was chosen according to the following criteria: i) individual Kaiser-Meyer-Olkin measure of sampling adequacy (KMO >0.5); ii) Eigenvalue >1.0; and iii) account for >60 % of data. Sensitivity analysis was conducted based on separate analysis of biomarkers of effect with all exposure biomarkers and OHPAHs and metal(loid)s separated, and by either using relative differences of paired samples (post-exposure to baseline) or individual concentrations of each biomarker. Sample grouping was only used for data display in PCA graphs to facilitate the observation of group aggregation by tobacco consumption and/or exposure. A significance set at $p \leq 0.05$ (two tailed) was considered.

3. Results and discussion

3.1. Study population

The enrolled firefighters (Table 1) were aged between 21 and 55 years old and presented a median 16 years of service (3 months to 34 years). Regarding recent participation in forest fires, 59 % of the subjects reported being exposed <8 h to wildfire combat, while 41 % were enrolled in fire combat for >8 h (maximum of 38 h). No differences ($p > 0.05$) were observed between smokers and non-smokers regarding study population characteristics. Urinary creatinine varied 0.328–4.06 g/L, with only 2 samples with levels above the recommended range (*i.e.*, 0.3–3.0 g/L) (WHO, 1996; ACGIH, 2019).

3.2. Effect biomarkers

The inter- and intra-individual variability in hydration and urine dilution was reduced by normalizing biomarker concentrations with individual urinary creatinine. Moreover, the use of paired samples

allowed firefighters to act as their own control limiting confounding variables. Biomarker levels are also presented without normalization in Table S4 of Supplementary material and used to compare with previous studies, when applicable.

3.2.1. Clara cell 16

The median concentration of the lung injury biomarker in firefighters was 6.58 (1.54–43.5) and 9.16 (2.88–31.9) ng/mg creatinine at the baseline and post-exposure, respectively (Fig. 1A). Wildland

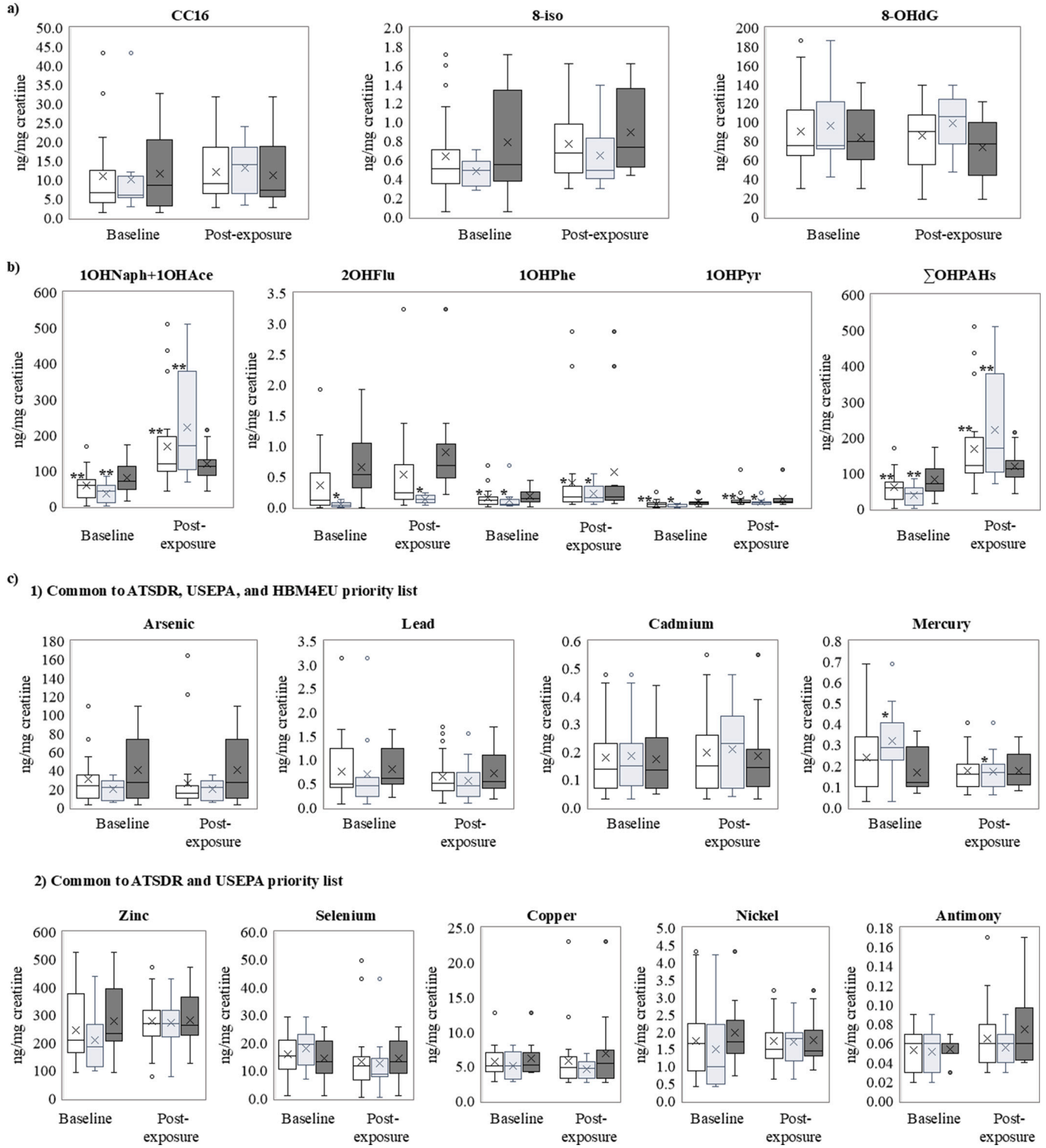

Fig. 1. Urinary creatinine-adjusted concentrations (ng/mg creatinine) of biomarkers of effect and exposure. a) Clara cell 16 (CC16), 8-isoprostane (8-iso), and 8-hydroxydeoxyguanosine (8-OHdG); b) polycyclic aromatic hydrocarbons metabolites (OHPAHs); c) metal(loid)s according to common inclusion on priority lists of: 1) ATSDR, USEPA, and HBM4EU; 2) ATSDR and USEPA.
1OHNaph + 1OHAce: 1-hydroxynaphthalene+1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhe: 1-hydroxyphenanthrene; 1OHPyr: 1-hydroxypyrene; ΣOHPAHs: Sum of polycyclic aromatic hydrocarbons metabolites.
Independent-samples Mann-Whitney *U* test: *Statistically significant difference ($p \leq 0.05$). **Statistically significant difference ($p < 0.001$).

firefighting was associated with elevated CC16 levels in paired samples, up to 39 % ($p > 0.05$) in creatinine adjusted levels (Figs. 1A and 2; Table 2) and up to 87 % ($0.017 < p < 0.026$; Tables S4 and S5) for unadjusted values. A study in firefighters' sputum showed increased CC16 after fire simulator exposure, linked to airway epithelial and inflammatory responses (Cordeiro et al., 2021). Post- and pre-exposure CC16 median concentrations herein determined were 3 and 1.93-times higher, respectively, than those reported for structural firefighters (3.2–3.8 ng/mg creatinine; Keir et al., 2017), suggesting a higher impact of wildfire combat on this biomarker. These raised levels could be the result of the less efficient personal protective equipment (PPE) opposed to the mandatory use of self-contained breathing apparatus (SCBA) by structural firefighters. Studies have shown that while respirators such as SCBA provide significant protection against the inhalation of pollutants, wildland firefighters' PPE (such as balaclavas and protective eyewear, used instead of SCBA) is less effective, allowing the penetration of toxic compounds (Chakr and Sav, 2024). As so, cell stress and inflammation in the lungs are potentiated, helping to explain the detected levels in wildland firefighters. Portuguese firefighters also presented 5- to 30-fold higher post-exposure CC16 levels than subjects exposed to woodsmoke-particular matter (range: 0.3–1.7 ng/mg creatinine (Stockfelt et al., 2012)). Previous studies suggested increased airway sensitivity (Gianniou et al., 2016; Cordeiro et al., 2021; Orysiak et al., 2022) and lung cancer risks exceeding the World Health Organization (WHO) guideline due to fire smoke exposure (Teixeira et al., 2024). Concerning tobacco consumption, greater post- to pre-exposure increments in urinary CC16 (Figs. 1A and 2) were observed in non-smokers (96–130 %; $p > 0.05$) compared to smokers (15 %), suggesting a stronger impact of firefighting in non-smokers, also influenced by firefighting duration (<8 h: 10.7 *versus* ≥8 h: 18.6 ng/mg creatinine; $p = 0.067$).

