

Biomonitoring of firefighters occupational exposure to polycyclic aromatic hydrocarbons during the 2014 hot season

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INTRODUCTION

Human biomonitoring is an important tool in environmental medicine that is used to assess the level of internal exposure to environmental pollutants. Firefighters are one of the most exposed and least studied occupations. During fire suppression, firefighters are heavily exposed to a wide range of chemicals.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that are considered as the largest known group of carcinogens due to their cytotoxic and mutagenic properties (IARC 2010, WHO 2013). Smoke and ashes released during a fire are important sources of PAHs. Firefighters can be also exposed to PAHs through smoking, via polluted ambient air, water, soil, and through consumption of food.

Metabolites of PAHs (OH-PAHs), such as 1-hydroxynaphthalene (1OHNapt), 1-hydroxyacenaphthene(1OHAce), 1-hydroxypyrene (1OHPy) and 3-hydroxybenzo[a]pyrene (3OHB[a]P) have been used as biological markers for measurements of human internal exposure to PAHs.

The present work aims to quantify the urinary metabolites of PAHs, namely 1OHNapt, 1OHAce, 1OHPy and 3OHB[a]P in study population of firefighters. Firemen exposed to fires that occurred during 2014 season were asked to fill a post-fire questionnaire and to collect urinary samples. A control study population group was selected to collect samples of urines during the pre-fires season (winter). Among all participating firemen only healthy no-smoking subjects were considered. OH-PAHs were analysed by high-performance liquid chromatography with fluorescence detection.

EXPERIMENTAL

Urine samples were collected from twenty wildland firefighters located in Bragança (North of Portugal). Urine sampling was performed in two different phases: first during a pre-fire season with no participation at any fire fighting activity within the last week, and the second one during the fire season and with active participation at the fire. Firefighters were asked to fill a structured questionnaire in order to identify other potential exposure routes to PAHs. After collection, urine samples were taken to the laboratory and were immediately frozen at -20 °C until analysis.

