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Associations between urinary biomarkers of oxidative stress and biomarkers of tobacco smoke exposure in smokers

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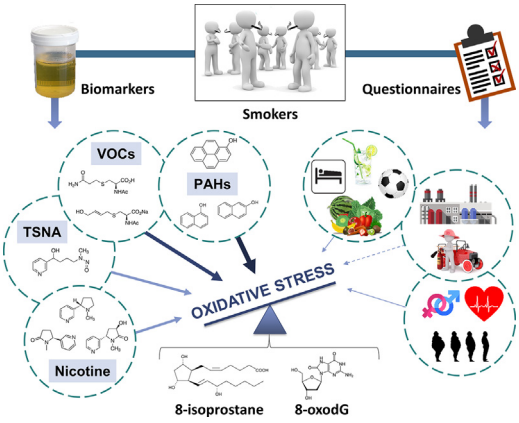
HIGHLIGHTS

- Oxidative stress biomarkers were associated with environmental pollutant biomarkers.
- 14 exposure biomarkers for environmental pollutants were quantified in 270 smokers.
- 6 urinary biomarkers of tobacco smoke exposure were quantified in 270 smokers.
- 8-OxodG concentrations were associated with PAH, VOC, and nicotine exposures.
- 8-Isoprostane were associated with PAH, VOC, nicotine, and nitrosamine exposures.

GRAPHICAL ABSTRACT



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ABSTRACT

Oxidative stress can contribute to the development of diseases, and may originate from exposures to toxicants commonly found in air pollution and cigarette smoke such as polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs). Yet, associations between these exposures and oxidative stress biomarkers are poorly characterized. We report here novel associations between 14 exposure biomarkers of PAHs and VOCs, and two oxidative stress biomarkers; 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-isoprostaglandin $F_{2\alpha}$ (8-isoprostane) in urine obtained from smokers participating in an ongoing clinical study (ESTxENDS, NCT03589989). We also assessed associations between six biomarkers of tobacco smoke exposure (metabolites of nicotine and tobacco-specific nitrosamines (TSNAs)) and both oxidative stress biomarkers. We then quantified the relative importance of each family of the 20 exposure biomarkers on oxidative stress. Participating smokers (153 men and 117 women, median age 44 years) had on average smoked 25 [2–62] years and smoked about 17 [5–40] cigarettes per day at the time of the study.

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Multiple linear regression results showed an association between 8-oxodG concentrations and the following metabolites in decreasing relative importance: PAHs (beta coefficient $\beta = 0.105$, p-value <0.001, partial $R^2 = 0.15$) > VOCs ($\beta = 0.028$, $p < 0.001$, partial $R^2 = 0.09$) > nicotine ($\beta = 0.226$, $p < 0.001$, partial $R^2 = 0.08$); and between 8-isoprostane concentrations and metabolites of PAHs ($\beta = 0.117$, $p < 0.001$, partial $R^2 = 0.14$) > VOCs ($\beta = 0.040$, $p < 0.001$, partial $R^2 = 0.14$) > TSNAs ($\beta = 0.202$, $p = 0.003$, partial $R^2 = 0.09$) > nicotine ($\beta = 0.266$, $p < 0.001$, partial $R^2 = 0.08$). Behavioral factors known to contribute to oxidative stress, including sleep quality, physical activity, and alcohol consumption, did not play a significant role. Exposures to PAHs and VOCs among smokers were significantly associated with oxidative stress.

1. Introduction

Excessive oxidative stress is associated with the onset of several diseases including cancers, inflammatory diseases, cardiovascular diseases, and diabetes (Sharifi-Rad et al., 2020). Environmental factors (e.g., air pollution) and behavioral factors (e.g., smoking) influence oxidative stress levels (Dröge, 2002). Human biomonitoring of oxidative stress can be assessed by measuring two well-studied biomarkers of oxidative stress: 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a marker of DNA damage, and 8-isoprostaglandin $F_{2\alpha}$ (8-isoprostane), a marker of lipoperoxidation (Cooke et al., 2000; Milne et al., 2005). Environmental exposures can be assessed using exposure biomarkers measured as urinary concentrations of the parent compound and their metabolites. Polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs) that may originate from air pollution, indoor air or habits such as smoking are known to produce oxidative stress (Weinstein et al., 2017). Associations between exposure to these chemicals and effect biomarkers of oxidative stress and their relative importance have not been elucidated. These associations can give insight into mechanisms of the development of diseases, and an understanding of exposures that produce oxidative stress. However, it is not simple to find a large number of individuals exposed simultaneously to high concentrations of PAHs and VOCs from environmental sources, not to mention resources needed to establish such cohorts. An alternative is to study the relationships between PAH and VOC exposures and oxidative stress in smokers exposed to tobacco smoke that contain both of these chemicals.

In a cohort of smokers, study participants are exposed to a known source (cigarettes) on a daily basis, making it easier to characterize the frequency, intensity and duration of exposure of these substances. Another advantage is that the exposure range is large (large variability in internal doses of PAHs and VOCs) depending on the number of cigarettes smoked per day. In addition to PAHs and VOCs, smokers are exposed to other carcinogens, such as tobacco-specific nitrosamines (TSNAs). A cohort of smokers can also give insight in the association between smoking, quantified as the internal dose of total nicotine equivalent (TNE), and reactive oxygen species (ROS) as this has been reported inconsistently (Church and Pryor, 1985).

PAHs are a family of fused aromatic rings generated during incomplete combustion of organic matter or pyrolysis processes (Lawal, 2017), and are commonly found in tobacco smoke (Vu et al., 2015). Occupational exposures can occur among others in the metal industry, in construction (e.g. roads), among professional drivers, chimney sweeps and firemen (Petit et al., 2019; Unwin et al., 2006; Stec et al., 2018). The general population is also exposed to PAHs through diet (grilled or smoked meat and fish) (Yebra-Pimentel et al., 2015). Biomonitoring of PAH exposures are assessed by measuring urinary concentrations of the respective hydroxylated PAHs (e.g., 1-hydroxypyrene (1-OHP), 1-naphthol and 2-naphthol, hydroxyfluorene, hydroxyphenanthrene). 1-OHP is a pyrene metabolite often used in occupational exposure studies. 1- and 2-naphthol are two metabolites of naphthalene, of which correlations between 2-naphthol and cotinine have previously been observed (Hecht, 2002).

VOCs are commonly found in tobacco smoke and are a mixture of chemical groups, including aldehydes, aliphatic hydrocarbons, amides, and epoxides. Occupational exposure to VOCs are found among others in nail stylists, printing workers, cleaning agents, and truck drivers (Lamplugh et al., 2019; Leung et al., 2005; Lin et al., 2021; Davis et al., 2007). Non-occupational sources of human exposure to VOCs include combustion processes, tobacco smoke, organic solvents, personal care products, cleaning agents, paints, adhesives, glues, wood preservatives, and air fresheners. Exposure to VOCs can be assessed using biomonitoring of urinary concentrations of mercapturic acids (Alwis et al., 2012). The mercapturic acid pathway is a major route for the biotransformation of electrophilic compounds and involves glutathione that plays a role in the regulation of oxidative stress (Hanna and Anders, 2019).

TSNAs are formed during the curing and processing of tobacco by the nitrosation of nicotine into N-nitrosonornicotine (NNN), nornicotine into nicotine-derived nitrosamine ketone (NNK), anatabine into N-nitrosoanatabine (NAT), and anabasine into N-nitrosoanabasine (NAB). Biomonitoring can be performed by measuring urinary concentration of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a metabolite of NNK. There are no other sources than tobacco for TSNAs, making NNAL ideal for discriminating smokers from non-smokers.

