Influence of smoking on levels of urinary 8-iso Prostaglandin F2α

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1. Introduction

Cigarette smoking is one of the most important preventable risk factors for the development of atherosclerosis and cardiovascular disease (CVD) [1]. The main mechanisms through which smoking increases the risk of CVD include the alteration of lipid levels [2], inflammation, and oxidative stress, among other pathways [3]. However, the precise causative biochemical mechanisms behind the increased risk for disease in smokers are not completely understood [4], and the relationship between specific tobacco constituents and mechanistic steps involved in these diseases remains unclear [5].

Alternative products to cigarettes that can potentially reduce exposure and risk to smokers who would otherwise continue smoking are being developed and marketed. In order to assess whether the use of these products will translate into a reduction in risk and harm caused by smoking cigarettes, the scientific community needs to identify and validate biomarkers that are predictive of a reduction in disease risk [6]. The search for biomarkers must consider molecules that are involved in biological pathways known to be affected by cigarette smoking and smoking cessation, such as those involved in the inflammatory response [7,8] and oxidative stress [3]. 8-iso prostaglandin F2α (8-epi-PGF2α)1 is an endpoint that could potentially be used, because it is part of the family of isoprostanes. Among these, 8-epi-PGF2α has been examined in more detail [9] and has been proven to be a potent vasoconstrictor [10], mitogen, and mild pro-aggregatory agent [11], promoting atherogenesis [9]. In arterial blood, 8-epi-PGF2α levels increase with hyperlipidemia, cigarette smoking, and diabetes [9], and the measurement of urinary 8-epi-PGF2α levels has been shown to be a reliable marker for in vivo oxidative stress [12,13].

Several studies have compared levels of 8-epi-PGF2α in smokers and nonsmokers [5,14] and found that smokers tend to have higher levels of 8-epi-PGF2α, although results vary by sex [15]. This research summarizes the available literature on 8-epi-PGF2α levels in smokers and nonsmokers as well as the influence of smoking cessation on 8-epi-PGF2α levels.

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2. Materials and methods

2.1. Search for articles

Literature searches in Medline were performed through PubMed and Scopus to identify studies that evaluated the relationship between smoking or smoking cessation and 8-epi-PGF2α levels. The final search was performed on March 5, 2018.

The PubMed query was: ("prostaglandins"[MeSH Terms] OR "prostaglandins"[All Fields] OR "prostaglandin"[All Fields]) AND alpha [All Fields] AND ("tobacco"[MeSH Terms] OR "tobacco"[All Fields] OR "tobacco products"[MeSH Terms] OR ("tobacco"[All Fields] AND "products"[All Fields]) OR "tobacco products"[All Fields]) OR ("smoking"[MeSH Terms] OR "smoking"[All Fields]) OR cessation[All Fields] OR quitting[All Fields]). The query used in Scopus was: Prostaglandin alpha AND (tobacco OR smoking OR cessation OR quitting).

Retrieval of articles was limited to studies conducted in humans and written in English. To ensure that all available studies were retrieved, the reference lists of the publications obtained through the original search were reviewed to identify additional articles.

2.2. Study selection

The following criteria were used to include publications in the review:
- Case control or cohort studies (observational and experimental studies)
- Adult, healthy human populations were studied
- Measurements of 8-epi-PGF2α by exposure group are presented as mean values by group with the standard deviation (SD) or standard error (SE) of the mean, sample size per group, or with enough information to allow for the calculation of the mean and SD

The following criteria were used to exclude publications from the review:
- Review articles, case reports, or editorials
- Results were not reported in urine
- Reports had incomplete data or included data that could not be incorporated into the review
- Articles included diseased populations
- Data were re-used in a more recent study

2.3. Data extraction

Two researchers extracted the data independently; when discrepancies were identified in the data, the discrepancies were discussed, and consensus was reached for all items. The following information was extracted from each study: first author’s name, year of publication, study design and population characteristics, number of participants per group, mean, SD or SE.