3.2.2. 8-isoprostane

8-iso has been identified as a standard biomarker of lipid peroxidation and oxidative stress (Nam, 2011; Van der Plas et al., 2019). In this study, for all firefighters, the baseline and post-exposure 8-iso concentrations were 0.519 (0.06–1.72) and 0.683 (0.300–1.62) ng/mg creatinine, respectively (Fig. 1A). Pre-exposure values are comparable with those reported for USA wildland firefighters (0.20–0.82 ng/mg creatinine; (Adetona et al., 2019; Wu et al., 2020)) while post-exposure levels were 73–147 % higher than those observed after training sessions, wildfire combat, and participation in prescribed burnings and regular work-shifts (Tables 3 and S6). Nevertheless, the present study observed lower urinary 8-iso levels compared to structural firefighters (-37 % to -46 %; (Keir et al., 2017)) and Midwest USA wildland firefighters exposed to prescribed burns (-28 % to -60 %%; (Wu et al., 2020)). In the characterized subjects, wildland firefighting augmented up to 33 % the 8-iso values (Figs. 1A and 2; $p > 0.05$; Table 2), and similar findings were observed for unadjusted levels (up to 68 % increase; Tables S4 and S5; $0.004 < p < 0.061$); no relation was observed between exposure duration (hours) to wildfire emissions and levels. Similar post-exposure increases in 8-iso have been reported in previous studies, namely +32 % in wildland firefighting *versus* training sessions (Gaughan et al., 2014), +110 % in structural firefighting when compared to office workers (Keir et al., 2017); +50–110 % in prescribed burn-days in comparison to pre-shift at non-burn days (Adetona et al., 2019; Wu et al., 2020). Overall, 8-iso data (Tables 3 and S6) collectively suggest the existence of common stressors among the different fire combat activities demonstrating the great potential of urinary 8-iso to monitor lipid peroxidation and oxidative stress in firefighters after occupational activities, irrespective of the fire combat type. When comparing smokers *versus* non-smokers, tobacco consumption had a non-significant positive impact on 8-iso both at baseline (+14 and + 55 % for normalized and not normalized values, respectively; $p > 0.05$) and after-exposure (+24–51 %; $p > 0.05$)

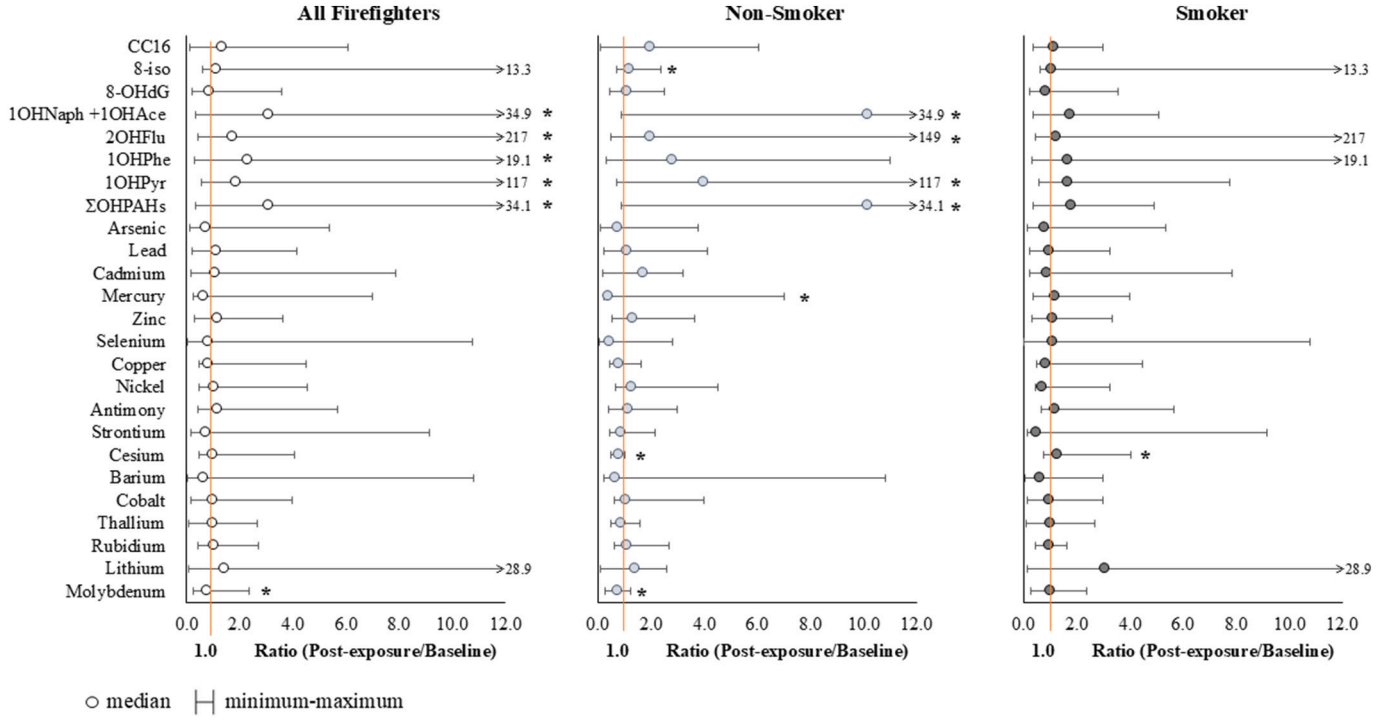

Fig. 2. Median ratio (minimum and maximum) of post-exposure to baseline creatinine-adjusted levels of Clara cell 16 (CC16), 8-isoprostane (8-iso), and 8-hydroxydeoxyguanosine (8-OHdG), polycyclic aromatic hydrocarbons metabolites (OHPAHs) and metals(loid)s in firefighters and according to their smoking status in paired samples. Vertical line represents the ratio equal to 1.
Wilcoxon Signed Ranks test for paired samples: *Statistically significant difference ($p \leq 0.05$).
1OHNaph + 1OHAce: 1-hydroxynaphthalene+1-hydroxyacenaphthene; 2OHFlu: 2-hydroxyfluorene; 1OHPhe: 1-hydroxyphenanthrene; 1OHPyr: 1-hydroxypyrene; ΣOHPAHs: Sum of polycyclic aromatic hydrocarbons metabolites.

Table 2
Significance of differences for repeated measurements (baseline and post-exposure) in creatinine-adjusted levels of urinary biomarkers of effect and exposure obtained by Wilcoxon Signed Ranks Test (significance set at $p < 0.05$).

	All firefighters	Non-smoker	Smoker
Biomarker of effect			
CC16	0.101	0.131	0.480
8-iso	0.140	0.047[a]	0.754
8-OHdG	0.648	1.00	0.388
Biomarker of exposure to polycyclic aromatic hydrocarbons			
1OHNaph +1OHAce	0.001[a]	0.004[a]	0.239
2OHFlu	0.014[a]	0.006[a]	0.209
1OHPhe	0.019[a]	0.05	0.182
1OHPyr	0.002[a]	0.004[a]	0.117
ΣOHPAHs	0.001[a]	0.004[a]	0.239
Biomarker of exposure to metal(loid)s			
Arsenic	0.274	0.155	0.695
Lead	0.637	0.722	0.666
Cadmium	0.858	0.721	1.00
Mercury	0.162	0.045[b]	0.724
Zinc	0.248	0.155	0.754
Selenium	0.224	0.155	0.875
Copper	0.403	0.689	0.480
Nickel	0.951	0.213	0.388
Antimony	0.266	0.682	0.237
Strontium	0.201	0.722	0.182
Cesium	0.605	0.021[b]	0.019[a]
Barium	0.117	0.328	0.182
Cobalt	1.00	0.593	0.759
Thallium	0.370	0.358	0.683
Rubidium	1.00	0.722	0.754
Lithium	0.144	0.328	0.158
Molybdenum	0.036[b]	0.021[b]	0.308

Wilcoxon Signed Ranks Test for paired samples: significance of differences for repeated measurements (baseline and post-exposure): a: increase; b: decrease; significance set at $p < 0.05$. 1OHNaph + 1OHAce: 1-hydroxynaphthalene+1-hydroxyacenaphtene; 2OHFlu: 2-hydroxyfluorene; 1OHPhe: 1-hydroxyphenanthrene; 1OHPyr: 1-hydrpxypyrene; 8-OHdG: 8-hydroxydeoxyguanosine; 8-iso: 8-isoprostane; CC16: Clara cell 16; ΣOHPAHs: sum of polycyclic aromatic hydrocarbons metabolites.