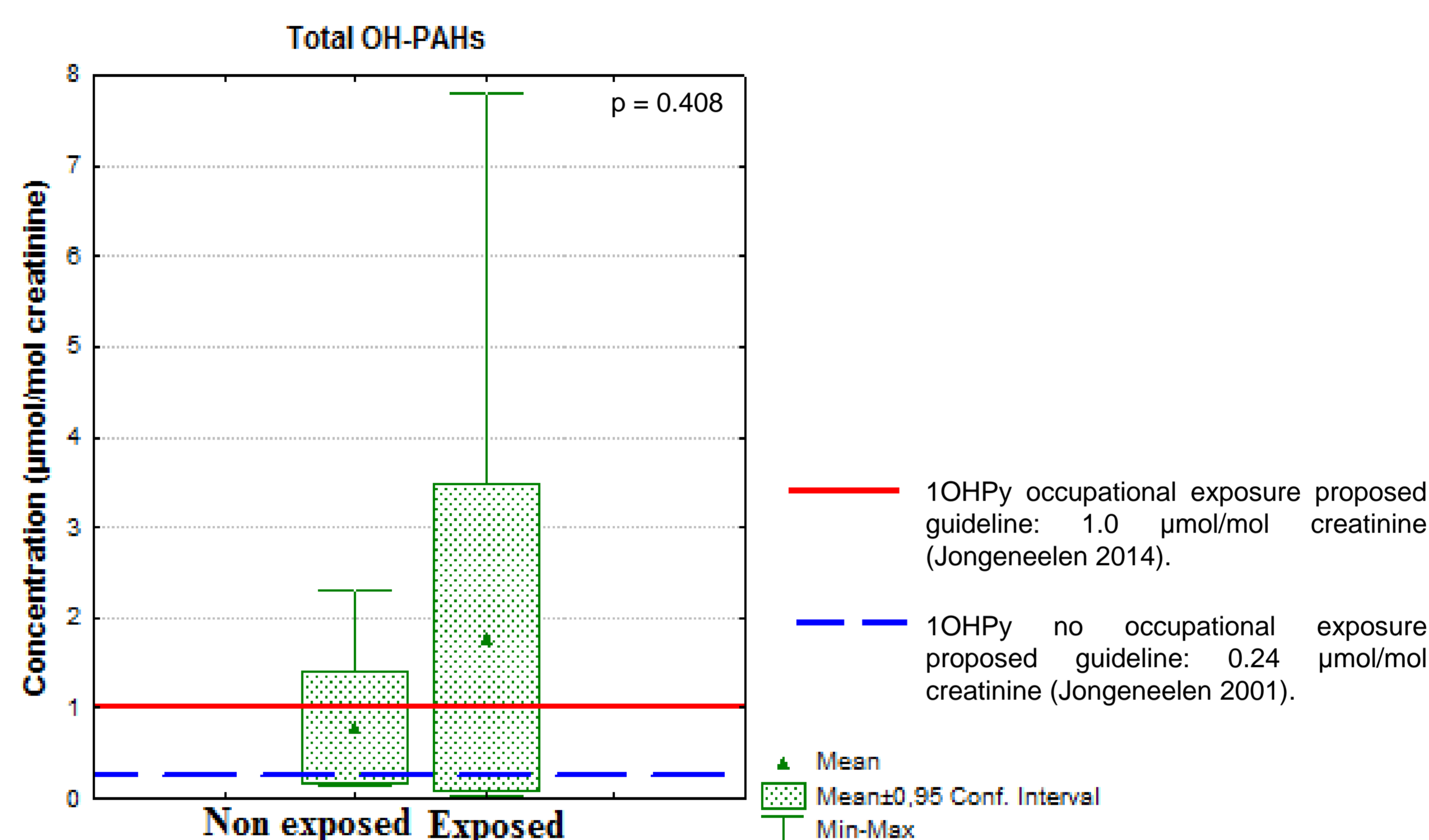
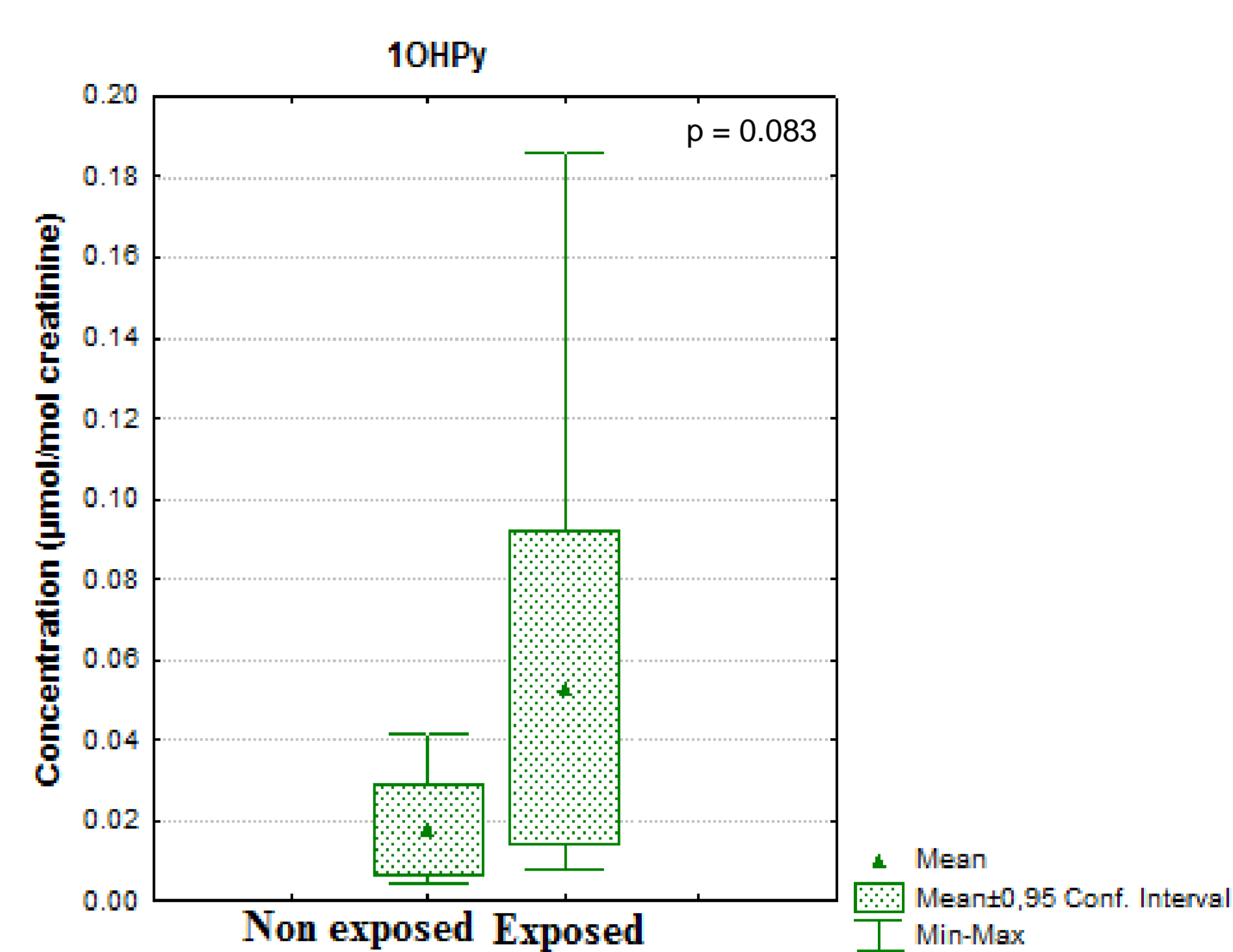
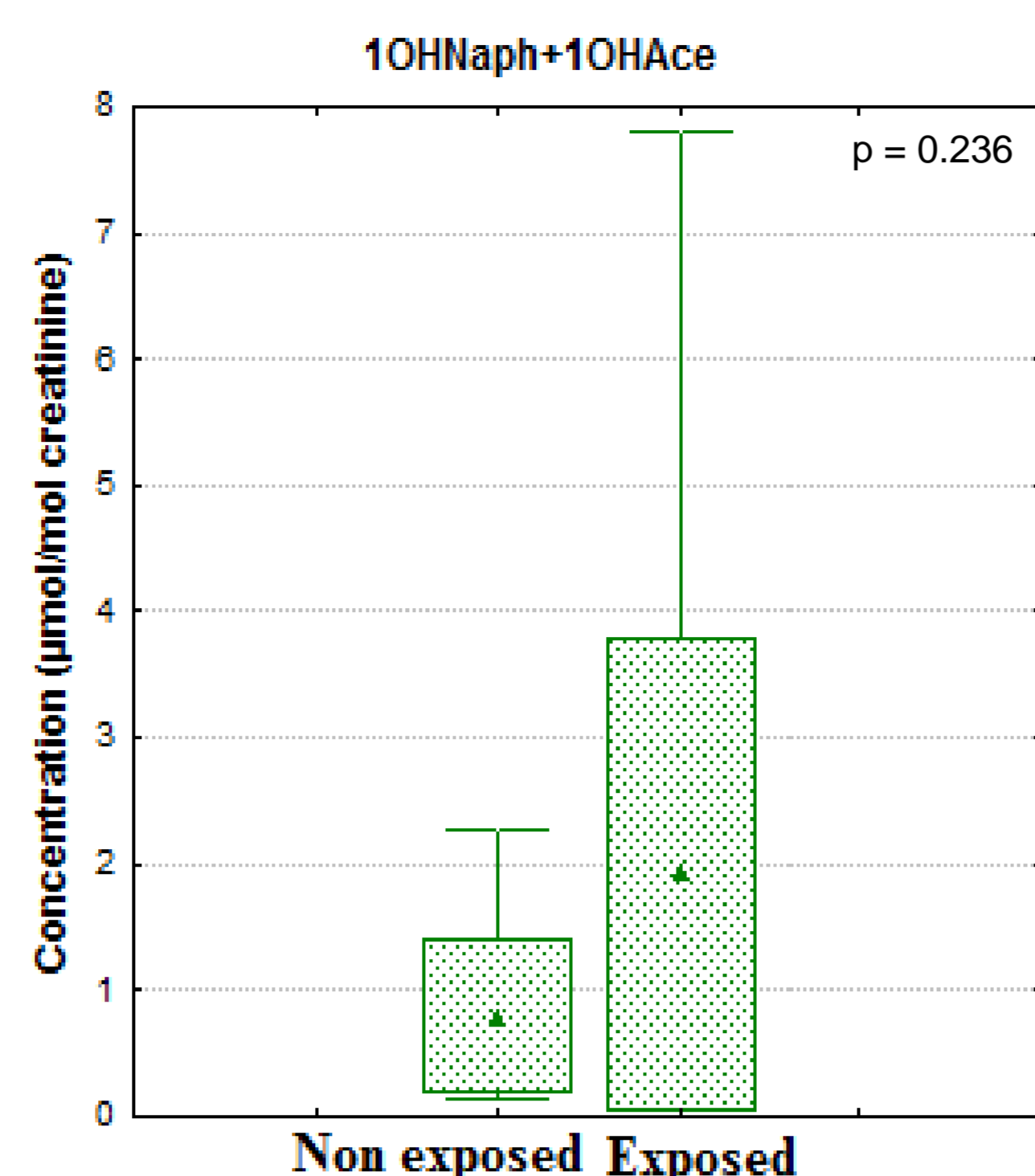
Briefly, urine samples were adjusted to pH 5.0 with HCl and then buffered with acetate buffer at pH 5.0. An aliquot of tert-butylhydroquinone and β-D-glucuronidase/arylsulfatase were added, being the mixture purged under nitrogen flow. Extracts were incubated with a constant stirring at 37 °C for 2 hours in the absence of light. The enzymatic reaction was stopped with the elution of urine extracts through solid-phase extraction (SPE) C18 cartridges that were preconditioned with water and methanol. After elution of the urine samples, C18 cartridges were sequentially washed with water and methanol and eluted with methanol and ethyl acetate. Extracts were evaporated to dryness and redissolved in methanol.