Several studies have reported a significant dose-response relationship between urinary PAH or VOC metabolites and 8-oxodG or 8-isoprostane (Kuang et al., 2013; Kho et al., 2015; Hong et al., 2009; Sun et al., 2017; Wang et al., 2020). In a large cohort study of residents exposed to air pollution, Cao et al. reported associations between the sum of 12 urinary PAH metabolites (ΣOH-PAHs) and two oxidative stress biomarkers (8-oxodG and 8-isoprostane) and concluded that oxidative stress might be involved in the reduction of the lung function observed (Cao et al., 2020). Similar relationships between VOC exposures and oxidative stress have previously been reported. Significant associations between 8-oxodG and 14 VOC metabolites in urine from asthmatic and healthy children exposed to air pollution was reported by Kuang et al. (2013), and between 8-oxodG and three VOC metabolites in urine from 154 elderly people by Yoon et al. (2010). Only one study (Weinstein et al., 2017) have analyzed simultaneously four PAH metabolites, eight VOC metabolites, and oxidative stress biomarkers (8-oxodG and 8-isoprotane) in urine samples from a small group of recently pregnant rural Guatemalan women who used woodstoves for cooking and heating. However, no dose-response relationships could be demonstrated. This might be related to the omission of individual and behavioral factors known to influence oxidative stress in their analysis. Moreover, none of the above studies investigated the relative importance of exposure biomarkers and other individual or behavioral factors on oxidative stress levels.

Typical individual and behavioral factors that influence oxidative stress biomarkers include age, gender, body mass index (BMI), health status, sleep quality, physical activity, alcohol consumption, fruit and vegetable consumption, place of residence, and occupations (Sharifi-Rad et al., 2020; Dröge, 2002). An increase of oxidative stress (8-isoprostane) has been associated with sleep disorders (e.g., obstructive sleep apnea), working night shifts, and poor sleep quality (e.g., sleep duration). Prolonged or high-intensity physical activity (i.e., 65 %–75 % maximal oxygen consumption (VO_{2max})) increases ROS production and may cause oxidative damage to skeletal muscles, while regular physical activity may be beneficial to protect the body from oxidative damages (Powers et al., 2020; Simioni et al., 2018). Elevated concentrations of 8-isoprostane after acute exercise were observed in blood, but not in urine (Mastaloudis et al., 2001; Nikolaidis et al., 2011). Urinary 8-oxodG was shown not to be sufficiently sensitive in detecting exercise-induced DNA oxidation (Karpouzi et al., 2016).

Alcohol consumption can increase oxidative stress because ethanol metabolism induce the formation of ROS (Albano, 2006; Galicia-Moreno and Gutiérrez-Reyes, 2014). Both 8-oxodG and 8-isoprostane have been associated with alcohol consumption (Irie et al., 2005; Sakano et al., 2009). Sleep disorders, strenuous physical activity, and alcohol consumption are factors that can modulate the associations between exposure biomarkers and oxidative stress biomarkers and therefore their influence need to be assessed.

Elevated concentrations of oxidative stress biomarkers among smokers are not consistent across studies. These equivocal results are probably related to the possibility that each of the many toxic compounds found in tobacco smoke can induce ROS formation. Determining which ones are associated with the formation of oxidative stress biomarkers would provide a better understanding of the underlying mechanisms. Furthermore, the usefulness of these biomarkers to elucidate relationships between smoking and diseases for which smoking is a main risk factor could be discussed.

Our objectives were to assess the associations between exposure biomarkers and oxidative stress biomarkers, and to evaluate simultaneously the effects of lifestyle related factors and their relative contribution to the urinary concentration of oxidative stress biomarkers.

2. Methods

2.1. Study population

Participants were selected from an on-going randomized controlled trial on smoking cessation: "Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: the ESTxENDS multicenter randomized controlled trial" (ClinicalTrials.gov Identifiers: NCT03589989) approved by the ethics committees of Bern, Geneva, and Lausanne (Project-ID: 2017–02332), Switzerland. The study was conducted in accordance with the Swiss law and the ethical principles of the World Medical Association Declaration of Helsinki and the International Committee on Harmonization for Good Clinical Practice. All participants provided written informed consent.

Inclusion criteria for the ESTxENDS study were: aged 18 or older, current smoker who had consumed five or more cigarettes per day for at least 12 months, and were willing to try to quit smoking within the next three months (exclusion criteria can be found in supplementary information). Participants were invited to a first clinical visit at baseline before their chosen quit date. The day of each clinical visit, participants self-collected their full first-void urine sample (first morning urine sample) before their first cigarette and brought the sample to the study center. Morning urine samples are the most concentrated. Due to logistical constraints, urine samples were stored at 4 °C for one to seven days (on average five days) and were transferred weekly to the laboratory in an icebox. Urinary metabolites (VOC, PAH, and nicotine metabolites, as well as NNAL) were shown to be stable during one week at 4°C (Centers for Disease Control and Prevention (CDC) et al., 2012; Centers for Disease Control and Prevention (CDC) et al., 2013; Xia and Bernert, 2010; McGuffey et al., 2014). They were then aliquoted and stored at −20 °C until analysis.

We included 270 smokers in our study. All participants completed their first clinical visit (baseline) between July 2018 and November 2019. Smoking status was verified by quantifying exposure biomarkers to tobacco smoke, such as exhaled carbon monoxide (CO; > 10 ppm), urinary anabasine (> 3 ng/mL), and urinary cotinine (> 30 ng/mL) (Benowitz et al., 2020a). Daily intake of nicotine was expressed as total nicotine equivalent (TNE 4). TNE 4 corresponds to the molar sum of nicotine, cotinine, norcotinine, and 3-OH-cotinine expressed in nmol/mg creatinine (Eq. (1)) (Benowitz et al., 2020b).

$$TNE = \frac{\frac{[nicotine]}{MW_{nicotine}} + \frac{[cotinine]}{MW_{cotinine}} + \frac{[3-OH-cotinine]}{MW_{3-OH-cotinine}} + \frac{[norcotinine]}{MW_{norcotinine}}}{[creatinine]} \quad (1)$$

where [compound] is the concentration in ng/mL (except for creatinine in mg/mL) and MWcompound the molecular weight in g/mol.

2.2. Analytical methods

All the biomarkers were quantified in first-void urine samples by liquid chromatography – tandem mass spectrometry (LC-MS/MS) methods. All analyses were performed at the Unit of Forensic Toxicology and Chemistry, University Center of Legal Medicine (Lausanne – Geneva, Switzerland), except the NNAL analysis, which was performed at the Central Environmental Laboratory (CEL) at École Polytechnique Fédérale de Lausanne (EPFL, Lausanne, Switzerland). Urinary creatinine was quantified with a routine clinical method based on Jaffe on a chemical analyzer (AU480 Chemistry Analyzer, Beckman Coulter, Nyon, Switzerland) (Jaffe, 1886). Exhaled CO was measured (Micro+ or piCO+ Smokerlyzer, Bedfont, Anif, Austria) during the participant's first clinical visit, which was conducted several hours after collecting the first-morning urine void. The laboratory is ISO17025 accredited and has participated in the German External Quality Assessment Scheme (G-EQUAS) n°65–68 within the time frame of this study (2020 − 2021).