(Fig. 1A; Table S4). Similarly, Gaughan et al. (2014) found non-significant differences in 8-iso according to smoking status in firefighters irrespective of wildfire exposure. It is possible that the number of smoked cigarettes (10–20 per day), compensatory mechanisms following wildfire-exposure, individual variability in metabolism and excretion rates of 8-iso, as well as the small sample size of smokers, could have contributed to these findings. However, when comparing the relative post-exposure to baseline difference in paired samples, statistical significance was detected in non-smokers (+18 %; $p = 0.047$; Fig. 2; Table 2), therefore suggesting that smoking may limit the use of 8-iso as a biomarker of exposure to environmental-PAHs as previously reported (Kang et al., 2005). These findings also evidence a greater impact of wildfire combat activities in 8-iso levels among non-smoking firefighters as observed with CC16. Additionally, 8-iso strongly correlated with the age of firefighters in exposed non-smokers only ($r = 0.813$, $p = 0.002$), which is consistent with age-related susceptibility to oxidative stress reported in previous studies (Yavuzer et al., 2016; Ali et al., 2022).

3.2.3. 8-hydroxy-2-deoxyguanosine

Urinary 8-OHdG is the most studied biomarker of DNA damage due to oxidative stress, and it has also been associated with lung cancer risk (Valavanidis et al., 2009; Graille et al., 2020). For all firefighters, 8-OHdG concentrations of 75.9 (30.1–187) and 85.7 (18.5–140) ng/mg creatinine were found at baseline and post-exposure, respectively (Fig. 1A). Pre-exposure 8-OHdG levels of Portuguese firefighters were similar to those of USA firefighters before exposure to prescribed burns [81–102 ng/mg creatinine; (Adetona et al., 2013; Wu et al., 2020)]. Nonetheless, Portuguese wildland firefighters presented up to 7-fold higher mean creatinine-adjusted 8-OHdG than Korean firefighters (Hong et al., 2000), and 21 to 40-times higher unadjusted levels than USA firefighters after wildfire combating and training sessions (Gaughan et al., 2014). In comparison to other occupations, 8-OHdG levels in this study were 3-times lower than those of coke oven workers after occupational exposure (Deng et al., 2019). However, direct comparison between different occupational settings must be interpreted with caution, as the specific composition of the exposure source can influence biomarker expression. For all firefighters, the median difference post-exposure to baseline varied -13 % (Fig. 2; $p > 0.05$) to +19 % (Fig. 1A; $p > 0.05$). Mixed results (*i.e.*, positive and negative variations) for 8-OHdG were also found in other studies in firefighters (Tables 3 and S6), *i.e.*, -14 % after exposure to prescribed burns, -19 to -34 % immediately after exposure to prescribed burns (with up to +63 % on the morning after), and +86 % (wildfire combat *versus* training), that was only significant when 8-OHdG levels were adjusted for urinary levoglucosan (woodsmoke exposure biomarker). DNA oxidation may result from wildfire pollutants, but using urinary 8-OHdG as a systemic effect biomarker for this requires further study. Regarding the impact of tobacco consumption, an inverse post-exposure response was observed, *i.e.*, 8-OHdG decreased 15 % in wildfire exposed smokers but increased 13 % in non-smokers (Fig. 2; $p > 0.05$). The reduced post-8-OHdG urinary concentrations can be due to a decrease in tobacco consumption during fire combat activities. However, in smoking firefighters, there was a significant positive moderate correlation between post-exposure 8-OHdG levels and the duration of the fire exposure ($r = 0.596$; $p = 0.041$). As previously discussed for CC16 and 8-iso, wildfire health impacts are more evident in non-smoking than in smoking firefighters. However, the increasing excretion of 8-OHdG with exposure hours in smokers supports the hypothesis that this biomarker could still reflect the effect of wildfire combat in these firefighters. Lastly, 8-OHdG correlated negatively with BMI in non-smokers at baseline ($r = -0.675$, $p = 0.032$), which is in line with a recent general population meta-analysis in which lower urinary 8-OHdG levels were observed among non-smokers with higher BMI (> 25 kg/m^2) (Graille et al., 2020).

3.2.4. Correlations within effect biomarkers

Urinary 8-OHdG was positively associated with CC16 only among exposed non-smokers ($r = 0.627$; $p = 0.039$), suggesting significant association between these two biomarkers and exposure to wildland firefighting. Evidence has been presented linking exposure to air pollution with higher urinary 8-OHdG content. Also, increased CC16 (mostly in serum) has been constantly and positively associated with air pollution (Coumans and Al Jaaidi, 2023). Oxidative DNA damage coupled with inflammatory response in the lungs (measured by serum 8-OHdG and CC16) have been observed in humans and cultured lymphoblasts exposed to traffic-related particles (Vattanasit et al., 2014). Wildfire emissions promote air quality deterioration (Barros et al., 2023b), therefore corroborating the observed associations between CC16 and 8-OHdG. Moreover, relative post-exposure to baseline differences of CC16 significantly correlated with those of 8-iso ($r = 0.483$; $p = 0.020$) and 8-OHdG ($r = 0.436$; $p = 0.038$), highlighting wildland firefighting as a common stressor to these three effect biomarkers, as a consequence of the promoted oxidative stress and inflammation in the lungs.

3.3. Exposure biomarkers

3.3.1. PAHs metabolites

1OHNaph + 1OHace (88.90–99.96 %) contributed the most to the sum of OHPAHs (∑OHPAHs), followed by 1OHPhe (0.01–10.1 %), 2OHFlu (0.002–3.2 %), and 1OHPyr (0.002–1.1 %), whereas 3OHBaP was always below the detection limit for all firefighters. Fire combat activities promoted a significant augment in ∑OHPAHs (Figs. 1B and 2, Tables 2, S4 and S5) (3-fold, $p < 0.001$). Urinary 1OHNaph + 1OHAce was by far the largest contributor to the increased post-fire levels (3-fold,

Table 3
Review of the existent studies reporting concentrations of urinary Clara cell 16 (CC16), 8-isoprostane (8-iso), and 8-hydroxydeoxyguanosine (8-OHdG) (geometric mean ± SD and range, ng/mg creatinine, except when indicated otherwise) in firefighters.