Not all articles reported the measurements in the same units, so values were transformed to either pg/mg of creatinine or μg/24 h. Transformations were used to convert from the median and range to the mean using the calculations postulated by Hozor et al. [16].

2.4. Statistical analysis

Pooled means were calculated for each exposure group (smokers and nonsmokers) by weighting the individual studies using their inverse pooled variance. To quantify the effects of smoking on 8-epi-PGF2α levels, pooled mean differences between smokers and nonsmokers and 95% confidence intervals (95%CI) were calculated using the fixed effects and random effects models in Review Manager 5.3 (RevMan 5.3) (Cochrane Collaboration, Oxford, UK). These two methods are used because while a fixed effect meta-analysis assumes that all studies are estimating the same (fixed) treatment effect, a random effects meta-analysis allows for differences in the treatment effect (or exposure) from study to study (inter-study heterogeneity) [17]. The degree of heterogeneity between the study results was tested by the inconsistency statistic (I²). Funnel plots were used to evaluate publication bias [18]. Statistical significance was assessed at α = 0.05.

Fig. 1. Flow diagram – article retrieval process.
| Reference          | Country                  | Study design  | Study participants | Smoking definition | Subgroup | Units       | Adjustment | Units       | Study participants | Smoking definition | Subgroup | Units       | Adjustment |
|--------------------|--------------------------|---------------|--------------------|--------------------|----------|-------------|------------|-------------|--------------------|--------------------|----------|-------------|------------|
| Reilly et al. [45] | U.S.                     | Cross-sectional | 5 heavy smokers and 14 nonsmokers. Men aged 20–47 years | None | Smokers | Mean ± SD | 553.10 ± 214.42 | 169.53 ± 31.66 | 383.57 (194.90, 572.24) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Obata et al. [46]  | Japan                    | Cross-sectional | 81 smokers aged 37.6 ± 11.1 years and 39 nonsmokers aged 38.6 ± 10.9 years | None | All | Mean ± SD | 605.20 ± 59.00 | 424 ± 70.40 | 181.20 (135.62, 246.72) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Dillon et al. [47]| U.K.                     | Cross-sectional | 10 smokers aged 41 ± 4.1 years and 10 nonsmokers aged 41 ± 4.1 years | None | All | Mean ± SD | 1579.37 ± 266.36 | 852.36 ± 166.10 | 726.71 (532.08, 921.34) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Liang et al. [48]  | U.S.                     | Cross-sectional | 41 men and women aged 32–80 years | None | All | Mean ± SD | 500 ± 370 | 160 ± 70 | 340 (148.24, 531.76) pg/mg creatinine | Mean difference | Δ (95%CI) |
| Jacob et al. [49]  | U.S.                     | Cross-sectional | 77 healthy men aged 35 ± 9 years and 34 ± 7 years | None | All | Mean ± SD | 830 ± 930 | 730 ± 60 | 100.00 (–261.03, 461.03) μg/24 hours | Mean difference | Δ (95%CI) |
| Harman et al. [50] | U.S.                     | Cross-sectional | 80 smokers and 96 nonsmokers aged 19–80 years | None | All | Mean ± SD | 1100 ± 894.43 | 510 ± 391.92 | 590 (378.90, 801.10) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Zedler et al. [51] | U.S.                     | Cross-sectional | 36 smokers aged 35.8 ± 11.1 years and 65 nonsmokers aged 36 ± 13.6 years | None | Men | Mean ± SD | 2140.75 ± 980.25 | 1183.75 ± 398.75 | 957.00 (483.21, 1431.72) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Yan et al. [52]    | U.S.                     | Interventional  | 32 smokers aged 44 ± 9 years and 12 nonsmokers aged 44 ± 7 years | None | All | Mean ± SD | 853 ± 545 | 730 ± 430 | 140 ± 70 | 330 ± 160 | 340 (148.24, 531.76) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Taylor et al. [53] | U.S.                     | Cross-sectional | 25 participants men and women aged 18–35 years | None | All | Mean ± SD | 430.00 ± 146.97 | 380.00 ± 24.49 | 50.00 (30.78, 269.22) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Takeshita et al. [54] | Japan                 | Cohort         | 11 smokers aged 24 ± 2.2 years and 12 nonsmokers aged 24 ± 3.6 years | None | All | Mean ± SD | 520.00 ± 484.94 | 480.00 ± 146.97 | 40.00 (–49.31, 229.31) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Basu et al. [55]   | Sweden, Italy, and Poland | Cross-sectional | 217 smokers and 89 nonsmokers aged 17–66 years | None | All | Mean ± SD | 918.95 ± 475.53 | 781.00 ± 329.50 | 137.95 (223.22, 455.24) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Sakano et al. [56] | Japan                    | Cross-sectional | 321 subjects aged 20–45 years | None | All | Mean ± SD | 933.00 ± 402.10 | 781.00 ± 329.50 | 152.00 (–79.39, 383.39) μg/24 hours | Mean difference | Δ (95%CI) |
| Ghanap et al. [57] | Italy                    | Cross-sectional | 20 smokers and 20 never-smokers aged 23 ± 13 years and 20 above | None | All | Mean ± SD | 918.95 ± 475.53 | 781.00 ± 329.50 | 137.95 (223.22, 455.24) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Lowe et al. [58]   | U.K.                     | Cross-sectional | 80 men and women aged 21–40 years and above | None | All | Mean ± SD | 1066.00 ± 498.25 | 1066.00 ± 498.25 | 0.92 ± 0.64 | 680.00 (–550.99, 1030.99) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Andreoli et al. [59] | Italy                 | Cross-sectional | 22 twin pairs, men and women aged 21–40 years and above | None | All | Mean ± SD | 332.32 ± 144.72 | 194.44 ± 72.71 | 137.88 (50.56, 225.20) pg/mgcreatinine | Mean difference | Δ (95%CI) |
| Frost Pineda et al. [60] | France             | Cross-sectional | 232 smokers aged 43 ± 14 years and 1044 nonsmokers aged 47 ± 12 years | None | All | Mean ± SD | 1066.00 ± 498.25 | 1066.00 ± 498.25 | 0.92 ± 0.64 | 680.00 (–550.99, 1030.99) pg/mgcreatinine | Mean difference | Δ (95%CI) |