2.2.1. TNE 4

TNE 4 included nicotine and three of its metabolites: cotinine, 3-OH-cotinine, and norcotinine. These metabolites, together with nicotine and anabasine, were analyzed with LC-MS/MS (Dionex Ultimate 3000 system + TSQ Quantiva, Thermo Scientific, Reinach, Switzerland; Thermo Fisher Scientific application note n°20709, 2013 (Jones, n.d.)). Sample preparation included a solid-phase extraction (SPE; Sola SCX 10 mg/1 mL, Thermo Scientific, Reinach, Switzerland). Limits of quantification (LOQs) were 1 ng/mL for all compounds. Two quality controls (QCs) were injected every 15 samples and the sample series were included if these were within 15 % of the given value. Performance of the method was assessed by inter-laboratory tests for nicotine and cotinine (G-EQUAS n°66–68).

2.2.2. PAH metabolites

Three PAH metabolites: 1- and 2-naphthol and 1-OHP, were analyzed with a routine laboratory method. Briefly, urine (3 mL) was mixed with a solution (3 mL) containing internal standards (1-OHP-d9, 1-naphtol-d7, and 2-naphtol-d7), enzyme (β-glucoronidase from *Helix pomatia* type HP-2 ≥ 100,000 U/mL, Merck KGaA, Buchs, St. Gallen, Switzerland), and hydrochloric acid (4 N) in sodium acetate buffer (32.8 g/L). The mixed solution was incubated at 37 °C overnight. The target analytes were extracted with SPE (ABN 60 mg/3 mL, Biotage, Uppsala, Sweden). The extract was dried and reconstituted in water/acetonitrile (500 μL, 80:20, *v*/v) for LC-MS/MS analysis. Limits of detection (LODs) were 0.1 ng/mL and LOQs were 0.2 ng/mL for each metabolite. A QC was injected every 15 samples and the sample series were included if these were within 15 % of the given value. Performance of the method was assessed by inter-laboratory tests (G-EQUAS n°66–67).

2.2.3. VOC metabolites

VOC metabolites were quantified with a LC-MS/MS according to the method of Alwis et al. (2012). Briefly, filtered urine samples were diluted 1:10 with buffer (100 μL urine +50 μL internal standards +850 μL 15 mM ammonium acetate pH 6.8). The diluted solution was directly injected in the LC-MS/MS. LOQs were 1.5 ng/mL for 3-HPMA, 2-HPMA and HPMMA, 2 ng/mL for AAMA, DHBMA, CYMA, 1 − /2-MHBMA, 3-MHBMA and PMA, and 3 ng/mL for GAMA and HEMA. 1-MHBMA and 2-MHBMA could not be separated chromatographically. We thus report the sum of these here. A QC sample was injected every 10 samples and the sample series were included if these were within 10 % of the given value (except for MHBMA: 15 %). Performance of the method was assessed by inter-laboratory tests (G-EQUAS n°67–68) for all analytes, except HPMMA.

2.2.4. NNAL

NNAL was quantified by LC-MS/MS (Acquity UPLC system + Xevo TQ MS, Waters, Baden-Dättwil, Switzerland). Samples were prepared at the department of occupational and environmental health (DSTE) of the Center for Primary Care and Public Health (Unisanté, Lausanne). The method was based on Biotage application note (n°AN884, 2017 (Biotage, n.d.)) and the publications of Byrd and Ogden (2003), Kavvadias et al. (2009), and Hu et al. (2014). Briefly, urine samples (1.3 mL) were centrifuged (4000 rpm, 5 min) and mixed with internal standards (100 μL), buffer (200 μL, phosphate buffer 1 M) and enzyme (400 μL of β-glucoronidase ~5 mg/mL or ~ 5200 U/mL; β-glucoronidase from *Escherichia coli* type IX-A 125KU, Merck KGaA, Buchs, St. Gallen, Switzerland). After one night in the dark at 37 °C, the mixture was extracted with a solid-supported liquid/liquid extraction (SLE cartridge; ISOLUTE® SLE+ 2 mL Sample, Biotage, Uppsala, Sweden) and the samples were analyzed by LC-MS/MS at EPFL. LOQ was 0.2 ng/mL. We quantified urinary NNAL in 103 of the 270 participants due to budgetary limits. Two TSNAs, NNN and NNK, were analyzed with the same analytical method but were below the LOQ. A QC sample was injected every 15 samples and the sample series were included if these were within 10 % of the given value.

2.2.5. 8-oxodG and 8-isoprostane

8-oxodG and 8-isoprostane were analyzed with a previously described LC-MS/MS (Dionex Ultimate 3000 system + TSQ Quantiva, Thermo Scientific, Reinach, Switzerland) method (Sambiagio et al., 2020). Sample preparation included a SPE (Chromabond C18ec SPE 500 mg 3 mL, Macherey-Nagel, Oensingen, Switzerland). LOQs were 0.5 ng/mL for 8-oxodG and 0.1 ng/mL for 8-isoprostane. Two QCs were injected every 15 samples and the sample series were included if these were within 10 % of the given value.

2.3. Health status, lifestyle and environmental exposure assessment

Self-reported questionnaires queried participants on health status and lifestyle, including smoking history (Cornuz et al., 2002; Cornuz et al., 1997), sleep quality (PSQI), physical activity (IPAQ), alcohol consumption (AUDIT-C questionnaire), and dietary habits (frequencies of vegetables and fruits consumption). We also asked participants to give their postal code and profession. The purpose of including these factors in the statistical analysis was to assess if they modulate the association between exposure biomarkers and oxidative stress biomarkers in our samples of smokers.

2.3.1. Self-reported previous health conditions

Participants were asked whether they had an infection, fever or acute illness the day of urine collection.

2.3.2. Smoking history

Three parameters were selected: daily cigarette consumption, years of smoking, and pack years. The latter was estimated by multiplying the number of cigarette packs per day (i.e. number of cigarettes per day divided by 20) by the number of years of smoking.

2.3.3. Sleep quality

Sleep quality can be assessed by the Pittsburgh sleep quality index (PSQI), which is a standardized self-report questionnaire (Buysse et al., 1989). Participants completed the PSQI questionnaire, and we calculated a score according to the authors' instructions (Buysse et al., 1989). Briefly, we grouped 19 items in seven components of sleep: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. Each component score ranged from 0 to 3, and all component scores were summed to yield a global score (from 0 to 21). A global score > 5 indicates poor sleep quality.

2.3.4. Physical activity

International physical activity questionnaire (IPAQ) is a validated self-report questionnaire, and the short IPAQ form has been recommended for use in national monitoring surveys (Craig et al., 2003). We included five IPAQ recommendations in our statistical analysis to assess physical activity in our cohort;

1) Excluded data reported as "do not know" and missing data,
2) Estimated Metabolic Equivalent Task minutes per week (MET-min/week) (a measure of total physical activity) by summing adjusted durations (minutes x days) for each type of activity (adjustment was performed with the following values: walking = 3.3 METs, moderate physical activity = 4.0 METs, and vigorous physical activity = 8.0 METs)
3) Excluded participants who reported a total activity time >960 min per day (i.e., intensive physical activity),
4) Excluded physical activity reported for <10 min per day in any domains, and
5) Truncated any domain exceeding 180 min to 180 min, as recommended by IPAQ (Craig et al., 2003).

We created a new variable including those with significant physical activity (vigorous MET-min/week >3000, corresponding to approximately 1 h of intense physical activity per day in one week; $n = 32$) to analyze this separately.