Country	Age (years)	Subgroup	Exposure		Collection time	*n*	CC16	8-iso	8-OHdG	Reference
			Type	Duration						
South Korea	33	Firefighters	Fire (does not specify)	<8 h	Post-exposure (up to 5 days)	36	–	–	12.3	(Hong et al., 2000)[d]
	35			≥8 h		13	–	–	14.1	
Canada	34 (25–50)	Structural Firefighters	Baseline	5 consecutive shifts (24 h) typically spanning 12 days	Before each work-shift	16	3.8 ± 0.2 (0.4–17.8)	1.0 ± 0.4 (0.2–5.9)	–	(Keir et al., 2017) £
			Firefighting	Multiple fires during 24 h shift	Post-exposure (within 18 h)	15[a]	3.2 ± 1.0 (0.6–15.8)	1.1 ± 0.1 (0.2–8.0)	–	
USA	29 ± 4.34	Wildland Firefighters	Training sessions	–	–	18	–	0.63 ± 2.57[b]	4.07 ± 2.45[b]	(Gaughan et al., 2014)[e]
			Sand Gulch fire combat ₮	2 days (12.5 h each day)	Post-exposure (4 days)	20	–	0.83 ± 2.63[b]	7.59 ± 1.95[b]	
USA	29.8 ± 6.1 (21–44)	Wildland firefighters	Baseline	n.a. (at 6 to 9 am at fire station)	Before work-shift	17[a]	–	–	81 ± 122	(Adetona et al., 2013)[e]
			Prescribed burns	–	Post-shift	17[a]	–	–	70 ± 90	
USA	33 ± 5.4	Non-burn days	Baseline	n.a. (days since last burn: 1–30)	Before work-shift	12	–	0.2 (0.2–0.3) | 0.5 (0.3, 1.0)[c]	–	(Adetona et al., 2019)[f] £
			Regular work shifts	3.9–7.8 h	Immediately after	12[a]	–	0.4 (0.3–0.5) | 1.0 (0.7, 1.6)[c]	–	
		Burn days	Baseline	n.a. (days since last burn: 3–30)	Before work-shift	12	–	0.4 (0.3–0.5) | 1.0 (0.7, 1.4)[c]	–	
			Prescribed burns	1.9–9.4 h	Immediately after	12[a]	–	0.3 (0.2–0.4) | 1.1 (0.9, 1.4)[c]	–	
USA	35 ± 7.2	Non-burn days	Baseline	n.a.	Before work-shift	19[a]	–	0.82 ± 0.13 (0.17–2.18) 0.75 ± 0.15 (0.17–2.40)[c]	¥ 102 ± 32.7 (8.67–502) 115 ± 26.6 (21.7–1147)[c]	(Wu et al., 2020) £
			Regular work shifts	n.a.	Immediately after	19[a]	–	0.50 ± 0.12 (0.13–1.64) 0.51 ± 0.18 (0.07–3.02)[c]	¥ 91.5 ± 38.3 (8.35–1180) 130 ± 39.2 (31.0–1069)[c]	
		Burn days	Baseline	n.a.	Before work-shift	19[a]	–	1.05 ± 0.21 (0.13–15.0) 1.30 ± 0.31 (0.09–15.2)[c]	¥ 83.0 ± 16.1 (12.6–902) 98.3 ± 14.0 (18.9–608)[c]	
			Prescribed burns	4.98 ± 1.34 h	Immediately after	19[a]	–	1.72 ± 0.36 (0.37–125.1) 3.18 ± 0.80 (0.15–17.3)[c]	¥ 67.5 ± 15.6 (10.2–1143) 117 ± 16.5 (24.9–509)[c]	
Portugal	36 (21–55)	Wildland Firefighters	Baseline	Work-shift	During work-shift	23[a]	7.34 ± 10.7 (1.54–43.5) | 11.4 ± 18.1 (3.69–78.6)[c]	0.54 ± 0.45 (0.056–1.7) 0.83 ± 0.70 (0.12–2.8)[c]	81.2 ± 41.5 (30.1–187) 126 ± 61.7 (37.2–299)[c]	This study £
			Wildland Firefighting	All	After arrival to the fire station	23[a]	9.76 ± 8.11 (2.88–31.9) | 18.9 ± 11.2 (5.46–46.6)[c]	0.69 ± 0.39 (0.30–1.6) 1.34 ± 0.94 (0.35–4.2)[c]	77.4 ± 32.4 (18.5–140) 150 ± 65.4 (56.5–313)[c]	

[a] Paired samples.
[b] Data originally expressed as Log_{10}.and presented as ng/mL.
[c] Data presented as ng/mL.
[d] Arithmetic mean.
[e] Arithmetic mean ± SD.
[f] Geometric mean (95 % confidence limits); n.a.: not applicable; SD: standard deviation. ₮: 495-acre wildland fire at 7000 ft elevation in high difficulty terrain; ¥: data refer to all oxidized guanine species. £: Additional information in Table S6 Supplementary material.

$p < 0.001$), followed by 1OHPhe (2-fold, $p = 0.016$), 1OHPyr (1.88-fold, $p = 0.001$), and 2OHFlu (1.75-fold, $p = 0.127$) (Fig. 1B). Similar results were found for individual OHPAH and ∑OHPAHs differences in repeated measurements of post- to pre-exposure paired samples for all firefighters ($p = 0.001$–0.021; Fig. 2; Table 2). This is in line with a recent study demonstrating that wildfires contributed to over 90 % of atmospheric naphthalene compared to baseline levels (Zhu et al., 2024). Irrespective of the fire combat type, the notorious contribution of fire emissions to PAHs exposure in this occupational context have been also demonstrated (Engelsman et al., 2020; Barros et al., 2023a). This study (Fig. 1B) presented 3- to 75-times higher ∑OHPAHs at baseline (60.3 ng/mg creatinine ~ 18.8 μmol/mol creatinine) and 1.7- to 23-times higher ∑OHPAHs after exposure (122 ng/mg creatinine ~ 44.3 μmol/mol creatinine) than other studies characterizing firefighters [0.25–6.96 μmol/mol creatinine; (Engelsman et al., 2020; Barros et al., 2023a)]. These findings indicate high exposure to PAHs even at fire stations. The current occupational limit for OHPAHs has only been established for 1OHPyr, *i.e.*, 2.5 μg/L, by the American Conference of Governmental Industrial Hygienists (ACGIH, 2019), which was not surpassed (maximum of 8.90×10^{-1} μg/L, Table S4). Smoking increased the baseline levels of ∑OHPAHs (3- to 4-fold, $p = 0.006$–0.01), 1OHNaph + 1OHAce (3- to 4-fold, $p = 0.006$–0.01), 2OHFlu (9- to 14-fold, $p = 0.002$–0.003), 1OHPyr (4- to 5-fold, $p = 0.002$–0.004), and 1OHPhe (2- to 3-fold, $p = 0.05$–0.09) when compared to non-smokers (Fig. 1B;

Table S4) in accordance with previous data (Helen et al., 2012; Wang et al., 2019; Barros et al., 2024). Naphthalene (272.16–11,100 ng/cigarette), fluorene (98.36–1900 ng/cigarette), phenanthrene (82.2–600 ng/cigarette), and pyrene (42–2800 ng/cigarette) are among the predominant PAHs in mainstream smoke (Adesina et al., 2022). Despite non-significant, smokers also present a median 81 % increase in post-exposure OHPAH levels in paired samples ($p > 0.05$), which contrasts with the 10-fold ($p < 0.001$) significant rise among exposed non-smoking firefighters (Fig. 2 and Table 2). Urinary 2OHFlu was the only metabolite that was significantly higher in exposed smokers than in exposed non-smokers (14-fold; $p < 0.001$), whereas only a borderline significant tendency was found for 1OHPyr (1.25-fold, $p = 0.069$) (Fig. 1B). On the other hand, 2OHFlu was significantly higher for all firefighters exposed ≥8 h in comparison to <8 h (3-fold, $p = 0.036$), which was no longer significant after stratifying by smoking status. These findings suggest a major source of exposure to fluorene in cigarette smoke, that mounts up with the contribution of fire emissions.