Urine samples were analysed by high-performance liquid chromatography with fluorescence detection (HPLC-FLD). Separation of the compounds was performed in a C18 column (Macherey-Nagel, Germany) at room temperature. Each compound was detected at its optimum excitation/emission wavelength pair: 232/337 nm for 1-OHNapt and 1-OHAce, 265/335 nm for 2-OHFlu, 263/363 nm for 1-OHPhe, 242/388 nm for 1-OHPy and 308/432 nm for 3-OHB[a]P. Each analysis was performed at least in triplicate.

The concentration of urinary creatinine was determined according to the Jaffe colorimetric method (WHO 2015).

Statistical analysis was performed using the SPSS (IBM SPSS Statistics 20) and Statistica software (v. 7, StatSoft Inc., USA). Mean values were compared through the nonparametric Mann-Whitney U test, since normal distribution was not observed by Shapiro-Wilk's test. Statistical significance was defined as $p < 0.05$.

RESULTS



- Globally mean urinary 1OHNaph+1OHAce and 1OHPy levels were slightly higher in exposed (1.92 ± 2.44 µmol/mol creatinine; 0.05 ± 0.05 µmol/mol creatinine) than in non exposed firefighters (0.78 ± 0.73 µmol/mol creatinine; 0.02 ± 0.01 µmol/mol creatinine), respectively. A great variability was found within the two groups.
- 3OHBaP was not detected in the firefighters urine. Some studies described that metabolites with five or more aromatic rings are mainly excreted in feces via bile rather than in urine (Likhachev et al. 1992; Toriba and Hayakawa 2007).
- Total OH-PAH concentrations ranged between 0.15 to 2.30 µmol/mol creatinine for non exposed and from 0.02 to 7.80 µmol/mol creatinine for those firefighters who participated in fire fighting activities.
- Overall, 1OHNapt and 1OHAce were the most abundant OH-PAHs in firemen urine samples, accounting with more than 90% for total OH-PAHs.
- No international guidelines are established for any OH-PAH metabolite. However Jongeneelen (2001) proposed a no occupational exposure limit for urinary 1OHPy (0.24 µmol/mol creatinine) and more recently the author recommended a guideline of 1.0 µmol/mol creatinine for occupational exposure (Jongeneelen 2014).
- Regarding 1OHPy it was observed that among the evaluated Portuguese firefighters those proposed limits were not exceeded.
- When the total OH-PAHs are considered it was found that firefighters not exposed to fires presented mean concentrations below the no occupational limit, still some firefighters exceeded that level, reaching a maximum of 2.30 µmol/mol creatinine.
- Concerning firefighters actively exposed to fires, mean concentrations of total OH-PAHs surpassed the occupational exposure guideline proposed by Jongeneelen (2014) for 1OHPy.

CONCLUSIONS

- For future work a higher number of both non exposed and exposed wildland firefighters should be considered.
- The quantification of other urinary OH-PAHs should be used in order to better characterize the firefighters occupational exposure.

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