2.3.5. Alcohol consumption

Alcohol consumption can be assessed by the alcohol use disorders identification test (AUDIT), a questionnaire developed in a multinational World Health Organization collaborative study and validated in different populations (Organization, 2001). A short form focusing on alcohol consumption (AUDIT-C) was proposed as a screening test for heavy drinking or alcohol dependence (Bush et al., 1998). Participants completed the AUDIT-C questionnaire, and a score from 0 to 12 was calculated by summing the score of each response (ranged from 0 to 4) (Organization, 2001). A score ≥ 3 for women or ≥ 4 for men can reflect a problematic alcohol consumption and higher scores may indicate dependence.

2.3.6. Daily consumption of fruits and vegetables

Participants were asked: "How often do you eat fresh vegetables (carrots, green beans, salad, etc.)?" and "How often do you eat fresh fruit or fresh fruit juice?". We separated fruit and vegetables into two categories: daily consumption and less than daily consumption.

2.3.7. Residence category (urban vs rural)

Participants were classified into two environmental categories according to their place of residence: urban or rural environment. We used the Swiss postal codes and classified them according to the FSO (Swiss Federal Statistical Office) system based on seven classes where 1 represents the city center up to 7 for the countryside (Table S1 in supplementary information). We grouped the classes 1 (in the city) and 2 (just outside city center) in the urban category and classes 3 to 7 in the rural category. This allowed discriminating the ones living in city from the others.

2.3.8. Professions

We selected several jobs in which possible occupational exposures to PAHs or VOCs may occur including city cleaner, cleaning agent, cook, cab, bus or streetcar driver, truck driver, Uber driver, garage worker, gas station employee, firefighter, hairdresser, mechanic, policeman, postman, nail stylist, chimney sweep, street vendor, chemical industry worker, construction worker (building/roads), and metal industry worker. We grouped the participants working in these occupations in one category (cat. 1 – potentially exposed to PAHs or VOCs) and the others in a second category (cat. 2 – non-exposed to these pollutants).

2.4. Data presentation and statistical analysis

Biomarker concentrations below limit of quantification (LOQ) were substituted with: LOQ/sqrt(2) (Goniewicz et al., 2018). We flagged biomarkers with >40 % of observations under LOQ and did not use it for statistical analysis. Oxidative stress biomarkers and biomarkers of exposure were creatinine-corrected, and presented as median with the interquartile range (IQR): 1st and 3rd quartiles (Q1–Q3).

We log-transformed data for each biomarker because they were all right-skewed. We conducted factor analyses on the (log-transformed) PAH and VOC biomarkers to assess the validity of summary scores labeled as ΣPAHs and ΣVOCs. The consistency of the different exposure biomarkers (cigarettes per day, exhaled CO, urinary TNE, urinary NNAL, as well as ΣPAHs and ΣVOCs) was assessed using linear and partial correlation coefficients (definitions and example in Fig. S1 in supplementary information). We defined the correlation as weak (r = 0.10–0.39), moderate (r = 0.40–0.69) or strong (r = 0.70–1.00) (Schober et al., 2018). The associations between ΣPAHs and ΣVOCs with the place of living and the professions was assessed using multiple linear regression models adjusting for smoking parameters.

We investigated the association between oxidative stress biomarkers and biomarkers of exposure: TNE, ΣPAHs, ΣVOCs, or TSNAs with multiple linear regression. We constructed one model per biomarker of exposure to avoid multicollinearity, adjusted for creatinine (spot urine samples are creatinine-adjusted to take into account the participants' hydration status) and several other factors (mentioned above). We added the variables into the model one at a time and if the p-values <0.05 were considered statistically significant, we kept the variable in the model. Consequently, the non-significant factors were not included in the models, except age, BMI, and gender. Associations between urinary concentrations of 8-oxodG and exposure biomarkers were calculated with Eq. (2) as a percentage increase (for log-transformed predictor only; more details on the calculation in the supplementary information).

$$\%_{\text{outcome}} = \left(\left(\frac{\%_{\text{covariate}}}{100} + 1\right)^{\beta} - 1\right) * 100 \quad (2)$$

where $\%_{\text{outcome}}$ is the percent increase or decrease in the concentration of an oxidative stress biomarker, $\%_{\text{covariate}}$ is the percent increase or decrease in the concentration of an exposure biomarker (or other log-transformed covariates), and β is the regression coefficient (estimate).

To compare the importance of the exposure biomarkers from the different models, we computed effect sizes. Effect sizes of the variables could then be compared by calculating the partial r-squared (partial R^2), which is the proportion of variance explained by each variable excluding the proportion of the variance due to the other variables in the model. In brief, this parameter allowed us to define the relative importance of each covariate, whether it is continuous or categorical, on the dependent variable. Partial R^2 of chemical groups composing the VOCs were also calculated: aldehydes (acrolein, crotonaldehyde), epoxides (ethylene oxide, propylene oxide), and others (acrylamide and 1,3-butadiene).

All calculations were performed with R version 4.0.2 (2020-06-22) - "Taking Off Again".

3. Results

3.1. Characteristics of participants

Participant demographics are presented in Table 1. We verified participants' smoking status with exhaled CO, anabasine, and cotinine. Fifteen participants had concentrations of exhaled CO lower than 10 ppm (sensitivity 94 %), and 42 had urinary anabasine concentration lower than 3 ng/mg creatinine (sensitivity 84 %). However, no participant had cotinine below 30 ng/mg creatinine (sensitivity 100 %). Therefore, all participants were considered as smokers.

In addition, participants reported if they had allergies (n = 101), hypertension (n = 34), hypercholesterolemia (n = 20), diabetes (n = 9), cardiovascular diseases (n = 33; CVD including previous myocardial infarction, percutaneous coronary intervention (PCI), heart failure, stroke, angina pectoris, peripheral arterial disease (PAD) or other self-reported CVD), and pulmonary diseases (n = 49; including pulmonary embolism, deep vein thrombosis (DVT), chronic obstructive pulmonary disease (COPD), chronic bronchitis, asthma or other self-reported pulmonary diseases).

Of 175 participants, only 59 reported a daily consumption of fruits and vegetables (information missing for 95 participants). Most of the participants lived in the city center of just outside the city center (n = 167), while 103 lived in the countryside. Only 20 participants had a job in which possible occupational exposures to PAHs or VOCs may occur.

3.2. Urinary biomarkers of exposure

Concentrations of biomarkers of tobacco smoke exposure are presented in Table 2 separately for men and women.

3.2.1. Correlation between exposure biomarkers and indicators of tobacco consumption

We computed ΣPAHs and ΣVOCs scores (correlations between the scores and their individual components can be found in Table S2 and S3 in supplementary information). SPMA was not included in the variable ΣVOCs, as >69 % of the observations were below the LOQ. Correlation coefficients and partial correlation coefficients (gray cells) between the variables cigarettes per day, exhaled CO, anabasine, TNE, ΣVOCs, ΣPAHs, and NNAL were calculated and can be found in supplementary information

Table 1
Summary of participant demographics (number, age, body mass index (BMI)), cigarette consumption and history (years of smoking and pack years), total nicotine equivalent (TNE), exhaled CO, sleep quality (Pittsburgh Sleep Quality Index (PSQI) score), physical activity (International Physical Activity Questionnaire (IPAQ) score), and alcohol consumption (Alcohol Use Disorders Identification Test – Consumption (AUDIT-C) score). Characteristics are presented for all participants, and separately for men and women. Except for participant number, all characteristics are presented as median with interquartile range (1st quartile – 3rd quartile).