3.3.2. Metal(loid)s

The most concentrated (ng/mg creatinine) metal(loid)s in the urine of firefighters at baseline were rubidium (847–3580) > zinc (94.0–528) > lithium (6.17–425) > strontium (4.41–276) > arsenic (2.89–110) > molybdenum (6.16–92.7) > selenium (1.13–29.5) > cesium (3.25–14.6) > copper (2.85–12.8) > barium (0.11–6.59) > nickel (0.43–4.32) > lead (0.07–3.15) > thallium (0.06–1.45) > cobalt (0.04–0.81) > mercury (0.03–0.69) > cadmium (0.03–0.48) > antimony (0.02–0.09) (Figs. 1C and S1). After firefighting (Fig. 2, Table 2), in paired samples, for all firefighters, higher post- than pre-values ($p > 0.05$) were displayed for lithium (43 %) > zinc (19 %) > antimony (17 %) > lead (13 %). Cadmium, rubidium, nickel, cesium, cobalt, and thallium were not altered after exposure (maximum variations of +8 %), whereas the remaining elements decreased (16–35 %; significantly for molybdenum ($p = 0.036$)). Out of the 16 elements studied by Wolfe et al. (2004) only arsenic and cesium were significantly associated with wildfire combat activities. However, samples were collected 2.5 weeks after exposure, which limited the study findings given the short half-lives of some metal(loid)s. Post-exposure urinary metal(loid)s were 57–200 % higher in this study (Figs. 1C and S1) for arsenic and cesium, but lower or similar for other metal(loid)s (Wolfe et al., 2004). Baseline levels in previous studies (Biomonitoring California, 2016; Gündüzöz et al., 2018) reported 13–70 % lower levels of arsenic, similar concentrations of cadmium, and 20 % higher mercury levels. However, compared to a recent Czech firefighter cohort (Pálešová et al., 2024), Portuguese firefighters presented 2- to 5-fold higher concentrations of arsenic and cadmium, yet similar or lower values were found for mercury, and lead. The divergence among studies could also be related to the type of vegetation burnt (for wildfire exposure), and/or other sources of exposure such as lifestyle and diet (highly variable between cultures). Dietary data should be integrated with paired sampling to reduce within-subject variability. Acceptable values for the occupational group were not surpassed for cadmium (5 μg/g creatinine), cobalt (15 μg/L), lead and inorganic compounds (200 μg/L), and mercury (20 μg/g creatinine) (ACGIH, 2019). However, 30 % and 48 % of firefighters presented baseline and post-exposure levels, respectively, above ACGIH recommendation for urinary arsenic (35 μg/L; (ACGIH, 2019)). Arsenic can be found in water, food [especially seafood; however, the characterized firefighters are all from the inland northern region of Portugal, where seafood consumption is very low (Barros et al., 2024; Paiva et al., 2024a, 2024b)], as well as in paints, wood preservatives, herbicides, and fungicides (Kaur et al., 2024). Low to moderate arsenic exposure can also cause other health problems such as type 2 diabetes, neurological problems, hepatic and renal dysfunctions, skin conditions, reproductive complications, chronic bronchitis, and cardiovascular diseases (Kakavandi et al., 2023). Given the known carcinogenic risks of this metalloid, these findings also indicate a high risk of developing lung, bladder, and skin cancer for these firefighters (ATSDR, 2011; IARC, 2024).

Tobacco consumption was not significantly associated with elevated metal(loid)s at baseline. After exposure, in smokers, there was a raised ($p > 0.05$) excretion of lithium (186–210 %), cesium (27–31 %), and mercury (20–33 %) (Figs. 1C, S1, and 2; Table 2). Exposure to metal(loid)s can lead to their retention in the kidneys, exerting nephrotoxic effects that, can be aggravated by cigarette smoking (El-Safty et al., 2003; ATSDR, 2004; Jin et al., 2018). Therefore, cumulative tobacco and wildfire exposure may synergistically increase nephrotoxicity, raising clearance of lithium, cesium, and mercury among wildfire exposed smokers. Additionally, smokers with >8 h of wildfire exposure showed an increment of 18–500 % in urinary lithium, nickel, rubidium, and cesium ($0.004 < p < 0.041$). For exposed non-smokers, there was a substantial post-exposure augment for lithium (43–118 %) > nickel (30–82 %) > cadmium (53–73 %) > zinc (33–45 %) > cobalt (10–23 %) > rubidium (10–17 %) (Figs. 1C, S1 and 2). Contrasting, cesium, and mercury decreased 18 to 57 % after exposure in non-smokers, which may also indicate a higher body burden of mercury and cesium due to kidney retention in these subjects. Moreover, urinary cadmium significantly raised with the number of hours of exposure to fire emissions, *i.e.*, 5-times higher in non-smoking firefighters with >8 h of exposure ($p = 0.017$), evidencing firefighter exposure as a significant source of cadmium. Lastly, the years of service as a firefighter correlated positively with zinc, molybdenum, and cesium ($0.607 < r < 0.713$; $0.014 < p < 0.048$), suggesting bioaccumulation of these elements and higher body burden in non-smoking exposed firefighters with longer careers.

3.3.3. Correlations within exposure biomarkers

After exposure to wildfire combat activities, in general, OHPAHs and metal(loid)s correlated well, indicating a common route of exposure to these pollutants. In non-smokers, the following correlations were found: 1OHNaph + 1OHAce and ∑OHPAHs with selenium ($0.618 < r < 0.691$, $0.004 < p < 0.043$); 1OHPhe with nickel ($r = 0.827$, $p = 0.002$), molybdenum ($r = 0.818$, $p = 0.002$), and cesium ($r = 0.636$, $p = 0.035$); 2OHFlu with cobalt ($r = 0.636$, $p = 0.035$), copper ($r = 0.791$, $p = 0.004$), and antimony ($r = 0.718$, $p = 0.013$); 1OHPyr with cobalt ($r = 0.708$, $p = 0.015$), nickel ($r = 0.661$, $p = 0.027$), zinc ($r = 0.765$, $p = 0.006$), molybdenum ($r = 0.743$, $p = 0.009$), and antimony ($r = 0.691$, $p = 0.019$). Thus, wildland firefighting seems to be a common source of naphthalene, acenaphthene, phenanthrene, fluorene, pyrene, selenium, nickel, molybdenum, antimony, cesium, cobalt, copper, and zinc in non-smokers. At baseline for non-smokers, the correlations found between urinary individual OHPAH and metal(loid)s point out to a mutual source for 1OHPhe and cobalt ($r = 0.720$, $p = 0.019$) and nickel ($r = 0.760$, $p = 0.011$), and a different exposure origin for 1OHPyr and mercury ($r = -0.744$, $p = 0.014$). Sousa et al. (2024) identified the presence of particulate-bound cobalt and nickel at the garage in Portuguese fire stations, whereas mercury was not detected. The correlations herein detected suggest vehicle exhausts as a possible source of exposure at fire stations, except for mercury (*e.g.* fish consumption, use of fungicides, preservatives, antiseptics). This study also reveals the need for identifying the potential sources of exposure to pollutants at the baseline.

For smoking exposed firefighters, positive correlations were found between 2OHFlu and 1OHPyr with mercury ($r = 0.755$, $p = 0.005$; $r = 0.720$, $p = 0.008$, respectively). This information is also supported by additional associations at baseline for 2OHFlu with copper ($r = 0.609$, $p = 0.047$) and molybdenum ($r = 0.618$, $p = 0.043$) suggesting that there is a shared source of exposure to these pollutants due to tobacco consumption. These data are also reinforced by the up to 14-fold increase in 2OHFlu in smokers in comparison to non-smokers, emphasizing tobacco consumption as the primary source of fluorene. However, negative correlations were observed for 1OHNaph + 1OHAce and ∑OHPAHs with lead ($r = -0.664$, $p = 0.026$) and antimony ($r = -0.720$, $p = 0.008$) in wildfire exposed smokers. Regarding lead, it is possible that contamination by tobacco smoking could have influenced this negative association since in exposed smokers lead correlated negatively with lithium

(r = -0.685, p = 0.014), but positively with arsenic (r = 0.811, p = 0.001). Pinto et al. (2017) evaluated 20 best-selling cigarette brands in Portugal and showed that both lead and arsenic are transferred at a high rate (33–60 %) to cigarette smoke, which may help to explain the results obtained. This also highlights a higher impact of these metal(loids) through tobacco consumption than wildfire emissions. Concerning antimony, a survey among the general population revealed that smokers present higher urinary antimony than non-smokers (Richter et al., 2009), therefore, like lead and arsenic, tobacco consumption could be the predominant source.