*Note: (continued on next page)*
### 3. Results

A flow diagram detailing the retrieval process of articles from the different sources used can be found in Fig. 1. There were 238 publications retrieved from the PubMed search and 705 retrieved from the Scopus search. Of these, 51 articles remained after screening for duplicates and review of the titles among the search results. The reference lists of these articles were reviewed, and 24 additional records were identified. In total, 75 abstracts were reviewed, and 54 articles remained for full review. For the analysis of smoking status and its association to 8-epi-PGF$_{2\alpha}$, a total of 46 publications that assessed the effect of smoking status were identified. Out of the 46 publications, 18 articles were included in the analyses.

Table 1 presents the characteristics for the 18 publications that were included in the analyses. The reasons for exclusion of 28 articles were that one evaluated the acute effects of smoking [19], one reported levels in bronchoalveolar lavage [20], two reported levels in exhaled breath condensate [21,22], one reported levels in lymphatic vessels [23], three reported plasma levels [24–26], one reported saliva levels [27], two reported levels in sputum [28,29], four reported data from diseased populations [30–33], eight had incomplete information [4,34–40], one presented log-transformed values [41], and four others reported units that could not be used [12,42–44]. A list of the 75 publications from which abstracts were screened can be found in Supplement 1. For the analysis to assess the effect of smoking cessation on 8-epi-PGF$_{2\alpha}$ levels, eight studies were identified, but only two had complete data that could potentially be used in a meta-analysis [45,60]. No meta-analysis was performed due to either incomplete information or lack of enough studies with the same follow-up time. Study characteristics can be found in Table 2.