Characteristic	Units	Total [median (Q1–Q3)]	Men [median (Q1–Q3)]	Women [median (Q1–Q3)]
Participants (−)	[number (%)]	270 (100)	153 (57)	117 (43)
Age	(years)	44 (32–54)	43 (32–54)	45 (33–54)
BMI	(kg/m^2)	25.1 (22.4–27.6)	25.5 (23.4–27.6)	24.5 (21.3–27.2)
Cigarette consumption	(cig/day)	17 (10 – 20)	20 (13 – 20)	15 (10–20)
Smoking history	(years)	25 (16–36)	25 (16–35)	26 (16–36)
Pack years	(−)	19.0 (11.2–31.5)	20.0 (13.5–32.3)	16.1 (9.3–29.7)
Anabasine	(ng/mg creatinine)		7.50 (4.40–10.70)	7.34 (3.83–11.73)
TNE	(nmol/mg creatinine)	26.5 (17.3–37.3)	24.5 (17.3–36.7)	27.6 (17.9–38.4)
Exhaled CO	(ppm)	25 (16–33)	26 (17–33)	23 (14–34)
PSQI score	(−)	5 (3–7)	5 (3–7)	5 (3–8)
IPAQ	(MET-min/week)	3126 (1436–5118)	3552 (1388–5172)	2886 (1388–4981)
AUDIT-C score	(−)	3 (2–5)	4 (2–6)	3 (2–4)

Table 2
Metabolites concentrations for urinary nicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), volatile organic compounds (VOC), and polycyclic aromatic hydrocarbons (PAH) in smokers (n = 270) as well as the percent of samples above the limit of quantification (>LOQ), elimination half-life (h: hours, d: days, NK means not known), corresponding parent compound.

	Parent compound	Biomarker	Half-life	>LOQ (%)	Men (ng/mg creatinine) [median (Q1–Q3)]	Women (ng/mg creatinine) [median (Q1–Q3)]
Nicotine	Nicotine[a]	Nicotine	1–2 h (Benowitz et al., 2009)	100	685 (376–1433)	746 (326–1240)
		Cotinine	16–18 h (Benowitz et al., 2009)	100	1230 (858–1784)	1194 (664–1617)
		Norcotinine	NK	100	124 (89–187)	140 (82–170)
		3-OH-cotinine	6.4 h (Benowitz and Jacob, 2001)	100	2272 (1452–3429)	2853 (1462–3728)
NNAL	NNK	NNAL[b]	42 d (Benowitz et al., 2009)	99	234 (147–383)	246 (107–337)
VOCs	1,3-butadiene	DHBMA	NK	100	420 (314–553)	404 (339–505)
		1 − /2-MHBMA[c]	> 9 h (van Sittert et al., 2000)	70	3.22 (<LOQ–6.39)	2.94 (<LOQ–5.89)
		3-MHBMA	NK	100	27.1 (17.0–38.91)	23.5 (15.90–36.88)
	Acrolein	3-HPMA	9–12 h (Watzek et al., 2012)	100	1355 (899–1979)	1026 (633–1787)
	Acrylamide	AAMA	12–14 h (Watzek et al., 2012)	100	156 (112–208)	166 (108–226)
		GAMA	22 h (Watzek et al., 2012)	99	17.1 (13.0–25.6)	16.1 (13.3–24.0)
		CYMA	8 h (Jakubowski et al., 1987)	100	177 (102–306)	203 (103–448)
	Benzene	SPMA	9 h (Benowitz et al., 2009)	31[d]	<LOQ (<LOQ–3.01)	<LOQ (<LOQ–6.43)
	Crotonaldehyde	HPMMA	NK	100	908 (642–1260)	762 (483–1127)
	Acrylonitrile, ethylene oxide, vinyl chloride	HEMA[e]	> 5 h (Haufroid et al., 2007)	68	3.69 (<LOQ–5.82)	4.76 (<LOQ–8.65)
	Propylene oxide	2-HPMA	NK	100	59.8 (40.2–82.0)	51.5 (31.6–78.0)
PAHs	Naphthalene	1-Naphtol	4.3 h (Li et al., 2012)	100	8.83 (5.83–13.23)	8.27 (4.59–12.52)
		2-Naphtol[f]	2.5 h (Li et al., 2012)	100	16.1 (11.9–20.7)	16.4 (12.5–23.2)
	Pyrene	1-OHP	20 h (Benowitz et al., 2009)	81	0.27 (0.20–0.42)	0.33 (0.23–0.45)

[a] Nicotine and its metabolites can also be found in individuals using nicotine replacement therapy or electronic nicotine delivery systems, thus not solely specific to tobacco exposure.
[b] NNAL was analyzed for 103 participants only.
[c] LOQ for 1 − /2-MHBMA was 2 ng/mL.
[d] the majority of the observations of SPMA (69 %) were under LOQ (2 ng/mL).
[e] LOQ for HEMA was 3 ng/mL.
[f] Four values of 1-naphthol were not included in the statistical analysis because they were higher than 1000 ng/mg creatinine.

(Table S4). Pearson's correlation coefficients between all the urinary biomarkers were high ($r > 0.5$) signifying a common source: cigarette smoking. We found weak/moderate correlations between the exposure biomarkers and the number of cigarettes per day (r ~ 0.3–0.5) and exhaled CO. Partial coefficients were greatly reduced compared to correlation coefficients for most relationships, although some were still moderate (r ~ 0.3–0.5).

3.2.2. Potential other sources of PAHs and VOCs

Covariates with significant positive associations were TNE and exhaled CO for both ΣPAHs and ΣVOCs. Residence and profession categories did not show any significant influence in our sample of smokers.

3.3. Urinary biomarkers of oxidative stress

The correlation coefficient between 8-oxodG and 8-isoprostane was high ($r = 0.69$). Table 3 presents the urinary oxidative stress biomarker concentrations.

Table 3
– Concentrations of 8-oxodG and 8-isoprostane in smokers, and separately in men and women (normalized for creatinine, median with interquartile range (1st and 3rd quartiles); $n = 270$). Percentages of samples above the limit of quantification (>LOQ) are also reported.

Biomarkers	>LOQ (%)	Total	Men	Women
8-oxodG (ng/mg creatinine) [median (Q1–Q3)]	100	4.33 (3.46–5.76)	4.15 (3.30–5.76)	4.42 (3.57–5.76)
8-isoprostane (ng/mg creatinine) [median (Q1–Q3)]	100	0.22 (0.17–0.31)	0.23 (0.17–0.32)	0.22 (0.16–0.30)

Biomarkers: 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-isoprostaglandin F2α (8-isoprostane).

3.3.1. Urinary 8-oxodG

Table 4 shows the results from the four regression models for 8-oxodG. We read from that table that a 50 % increase in the ΣVOC and ΣPAH scores yielded 1 % (β = 0.028) and 4 % (β = 0.105) increases in 8-oxodG concentration, respectively. TNE, ΣPAHs and ΣVOCs were all associated with urinary 8-oxodG.

We did not find any change of the measures of association with age and gender in our study sample, but found a negative association between BMI and 8-oxodG for the ΣPAHs regression model only. Other covariates, such as residence category, professions, diseases, sleep quality, physical activity, alcohol consumption, vegetable and fruit consumption, did not change the measures of association with 8-oxodG concentrations (not included in the final models).

Fig. 1 shows the relative importance of the covariates for 8-oxodG formation (effect sizes of the covariates with partial R^2). Besides creatinine, ΣPAHs with the greatest association (partial $R^2 = 0.15$) followed by ΣVOCs (partial $R^2 = 0.09$), and TNE (partial $R^2 = 0.08$).