3.4. Associations between urinary biomarkers

3.4.1. Correlations between effect and exposure biomarkers

The lung injury biomarker, CC16, correlated with copper (r = 0.764, p = 0.006), cadmium (r = 0.749, p = 0.008), and barium (r = 0.647, p = 0.031) among wildfire exposed non-smokers (Fig. 3). These results demonstrate that exposure to these elements during wildland firefighting may induce local airway inflammation in the lungs. A lung function decline of 15 % was also observed after fire exposure in wildland firefighters (Panumasvivat et al., 2024), supporting the importance of biomonitoring. For exposed smokers, correlations were found between CC16 and 1OHPyr (r = 0.755, p = 0.005), cesium (r = -0.615, p = 0.033), and cadmium (r = -0.608, p = 0.036) (Figs. 3 and S2). Given the bioaccumulation of cadmium and cesium in smokers due to tobacco consumption (ATSDR, 2004; Richter et al., 2009), there could be a reduced detoxification of these metals in the urine of smokers. The opposite correlations between CC16 and cadmium in wildfire exposed smokers *versus* exposed non-smokers may reflect differences in baseline lung damage and biomarker regulation. Chronic exposure to tobacco smoke may alter CC16 expression dynamics, leading to a divergent response compared to individuals without smoking-related lung stress (Lam et al., 2018). Moreover, the Wuhan-Zhuhai cohort study of large Chinese cities has reported an association between metal(loid)s and PAHs exposure with airway inflammation that, for metal(loid)s was stronger when plasma CC16 were lower showing the importance of this protein in the inflammatory response to these environmental pollutants (Zhou et al., 2018; Li et al., 2020). At baseline, CC16 correlated with exposure biomarker 1-OHPyr (r = 0.632, p = 0.05) in non-smoking firefighters, and it was positively associated with barium among smokers (r = 0.791, p = 0.004). Barium can be found in tobacco (1.15–188 μg/g; (NIPHE, 2015)), and once inhaled, barium can cause

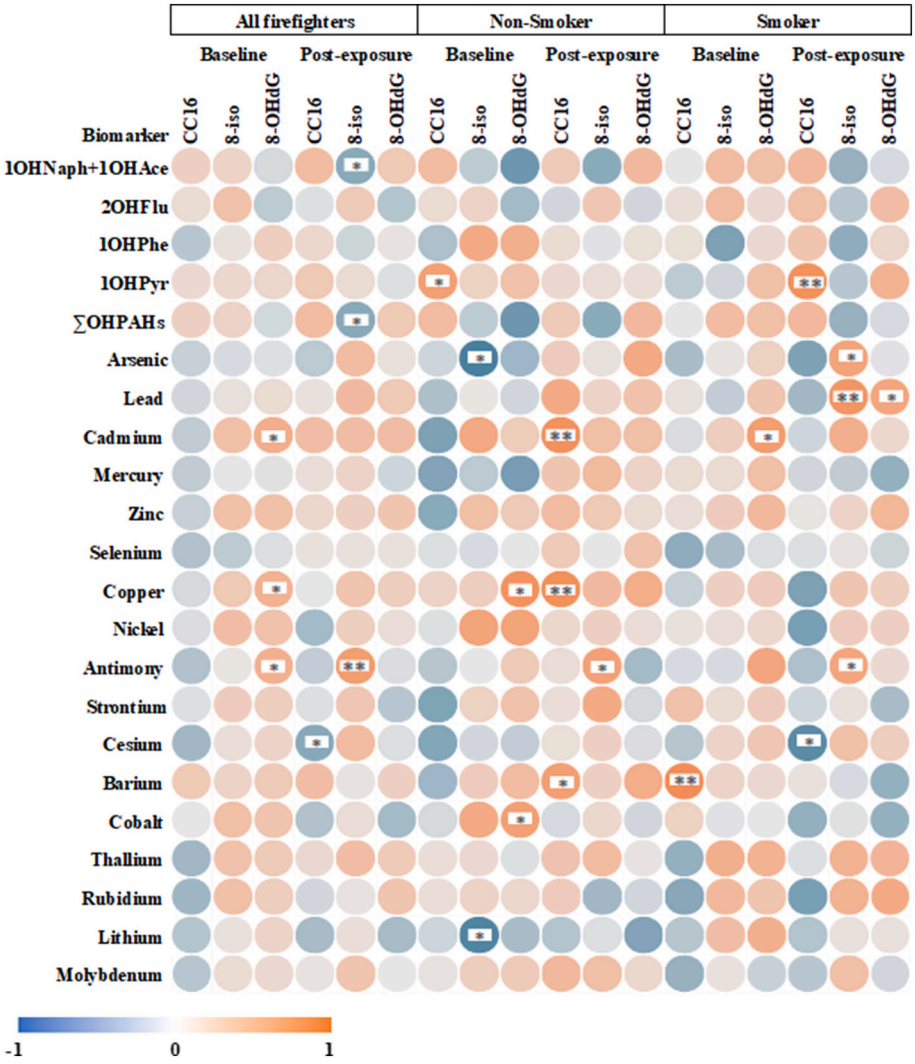

Fig. 3. Spearman's correlations between creatinine-adjusted urinary levels (ng/mg creatinine) of biomarkers of effect and biomarkers of exposure among Portuguese firefighters according with exposure and smoking status.
*Significant Spearman correlation ($p \leq 0.05$). **Significant Spearman correlation ($p < 0.01$).
1OHNaph + 1OHAce: 1-hydroxynaphthalene+1-hydroxyacenaphtene; 2OHFlu: 2-hydroxyfluorene; 1OHPhe: 1-hydroxyphenanthrene; 1OHPyr: 1-hydrpxypyrene; 8-OHdG: 8-hydroxydeoxyguanosine; 8-iso: 8-isoprostane; CC16: Clara cell 16; ΣOHPAHs: Sum of polycyclic aromatic hydrocarbons metabolites.

benign granulomatous pneumoconiosis, which is characteristic of inflammatory processes in the lung (Pappas, 2011), that could help to explain this correlation.

Lipid peroxidation, 8-iso, was positively associated with antimony ($r = 0.615$, $p = 0.044$), zinc ($r = 0.647$, $p = 0.031$), and cadmium ($r = 0.838$, $p = 0.001$) in wildfire exposed non-smokers (Figs. 3 and S2). The increase in lipid peroxidation biomarker with zinc was not expected since this essential metal has been known for its antioxidant benefits (Olechnowicz et al., 2018). However, wildland fire smoke pollutants can included other metal(loid)s like antimony and cadmium that may disrupt zinc's function, causing an oxidant/antioxidant imbalance. Moreover, cadmium exposure has been associated with intra-cellular zinc-depletion (Renu et al., 2022) whereas antimony has been suggested to interact with zinc finger domains (Lai et al., 2022). Therefore, simultaneous exposure to these elements may increase lipid peroxidation and zinc excretion, explaining the observed correlations in exposed non-smoking firefighters. Recently, blood cadmium has also been linked to an increased risk of metabolic syndrome in non-smoking male Korean firefighters and negatively associated with blood high density lipoproteins, further emphasizing the impact of occupational exposure to metal (loid)s on cardiometabolic profile (Choi et al., 2023; Pálešová et al., 2024).

Associations of lipid peroxidation with metal(loid)s were also determined at baseline. The urinary levels of 8-iso correlated with lithium (r =-0.661, $p = 0.038$), arsenic (r =-0.685, $p = 0.029$), cesium ($r = 0.673$, $p = 0.033$), and lead ($r = 0.709$, $p = 0.022$) in non-smokers (Figs. 3 and S2). Lithium has an important positive influence in antioxidant activity (Machado-Vieira, 2011) and its administration has been suggested as a therapeutic measure to reduce oxidative stress (Vošahlíková et al., 2021). Arsenic seems to have an indirect effect on 8-iso levels by reducing the activity of antioxidant proteins, rather than influencing 8-iso formation (Wang and Xu, 2006). These mechanisms of action could explain the negative associations between 8-iso with lithium and arsenic. Conversely, 8-iso has been recently highlighted as a mediator of cesium-linked pulmonary function decline in chronic obstructive pulmonary disease patients (Tang et al., 2023), whereas lead can both directly and indirectly affect the increase of lipid peroxidation products (Renu et al., 2022), supporting the positive correlations found here. For wildfire exposed smokers (Figs. 3 and S2), urinary 8-iso was positively associated with arsenic ($r = 0.601$, $p = 0.039$), antimony ($r = 0.587$, $p = 0.045$), lead ($r = 0.715$, $p = 0.009$) and copper ($r = 0.629$, $p = 0.028$). Arsenic and lead are among the metal(loid)s with the highest transfer from tobacco to cigarette smoke, and high levels of these chemicals were found in the lung tissue of smokers (Pinto et al., 2017); copper (up to 0.013 μg/g) and antimony (up to 1018 μg/g) can also be present in tobacco smoke (NIPHE, 2015). These metal(loid)s have also been linked to lipid peroxidation (Schaich, 1992), thus suggesting that cumulative exposure to cigarette smoke and wildland fire emissions may increase lipid peroxidation levels. Nevertheless, at baseline, 8-iso correlated negatively with 1OHNaph + 1OHAce/∑OHPAHs (r = -0.618, $p = 0.043$; Fig. S2) in smokers. PAHs are known for exerting their toxicity through oxidative stress (Zhu et al., 2021). However, this negative correlation may result from PAHs' limited effect on antioxidant activity, keeping urinary 8-iso levels stable or reduced, as seen in human lung fibroblasts exposed to benzo(*a*)pyrene from $PM_{2.5}$ (Rossner et al., 2019). In these cells, exposure (4–24 h) resulted in decreased isoprostanes. Thus, a possible reason for the reduced urinary 8-iso found in firefighters who smoke and are more exposed to PAHs released during tobacco consumption.