#### 3.1. Effects of smoking status on 8-epi-PGF$_{2\alpha}$ levels

Due to studies reporting different measurement units, there were two meta-analyses performed. The first meta-analysis used concentrations adjusted for creatinine concentration (pg/mg creatinine), and the second used daily excretion (µg/24 h). The results of the meta-analyses can be found in Table 2. The meta-analysis included 15 studies reporting 18 comparisons [5,15,45–56,59]. The pooled analysis showed increased levels of 8-epi-PGF$_{2\alpha}$ in smokers compared with nonsmokers (mean difference: 172.38, 95%CI: 152.75, 192.01 pg/mg creatinine), and it showed significant heterogeneity ($I^2$: 89%, $p < 0.001$). The Forest plot for this meta-analysis can be found in Fig. 2. The random effect analysis confirmed the results (mean difference: 274.51, 95%CI: 186.16, 359.86 pg/mg creatinine). The Forest plot for this meta-analysis can be found in Fig. 3. The meta-analysis looking at daily excretion of 8-epi-PGF$_{2\alpha}$ included five studies with six comparisons [5,14,52,57,58], with the pooled mean difference showing increased levels in smokers compared with nonsmokers (mean difference: 0.16, 95%CI: 0.14, 0.19 µg/24 h). The Forest plot for this meta-analysis can be found in Fig. 4. The heterogeneity in this analysis was also significant ($I^2$: 98%, $p < 0.001$). The random effect analysis rendered the results not statistically significant (mean difference: 0.24, 95%CI: −0.05, 0.53 µg/24 h). The Forest plot for this meta-analysis can be found in Fig. 5. After inspection of the funnel plots (Figs. 6 and 7), there

#### Table 1

| Study design | Smoking definition | Smoking status | Study participants | Study participants | Study design | Country | Smoking definition | Reference |
|--------------|--------------------|----------------|--------------------|--------------------|--------------|---------|--------------------|-----------|
| Cross-sectional | Smokers aged 37 ± 14 and 38 ≤ 21 yrs | All | 22 smokers | Campos et al. [59] |
| Cross-sectional | Nonsmokers aged 21–65 yrs | All | 22 nonsmokers | Campos et al. [59] |
| Cross-sectional | Smokers aged 37 ± 14 and 38 ≤ 21 yrs | All | 204 men and women | Haswell et al. [14] |
| Cross-sectional | Nonsmokers aged 37 ± 14 and 38 ≤ 21 yrs | All | 204 non-smokers | Haswell et al. [14] |

#### Table 2

| Meta-analyses | Studies (estimates) | Mean difference (95%CI) |
|---------------|---------------------|-------------------------|
| µg/24 h       | 5 (6)               | 0.16 (0.14, 0.19)       |
| pg/mg creatinine | 15 (18)          | 172.38 (152.75, 192.01) |

| Mean difference (95%CI) |
|-------------------------|
| Fixed effects | $I^2$ (%) | Random effects |
| µg/24 h       | 0.24 (0.05, 0.53) |
| pg/mg creatinine | 274.51 (189.16, 359.86) |
was no evidence of publication bias in the meta-analyses.

3.2. Effects of smoking cessation on 8-epi-PGF$_2\alpha$ levels

The searches in PubMed and Scopus and the review of the reference lists yielded eight studies assessing the influence of smoking cessation on 8-epi-PGF$_2\alpha$ levels. Out of these eight studies, only two reported complete information. Therefore, no meta-analysis could be performed [45,60]. The results of these studies can be found in Table 3. The rest of the studies were performed in diseased populations [30,61], did not provide complete information [42,62,63], or compared differences between smokers and ex-smokers with unknown follow-up time [64].