3.3.2. Urinary isoprostane

Table 4 shows the results from the four regression models for 8-isoprostane. All biomarkers of exposure had a positive relationship with urinary 8-isoprostane concentrations. A 50 % increase in the following exposure biomarkers: TNE, NNAL, ΣVOC, and ΣPAH was associated with 11 %, 8 %, 2 %, and 5 % increase in 8-isoprostane concentrations, respectively. In our smoker cohort, we observed a positive association between 8-isoprostane concentration and BMI, but no change of the measure of association with age and gender. Daily consumption of fruits and vegetables was negatively associated with 8-isoprostane (concentration were reduced by 16 %).

We found that ΣPAHs and ΣVOCs had the greatest association with 8-isoprostane concentrations (partial $R^2 = 0.14$ for both) followed by NNAL and TNE (partial $R^2 = 0.09$ and 0.08, respectively), as shown in Fig. 1. The effect size of fruit and vegetable consumption was low (partial $R^2 = 0.03$; insignificant for the NNAL model) and similar to BMI (partial

Table 4
– Multiple linear regression models for 8-oxodG and 8-isoprostane (estimate (coefficient beta (β)) [95 % confidence interval] and *p*-value (* means significant: <0.05)). All models were adjusted for biomarker of exposure (adjusted for creatinine), age, gender, body mass index (BMI), and other significant covariates if any. Covariates significantly associated with the outcome (*) are in bold.

	TNE	ΣPAHs	ΣVOCs	NNAL
No. of Samples	270	266	270	103
8-oxodG				
Biomarker of exposure	**0.226 [0.134, 0.318] ***	**0.105 [0.075, 0.135] ***	**0.028 [0.017, 0.038] ***	0.037 [−0.084, 0.159]
Age	0.001 [−0.003, 0.005]	0.002 [−0.002, 0.005]	0.000 [−0.003, 0.004]	0.001 [−0.006, 0.008]
Gender	−0.017 [−0.120, 0.086]	0.005 [−0.093, 0.103]	−0.009 [−0.110, 0.093]	−0.051 [−0.227, 0.126]
BMI	−0.007 [−0.018, 0.004]	**−0.013 [−0.023, −0.003] ***	−0.008 [−0.019, 0.002]	−0.011 [−0.029, 0.006]
8-isoprostane				
Biomarker of exposure	**0.266 [0.158, 0.374] ***	**0.117 [0.081, 0.153] ***	**0.040 [0.028, 0.052] ***	**0.202 [0.068, 0.335] ***
Age	0.003 [−0.002, 0.007]	0.003 [−0.002, 0.007]	0.001 [−0.003, 0.006]	0.000 [−0.008, 0.008]
Gender	0.055 [−0.067, 0.176]	0.090 [−0.022, 0.209]	0.058 [−0.059, 0.175]	0.110 [−0.084, 0.303]
BMI	**0.020 [0.007, 0.033] ***	**0.014 [0.001, 0.026] ***	**0.019 [0.007, 0.031] ***	**0.022 [0.003, 0.041] ***
Fruits and vegetables[a]	**−0.170 [−0.326, −0.015] ***	**−0.170 [−0.324, −0.015] ***	**−0.181 [−0.334, −0.028] ***	−0.020 [−0.266, 0.225]

Biomarker concentrations were log-transformed. Creatinine concentration (log-transformed) was added as covariate in the models (not showed).
Biomarkers: 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-isoprostaglandin F2α (8-isoprostane), total nicotine equivalent (TNE), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL).
[a] The effect of the daily consumption of fruits and vegetables was added separately as 95 observations were missing.

$R^2 \sim 0.03$; for all models). The relative importance of smoking was thus much larger than the potential protective effect from fruit and vegetable consumption.

4. Discussion

In this analysis of urinary biomarkers of oxidative stress and biomarkers of tobacco smoke exposure among 270 longtime smokers, exposures to PAHs and VOCs were strongly associated with both urinary 8-oxodG and 8-isoprostane concentrations.

Our participants had age and gender distributions comparable to the 2017 Swiss Health Survey of tobacco consumption population (Swiss Federal Statistical Office – FSO) and thus we deemed our sample to be representative of the smoking population of Switzerland (Table S5 available in supplementary information). We estimated the daily nicotine exposure with total nicotine equivalent (TNE), as it accounts for differences in metabolic rate and genetic variation among smokers (Taghavi et al., 2018). The gold standard to estimate daily nicotine intake is TNE 7, which is the molar sum of nicotine and six of its metabolites (cotinine, 3-OH-cotinine, nornicotine, norcotinine, nicotine N-oxide, cotinine N-oxide, including their glucuronide conjugates). In our study, we used TNE 4 (nicotine, cotinine, 3-OH-cotinine, and norcotinine), as it was previously showed that TNE 3 (molar sum of nicotine, cotinine, and 3-OH-cotinine) strongly correlated to TNE 7 ($r = 0.99$) and was insensitive to individual metabolic variation (Benowitz et al., 2020b).

The exposure biomarker concentrations (nicotine, TSNA, VOC, and PAH metabolites) were in the same range as reported by Goniewicz et al. (2018) who analyzed 2411 urine samples from smokers. All biomarkers were detected in all of our samples, except for three: SPMA (benzene metabolite; 31 %), HEMA (metabolite of acrylonitrile, ethylene oxide, vinyl chloride; 68 %), and 1−/2-MHBMA (1,3-butadiene metabolite; 70 %). This was because LOQs (2 ng/mL for SPMA and 1−/2-MHBMA, and 3 ng/mL for HEMA) for these were not sufficiently sensitive. Consequently, we do not recommend analyzing SPMA (benzene metabolite) if LOQ is higher than 0.6 ng/mL (corresponding to ~70 % > LOQ in smokers) (Goniewicz et al., 2018), and including the two biomarkers 1−/2-