Concerning the biomarker of oxidative DNA damage in wildfire exposed non-smokers, 8-OHdG correlated with 1OHNaph + 1OHAce/∑OHPAHs ($r = 0.609$, $p = 0.047$), selenium ($r = 0.682$, $p = 0.021$), and rubidium ($r = 0.700$, $p = 0.016$) in (Figs. 3 and S2). In other studies with firefighters, 8-OHdG did not correlate with $PM_{2.5}$ exposure from prescribed burns (Adetona et al., 2013), but correlated with levoglucosan (wood smoke exposure) (Gaughan et al., 2014). DNA oxidation in the blood of wildland firefighters correlated with ∑OHPAHs in urine (Oliveira et al., 2020). Moreover, the associations between OHPAHs and 8-OHdG have also been reported for the general population (Zhu et al., 2021; Wu et al., 2022) and coke-oven workers (Kuang et al., 2013; Deng et al., 2014), thus corroborating the obtained correlations found herein. Regarding selenium, its use as a supplement has been highlighted by Bera et al. (2013) for reducing cancer risks probably by activating DNA repair mechanisms, thus increasing 8-OHdG excision and consequent excretion. As for rubidium, there is a lack of information, yet Busby (Busby, 2013) has proposed a mechanism *via* membrane affinity that may lead to an augmented risk of damage to DNA due to internal rubidium. Moreover, rubidium chloride has been used in science to induce cells to replicate DNA (Chemistry Dictionary, 2023), thus increasing DNA repair activity, which may support its positive correlation with 8-OHdG after wildfire exposure. In non-smokers at baseline, 8-OHdG was correlated with copper ($r = 0.745$, $p = 0.013$), cobalt ($r = 0.632$, $p = 0.05$), strontium (r = -0.709, $p = 0.022$), and cadmium (r = -0.636, =0.048) (Figs. 3 and S2). Copper and cobalt's lower redox activity can lead to reactive oxygen species formation that could be DNA-damaging (Angelé-Martínez et al., 2014, 2023), whereas the negative correlations of 8-OHdG with strontium could be related with its ability to mimic calcium within the body (Marx et al., 2020), thus, less likely to exert its toxicity through DNA oxidation. On the other hand, cadmium DNA toxicity was showed to be dose-dependent in a study performed in human lymphoblastoid cells, leading to formation of DNA strand breaks and 8-OHdG even at low cadmium concentrations (Mikhailova, 1997), thus a possible explanation for the obtained negative correlations. For wildfire exposed smokers, 8-OHdG correlated positively with lead ($r = 0.578$, $p = 0.049$). Lead bioaccumulation from tobacco and firefighting exposure may additively increase its genotoxicity in firefighters (Jaishankar et al., 2014; Hemmaphan and Bordeerat, 2022). For smokers, there were additional correlations at baseline for 8-OHdG with cadmium ($r = 0.661$, $p = 0.027$), copper ($r = 0.691$, $p = 0.019$), strontium ($r = 0.782$, $p = 0.004$), cesium ($r = 0.755$, $p = 0.007$), barium ($r = 0.682$, $p = 0.021$), and thallium ($r = 0.691$, $p = 0.019$) (Figs. 3 and S2). These positive associations could be related to chronic exposure to the mixture of these elements from tobacco consumption (NIPHE, 2015). Interactions could occur by either synergism/antagonism on the biological concentrations of barium, cadmium, cesium, copper, and thallium exerting genotoxic effects that promoted elevated urinary excretion of 8-OHdG (ATSDR, 2004; Genter, 2012; Angelé-Martínez et al., 2014; NIPHE, 2015; Drago et al., 2018). For strontium, a synergetic impact on the concentrations of the other metals (*i.e.*, cadmium) leading to their bioaccumulation in smokers (Bernhard et al., 2006) or strontium complexation (Jaishankar et al., 2014) could explain its positive correlation with 8-OHdG only in this group. Mixed exposure in metal carpentry workers exposed to welding fumes resulted in a correlation of barium, mercury, lead, and strontium with 8-oxo-7,8-dihydroguanosine [8-oxodG, oxidized form of 8-OHdG; (Buonaurio et al., 2021)], thus corroborating the associations of 8-OHdG with a mixed metal exposure during cigarette smoking in firefighters.

3.4.2. PCA analysis

A first PCA was conducted using the creatinine-adjusted relative differences in paired samples (post-exposure to baseline) according to smoking status based on the urinary levels of CC16, 8-OHdG, 1OHNaph + 1OHAce, ΣOHPAHs, 1OHPyr, and copper (Fig. 4A). This PCA is expressed with two functions, F1 and F2, representing 74.86 % of original data with a Kaiser-Meyer-Olkin measure of sampling adequacy (KMO) of 0.663. A positive association was found between post-exposure to baseline relative differences of urinary CC16, 8-OHdG, OHPAHs, and copper, especially in non-smoker firefighters due to the effective group separation by F1 (~67 % of non-smoker pairs gathered on the F1-positive quadrants). Supporting these data, two additional PCAs were performed for relative post-exposure to baseline differences in the effect biomarkers separately, namely, for urinary CC16 combined

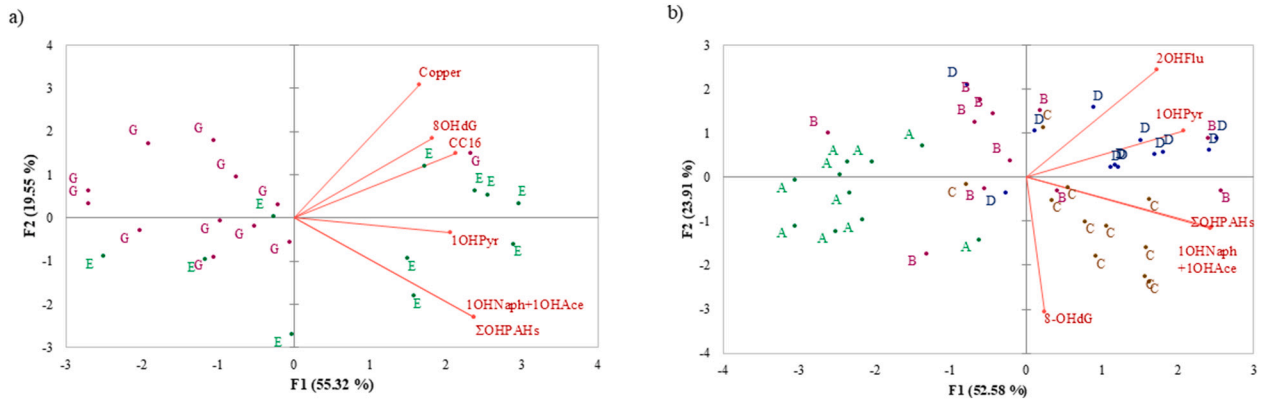

Fig. 4. Principal component analysis (PCA) plot based on: a) the relative differences of creatinine-adjusted urinary levels of effect and exposure biomarkers in paired samples: E: non-smoker pairs; G: smoker pairs. PCA: Clara cell 16 (CC16), 8-hydroxydeoxyguanosine (8-OHdG), and polycyclic aromatic hydrocarbons metabolites (OHPAHs, *i.e.*, 1OHNaph + 1OHAce, 1OHPyr, ΣOHPAHs), and copper; b) the creatinine-adjusted urinary levels of biomarkers of effect and exposure to PAHs according to exposure and smoking status subgroups: A: non-smoker at baseline; B: smoker at baseline; C: non-smoker at post-exposure; D: smoker at post-exposure. PCA: 8-OHdG and OHPAHs (*i.e.*, 1OHNaph + 1OHAce, 1OHPyr, 2OHFlu, and ΣOHPAHs).
1OHNaph + 1OHAce: 1-hydroxynaphthalene+1-hydroxyacenaphtene; 2OHFlu: 2-hydroxyfluorene; 1OHPyr: 1-hydrpxypyrene; ΣOHPAHs: Sum of polycyclic aromatic hydrocarbons metabolites.