Of the two studies reporting results, the study by Reilly et al. [45]
reports decreasing levels of 8-epi-PGF$_{2\alpha}$ after two to three weeks of smoking cessation, whereas the study by Lüdicke et al. [60] reports that there was an increase in 8-epi-PGF$_{2\alpha}$ levels 90 days after cessation.

4. Discussion and conclusions

The present study summarizes the evidence that smokers have higher 8-epi-PGF$_{2\alpha}$ levels compared with non-smokers via two meta-analyses, which had not been done previously. We performed meta-analyses of published articles on the association of smoking and 8-epi-PGF$_{2\alpha}$ levels. The retrieved studies presented data in different units; therefore, two meta-analyses were performed. The relationship of smoking cessation to 8-epi-PGF$_{2\alpha}$ levels could not be evaluated through meta-analyses, as not enough articles with complete information were identified. The results of the smoker to nonsmoker comparisons show that smokers had statistically significant higher levels of 8-epi-PGF$_{2\alpha}$. There was, however, very high inter-study heterogeneity, and after running random effects model meta-analyses, one of the results was no longer statistically significant. Because the random effects model takes into account the variability of the exposure effect, analyses under this model result in an estimate of the average effect rather than the common effect of smoking on 8-epi-PGF$_{2\alpha}$ levels [17]. Performing sensitivity analysis (looking into the heterogeneity that single studies contribute to the meta-analysis) in the pg/mg creatinine analysis, the studies by Basu et al. [15], Harman et al. [50], Takeshita et al. [54], Lowe et al. [5], Dillon et al. [47], and Zedler et al. [51] accounted for most of the heterogeneity, and excluding these studies lowered the inter-study heterogeneity significantly without changing the results of the meta-analysis (mean difference: 183.72, 95%CI: 160.70, 206.74, $p < 0.001$, $I^2$: 13%). Limiting the number of studies to Asian or Western countries did not decrease the heterogeneity $I^2$ value. Finally, in the meta-analysis using µg/24 h values, the studies by Frost Pineda et al. [65] and Lowe et al. [5] accounted for most of the inter-study heterogeneity, most likely because the reported values corresponded to the two highest [5,65] from the studies. Excluding these studies did not change the results of the meta-analysis (mean difference: 0.08, 95%CI: 0.05, 0.11, $p < 0.001$, $I^2$: 21% versus 0.16, 95%CI: 0.14-0.19, $I^2$: 98%), and the heterogeneity was no longer significant.

Despite the high heterogeneity found in the meta-analyses, these showed increased levels of 8-epi-PGF$_{2\alpha}$ in smokers compared with nonsmokers. On the other hand, 8-epi-PGF$_{2\alpha}$ levels do not seem to be affected by smoking cessation, as out of the two studies with complete data retrieved, one showed decreased levels after two to three weeks of quitting [45], while the second reported higher levels of 8-epi-PGF$_{2\alpha}$ 90 days after cessation [60].

Cigarette smoking is a strong risk factor for pulmonary disease as
well as CVD [66]. Smoking cessation is the recommended method of avoiding such increased risk [5], but cessation is also difficult to achieve [67]. Because of these facts, the U.S. Food and Drug Administration published draft guidelines for the tobacco industry for the marketing authorization of tobacco products that would decrease the exposure to tobacco toxicants and/or reduce the risk to tobacco-associated diseases [68]. One of the ways to approach the evaluation of risk reduction is through the usage of clinical risk endpoints [5]. Such endpoints should, in principle, be associated with smoking as well as influenced by smoking cessation, such as 8-epi-PGF2α.

These meta-analyses showed that 8-epi-PGF2α levels are elevated in smokers versus nonsmokers, while more studies assessing the changes in 8-epi-PGF2α after smoking cessation are needed to evaluate the reversibility of this marker as a clinical risk endpoint.

**Conflict of interest**

All authors are employees of Philip Morris International.

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**Transparency document**

The Transparency document associated with this article can be found in the online version.

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