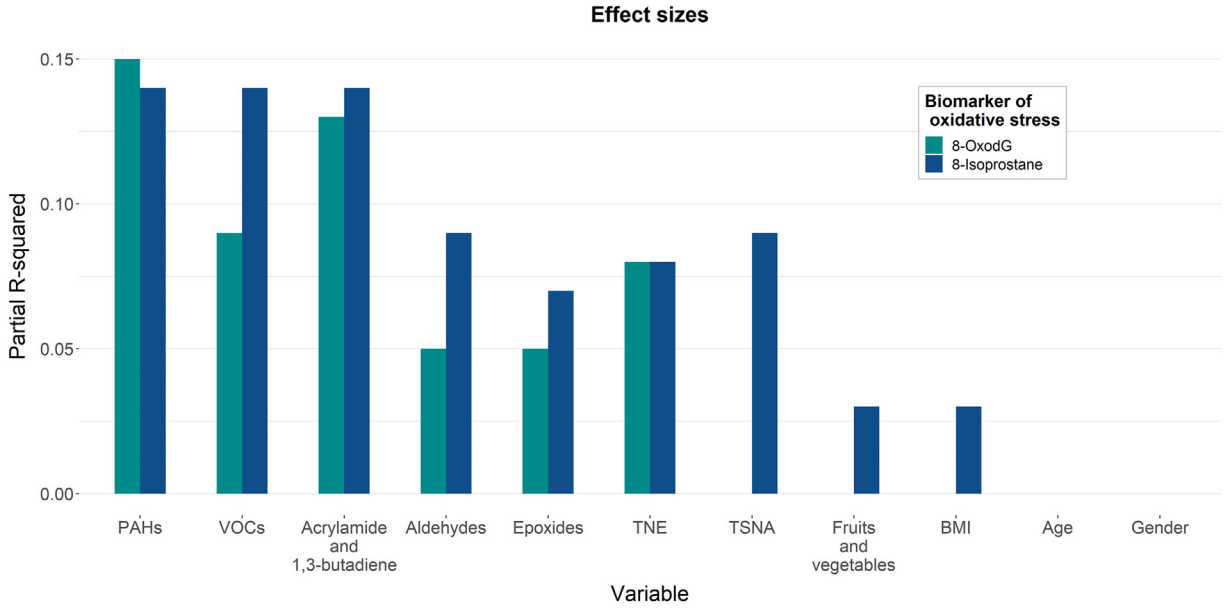

Fig. 1. – Relative importance of exposure biomarkers (metabolites of polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), total nicotine equivalent (TNE), and tobacco-specific nitrosamines (TSNAs)), as well as individual (body mass index (BMI), age, and gender) and behavioral (daily fruit and vegetable consumption) factors on oxidative stress biomarkers, expressed as partial R-squared, an effect size indicator. Effect sizes were also calculated for the groups composing the VOCs: aldehydes (acrolein, crotonaldehyde), epoxides (ethylene oxide, propylene oxide), and others (acrylamide and 1,3-butadiene).

MHBMA and HEMA unless in conjunction with others in the statistical analysis.

4.1. Linear relationships between biomarkers

VOC metabolites (except SPMA) showed moderate to strong correlations with each other (average $r = 0.45–0.50$; Table S3), which was expected because they are all tobacco smoke exposure biomarkers. VOC metabolites have similar phase two elimination pathways (mercapturic acid pathway), but different half-lives, which could explain the moderate correlations. This reasoning is also valid for the three PAH metabolites, which also showed moderate correlations (Table S2). The strength of these correlations justified the use of ΣVOCs and ΣPAHs score.

Many studies, including ours, have shown weak to moderate correlations between cigarettes per day and urinary nicotine metabolites, and the reasons could potentially be explained by different factors, such as smokers' puffing behaviors, inter-individual differences in metabolism, timing between the last cigarette and the urine collection, and inaccurate number of cigarettes reported (Murphy et al., 2004). Here, we take into account these inter-individual differences in metabolism by summing the different metabolites and expressing them as TNE 4, but we had limited influence on the other factors. Weak correlations between exhaled CO and other biomarkers of exposure ($r = 0.3–0.35$) could be explained by the delay between the urine collection at home (first-void urine) and exhaled CO measurement at the clinical visits (usually several hours afterwards). Participants may have smoked in-between. Exhaled CO measurement is not essential if anabasine and cotinine are used for biochemical verification of tobacco exposure.

4.2. Degree of association between variables

The moderate partial correlations we observed between ΣPAHs and ΣVOCs scores ($r = 0.51$) could suggest the presence of other exposure sources than cigarette, such as environmental sources (e.g., traffic, industrial activities). Although these environmental exposures were likely present, we deemed them as less contributing to overall VOCs and PAHs than cigarette, because the correlations between anabasine (tobacco-specific biomarker) and ΣVOCs as well as ΣPAHs were strong. Explanations of the moderate partial correlation may include the origins of the compounds (incomplete combustion processes for PAHs and VOCs, while nicotine and anabasine are inherent in tobacco leaves), puffing behavior, and varying amount of nicotine in different brand of cigarettes. These hypothesis were supported by the strong correlation between ΣVOCs and ΣPAHs ($r = 0.78$) that was higher than those with the other exposure biomarkers.

We tested the effect of some professions where exposures to VOCs and PAHs are possible (list of 19 professions, grouped together) but did not find any association. This was probably due to the low number of participants included in the exposed category ($n = 20$), and furthermore, the activities were diverse (12 different professions). Moreover, we found no association with residence category of the participants (urban vs rural). These results were not surprising, as tobacco smoke remains the major well-known source of these compounds, and air pollution exposures are in general lower than smoking (Gao et al., 2020).

4.3. Oxidative stress biomarker concentrations in smokers

We compared urinary concentrations of oxidative stress biomarkers (normalized for creatinine) with the ones reported in two recent systematic literature reviews (Graille et al., 2020a; Graille et al., 2020b). For 8-oxodG, the first systematic review reported urinary concentration of 22.2 (3.0–41.4) ng/mg creatinine (median with interquartile range (1st quartile – 3rd quartile)) for smokers with a BMI ≤ 25 and 4.0 (3.5–4.5) ng/mg creatinine (median with interquartile range (1st quartile – 3rd quartile)) for smokers with a BMI > 25. This was contrary to our results, which showed similar 8-oxodG concentrations (Table 3) for smokers with a BMI above and below a BMI of 25. Interestingly, our results showed 8-oxodG concentrations to be four times lower for smokers with BMI ≤ 25 compared to the systematic review. However, Graille et al. (2020a) specified that the high 8-oxodG concentrations for smokers with BMI ≤ 25 in the meta-analysis originated from only one study and thus these results need to be confirmed. Contrary to the 8-oxodG results, no consistent effect of smoking was observed for 8-isoprostane in the second systematic review. However, BMI influenced 8-isoprostane concentrations; they reported that subgroups with a BMI > 25 had urinary 8-isoprostane of 0.55 (0.51–0.65) ng/mg creatinine (median with interquartile range (1st quartile – 3rd quartile)) while subgroups with a BMI ≤ 25 had half of this concentration (0.25 (0.24–0.41) ng/mg creatinine (median with interquartile range (1st quartile – 3rd quartile)). We did not have an effect of BMI in our study, but we did show that BMI had a significant, although week (partial $R^2 = 0.03$), positive association with 8-isoprostane.

4.4. Multiple linear regression models

None of the tested factors (the place of residence, diseases, sleep quality, physical activity, and alcohol consumption) had any significant influence on the oxidative stress biomarkers in addition to cigarette smoking. Lifestyle factors and health status were investigated as potential confounding factors in our study restricted to smokers. Statistical power was also limited by the sample size to explore such associations. We urge for careful interpretation when extrapolating results to other population. For 8-oxodG, results from the multiple linear regression showed that NNAL was not associated with 8-oxodG. Even if we had fewer observations than for the other exposure biomarkers ($n = 103$), the effect size was very small (partial $R^2 < 0.01$) and therefore, we would not expect to observe a consistent association with 8-oxodG with more participants. We did not observe any change of the measure of association with gender or age, but found a small negative association with BMI (significant for ΣPAHs model only), i.e. an 8-oxodG decrease of 1 % when BMI increased by one unit. The link between BMI and 8-oxodG is not clearly established, as conflicting results are reported in several studies (Irie et al., 2005; Zanolin et al., 2015). We only found an association with BMI in the PAH model. One hypothesis would be that some of the inhaled PAHs are stored in the adipose tissue due to their high lipophilicity and are gradually released (Abdel-Shafy and Mansour, 2016). The body's defense mechanisms would then be less likely overwhelmed. For 8-isoprostane, BMI was significant in all models of the regression analysis. Several studies have reported that oxidative stress increases with BMI (Keaney et al., 2003). Although, we observed an association with hypercholesterolemia, we did not include this factor in the multiple linear regression model because it was highly correlated with BMI, which was already included in the analysis.