with barium, cadmium, cobalt, copper, nickel, and strontium (F1 and F2: 73.26 % of original data; KMO: 0.719; Fig. S3A), and for urinary 8-OHdG as well as antimony, barium, cadmium, cobalt, copper, lead, nickel, strontium, and zinc (F1 and F2: 61.25 % of original data; KMO: 0.671; Fig. S3B). The observation of an agglomeration of non-smokers (2/3 of samples) on the positive F1 and F2 quadrant of Fig. S3A along with a high correlation between relative differences of CC16, copper, and cadmium, support the findings of the Spearman's correlation matrix in exposed non-smokers (Fig. 3). Moreover, it is possible to observe a higher correlation between post-exposure to baseline relative differences of 8-OHdG, antimony, cadmium, copper, lead, and zinc (positive F1 and F2 quadrant of Fig. S3B), thus associating 8-OHdG with genotoxic metals, especially lead (shortest angle between these variables), therefore in agreement with the Spearman's correlation matrix (Figs. 3 and S2). Moreover, the analysis of individual creatinine-adjusted concentrations yielded similar results. Urinary DNA oxidation levels (Fig. 4B) were associated with 1OHNaph + 1OHAce and ΣOHPAHs in a PCA model representing 76.48 % of raw data (KMO: 0.574). For exposed non-smokers, 82 % of observations gathered on the negative-F2 and positive-F1, contrasting with a greater agglomeration of exposed smokers on the positive F1 and F2 quadrant nearer to 2OHFlu and 1OHPyr, which were more associated with cumulative exposure to both wildfire combat and tobacco consumption (Fig. 4B). Similar results were obtained for individual concentrations of CC16 (Fig. S3C); lung injury was also associated with all individual OHPAHs, 1OHNaph + 1OHAce, and ΣOHPAHs (F1 and F2 accounting for 69.74 %; KMO: 0.629). Together (Figs. 4 and S3), these findings point out that exposure to wildland fire combat emissions induces lung injury and DNA oxidation in firefighters and that urinary CC16 is the biomarker with the greatest potential and reliability to be applied in future biomonitoring campaigns in this occupational context.

PCA results based on relative differences of paired samples that included 8-iso with both OHPAHs and metal(loid)s (and separately) did not meet the predefined criteria, nor showed a good separation of sample groups. Nevertheless, when analyzing unpaired concentrations (Fig. S3D), non-smokers at baseline are clearly separated from the exposed groups (non-smoker and smoker), corroborating the associations of 8-iso and exposure to wildland firefighting. However, this is more relevant within the group of exposed smokers with OHPAHs such as 2OHFlu and 1OHPyr (more associated with tobacco consumption) in the same quadrant as 8-iso and 92 % of observations. These results corroborate the combined impact of participation in wildland firefighting and tobacco consumption on lipid peroxidation. Collectively, these findings also highlight the negative effects of tobacco consumption and increased susceptibility of smokers to lipid peroxidation induced by exposure to wildfire combat activities.

This study provided valuable insights into both exposure and effect biomarkers in the context of wildfire combat. Nevertheless, the small sample size is a limitation of this study, as it reduces the statistical power and, consequently, the generalizability of the findings to broader firefighter populations. Furthermore, while each firefighter served as their own control through pre- and post-exposure comparison, the absence of an external reference group limits the ability to fully isolate the effects of wildfire exposure from other environmental or lifestyle factors (such as age, sex, BMI, cigarette smoking use intensity, years of service, diet, among others). Future studies should consider including a reference group to strengthen causal inferences and analyze the impacts of population characteristics on these biomarkers to improve generalizability.

4. Conclusions

This study is the first attempt to simultaneously characterize the impacts of wildland firefighting in urinary CC16, 8-iso, and 8-OHdG while exploring correlations with exposure biomarkers of PAHs and metal(loid)s. CC16 revealed to be the most powerful biomarker for characterizing the health effects of wildland firefighting, also correlating with post- to pre-exposure relative concentration differences of urinary 8-iso and 8-OHdG. Altogether, the results indicate an important contribution of wildland firefighting to lung injury, DNA oxidation, and lipid peroxidation, as well as an higher exposure to OHPAHs and several metal(loid)s (lithium, zinc, antimony, and lead). None of the available occupational limits for exposure biomarkers was surpassed, except for arsenic. Wildland firefighting impacts on both exposure and effect biomarkers were more evident among non-smoking firefighters. After exposure, in non-smokers, there were higher post- to pre-exposure differences in urinary levels of ∑OHPAHs, metals (lithium, nickel, cadmium, zinc, cobalt, and rubidium), CC16, 8-iso, and 8-OHdG. Moreover, in non-smoking wildfire-exposed subjects, positive associations of individual OHPAHs and/or ∑OHPAHs with all three effect biomarkers were detected, as well as some metal(loid)s positively correlated with CC16 (copper, cadmium and barium), 8-iso (antimony and cadmium), and 8-OHdG (antimony, cadmium, copper, lead, zinc, selenium, rubidium). For smoking firefighters after exposure to wildfires, an increased excretion of 2-OHFlu, lithium, cesium, and mercury were observed. Moreover, metal(loid)s-induced lipid peroxidation (by arsenic, antimony, lead, copper) and DNA oxidation (by lead) were more evident

among wildfire exposed smokers.

Regarding the baseline values, positive correlations were found between CC16 and 1-OHPyr; among 8-iso and cesium, and lead; and between 8-OHdG with copper and cobalt. Additionally, cigarette smoking (in non-exposed subjects) increased the levels of individual OHPAHs (2-OHFlu > 1-OHPyr >1OHNaph + 1OHAce/∑OHPAHs > 1-OHPhe), but metal(loids) were not significantly affected. Tobacco consumption was positively associated with lung injury induced by barium and with DNA oxidation caused by cadmium, copper, strontium, cesium, barium, and thallium at baseline.

Further research should include a higher number of individuals to understand if the patterns reported here are also observable in larger groups. Moreover, considering the different exposure routes during fire suppression, knowledge gaps concerning the half-lives of exposure biomarkers for inhalation and dermal contact present a significant challenge in determining optimal sampling times, especially when multiple biomarkers are being assessed. Given the carcinogenic classification of benzo(*a*)pyrene, nickel, arsenic, cadmium, lead, mercury, and their compounds by IARC, the establishment of a well-defined panel of biomarkers (effect and exposure), preferentially measured in non-invasive matrices, is essential to comprehensively characterize and improve risk assessment.

CRediT authorship contribution statement

Bela Barros: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ana Margarida Paiva:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rui Azevedo:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sara Alves:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Filipa Esteves:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Adília Fernandes:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Josiana Vaz:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Maria José Alves:** Writing – review & editing, Software, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Klara Slezakova:** Writing – review & editing, Validation, Formal analysis, Data curation, Conceptualization. **João Paulo Teixeira:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Solange Costa:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Agostinho Almeida:** Writing – review & editing, Validation, Methodology, Investigation, Data curation, Conceptualization. **Marta Oliveira:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Simone Morais:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Ethics approval

This work received approval for research ethics by the Accredited Ethics Committee of the University of Porto, Portugal, Report Nr. 92/CEUP/2020, under the project BioFirEx project (PCIF/SSO/0017/2018): “A panel of (bio)markers for the surveillance of firefighter’s health and safety”.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180012.

Data availability

The authors do not have permission to share data.

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