We observed a significant negative association of 8-isoprostane concentrations with the daily consumption of fruits and vegetables, which reduced the 8-isoprostane concentrations of about 15–16 % in smokers (Table 4). This reduction is likely due to their natural antioxidant content (Tan et al., 2018). This result has support from other studies. Excretions of 8-isoprostane in women ($n_{tot} = 246$) were reduced with consumption of fruits and vegetables, and an inverse association between urinary 8-isoprostane concentration and fruit consumption in a study on polyphenols contained in the Mediterranean diet has also been reported (Ruiz et al., 2019; Thompson et al., 2005). The link between fruit and vegetable consumption and oxidative stress is still being studied as several studies have not found conclusive results (Peluso et al., 2018; Vetrani et al., 2013; Ávila-Escalante et al., 2020).

4.5. Effect size indicator

The contribution from each exposure biomarker to the oxidative stress biomarkers varied greatly. A 50 % increase in urinary hydroxypyrene (1-OHP) concentrations, which is the biomarker representative of the overall PAH exposures, would lead to an 11–13 % increase of 8-oxodG and 8-isoprostane concentrations. Contrary to PAHs, no VOC metabolite is an indicator of overall VOC exposures. Therefore, we regrouped the VOC

metabolites from aldehydes (acrolein, crotonaldehyde), epoxides (ethylene oxide, propylene oxide), and others (acrylamide, 1,3-butadiene) and calculated the effect sizes (Fig. 1) for these to understand if any of these known carcinogens were in particular related to oxidative stress.

Acrylamide and 1,3-butadiene had an effect size twice that of the other groups (supplementary information; Table S6), which meant that they were the most associated with increased concentrations of oxidative stress biomarkers. 1,3-Butadiene and acrylamide are oxidized by cytochrome P450 enzymes, known to generate free radicals, before conjugation with glutathione (Kakehashi et al., 2013; Henkler et al., 2010). The others are not. This might explain the larger effect sizes for 1,3-butadiene and acrylamide compared to aldehydes or oxides. Both aldehydes and oxides react directly with glutathione and cause an indirect increase of free radicals by decreasing the antioxidant defenses of the body (Armstrong et al., 2002). This would explain why their effect sizes were similar. Although acrylonitrile can also be oxidized by cytochrome P450 enzymes, the effect size was lower than most of the other compounds (not included in Fig. 1). One hypothesis is that CYMA (one of acrylonitrile metabolites) results from the direct conjugation to glutathione, and it might not be linked to the cytochrome P450 enzyme activity (Li et al., 2021). Moreover, the second metabolite HEMA, resulting from oxidation by the cytochrome P450 enzymes, is not specific to acrylonitrile, as it is also a metabolite of ethylene oxide and vinyl chloride. Thus, the importance of this compound on the formation of oxidative stress biomarkers is probably masked.

Exposures to environmental pollutants such as VOCs and PAHs nor their corresponding exposure biomarkers can predict the development of diseases. Indeed, exposure biomarkers are measures of the dose in the body or internal exposures, not a measure of a health effect. Oxidative stress biomarkers are effect biomarkers, which can be used to detect early development of diseases. Among the many oxidative stress biomarkers (Frijhoff et al., 2015), we selected two that could be quantified in urine with the same chemical analytical method (Sambiagio et al., 2020) and where background concentrations had been reviewed (Graille et al., 2020a; Graille et al., 2020b). Oxidative stress is a mechanism on the pathway to several diseases. Our selected oxidative stress biomarkers reflect two mechanistic pathways: reaction of reactive oxygen species (ROS) with DNA (8-oxodG) and oxidation of membrane lipids (8-isoprostane). In our biomonitoring study, we found significant associations between exposure biomarkers and oxidative stress biomarkers, which suggested that exposure to harmful compounds increased the oxidative damages in the human body. We also observed a linear relationship between the number of cigarette per day (median 17 cig/day in our cohort) and the concentrations of ΣPAH and ΣVOC metabolites in our cohort. For example, smoking 1 cigarette per day would increase urinary PAH metabolite concentrations by about 2 %, but this exposure was not detected for the effect biomarkers. Therefore, the oxidative stress biomarkers were far less sensitive. Further longitudinal cohort studies investigating a causal relationship between exposure, oxidative stress, and health effect outcomes are needed. We would suggest newer studies to target occupational cohorts that are often associated with greater and consistent exposures as opposed to temporal and spatial exposures associated with cigarette smoking. The great difficulty that is currently encountered with these oxidative stress biomarkers is their lack of specificity and sensitivity to exposure as well as to disease types. This limit currently hampers their use in health outcome studies.

4.6. Study strengths and limitations

Our study has several strengths. We measured a large number of exposure biomarkers (20 biomarkers of tobacco smoke exposure) and two oxidative stress biomarkers. We also had data on factors that influence relationships between the exposure biomarkers and effect biomarkers, such as health status and lifestyle habits. This was particularly important as the oxidative stress biomarkers are not just related to chemical exposures but also lifestyle factors, and can vary greatly between individuals. We were able to characterize exposure biomarkers and their variability from a single exposure source due to our cohort of smokers, and allowed us to assess the relative importance of factors influencing oxidative stress levels. This study had a convenience sampling design. We continuously included participants, as they were recruited to the larger clinical trial. Consequently, we did not perform power calculations before the study but our statistical analyses were finally robust. We had sufficient participants ($n = 270$) to detect possible associations between exposure and oxidative stress. One limitation is that urine collections and exhaled CO measurements were not collected at the same time thus possible relationships between the biomarkers in different biological media are still unknown. The non-inclusion of other known PAH metabolites such as hydroxyphenanthrene and hydroxyfluorene previously associated with exposure to cigarette smoking is another limitation, due to the lack of robust analytical methods (St Helen et al., 2012). The lack of information on cigarette brand and puff parameters for each participant is another limitation making the interpretation of partial correlation analyses between biomarkers of tobacco smoke exposure difficult, which was not the case when assessing associations with oxidative stress biomarkers.

5. Conclusion

We found statistically significant associations between two oxidative stress biomarkers and four different families of tobacco exposure biomarkers (TNE, ΣVOCs, ΣPAHs, and NNAL) in smokers, and we defined relative importance of these biomarkers on oxidative stress levels (calculated with effect size indicators) for the first time. They reflected exposures from cigarette smoking and were associated with of 8-oxodG and 8-isoprostane concentrations. We also showed that BMI had an inverse association with 8-oxodG concentrations and a positive association with 8-isoprostane concentration. We observed that daily consumption of fruits and vegetables reduced the 8-isoprostane concentration in smokers, but only to a small extent compared to effect of smoking that increased oxidative stress. Consequently, exposures to carcinogens and irritants in cigarette smoke are greatly associated to oxidative stress levels, and in particular exposures to PAHs and VOCs.

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CRediT authorship contribution statement

Nicolas Sambiagio: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Aurélie Berthet:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **Pascal Wild:** Conceptualization, Formal analysis, Methodology, Writing – review & editing. **Jean-Jacques Sauvain:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Reto Auer:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Anna Schoeni:** Data curation, Funding acquisition, Project administration, Writing – review & editing. **Nicolas Rodondi:** Funding acquisition, Investigation, Resources, Writing – review & editing. **Martin Feller:** Funding acquisition, Investigation, Resources, Writing – review & editing. **Jean-Paul Humair:** Funding acquisition, Investigation, Resources, Writing – review & editing. **Ivan Berlin:** Funding acquisition, Investigation, Resources, Writing – review & editing. **Florian Breider:** Investigation, Resources,

Validation, Writing – review & editing. **Dominique Grandjean:** Investigation, Resources, Validation, Writing – review & editing. **Nancy B. Hopf:** Conceptualization, Funding acquisition, Methodology, Supervision, Visualization, Writing – review & editing.

Data availability

Data will be made available on request.

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.2022.158361.

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