Influence of smoking and smoking cessation on levels of urinary 11-dehydro thromboxane B₂

Angela van der Plas, Sandrine Poully, Guillaume de La Bourdonnaye, Wee Teck Ng, Gizelle Baker, Frank Lüdicke

Abstract

Background: Thromboxane is a key clinical risk endpoint of smoking-induced inflammation which has been associated in the pathogenesis of cardiovascular disease. The goal of this review is to quantify the effect of smoking and smoking cessation on one of its urinary metabolites, 11-dehydrothromboxaneB₂.

Methods: PubMed and SCOPUS were searched to identify publications which report urinary 11-dehydrothromboxaneB₂ levels in smokers and non-smokers, as well as articles reporting the effect of smoking cessation on urinary 11-dehydrothromboxaneB₂ excretion.

Results: We found ten studies assessing urinary 11-dehydrothromboxaneB₂ levels in smokers and non-smokers. Four papers reported the amount of urinary 11-dehydrothromboxaneB₂ excreted in 24 h while six reported the amount excreted adjusted for creatinine. The meta-analyses comparing the excretion of urinary 11-dehydrothromboxaneB₂ in current smokers to non-smokers report increased levels in current smokers (mean difference = 0.31 pg/24-h [95%CI: 0.27–0.34] and 166.45 pg/mg creatinine [95%CI: 120.51–212.40]). There were not enough publications to perform meta-analyses on the effects of smoking cessation on urinary 11-dehydrothromboxaneB₂ excretion.

Conclusions: Urinary 11-dehydrothromboxaneB₂ levels are increased in cigarette smokers, however, more data are needed to elucidate the effects of smoking cessation on urinary 11-dehydrothromboxaneB₂ excretion.

1. Introduction

Cigarette smoking is an important modifiable risk factors for cardiovascular diseases (CVD) such as myocardial infarction, sudden death and stroke [1–3]. For instance, women smokers of 25 or more cigarettes per day have a relative risk (RR) of 5.4 (95% CI: 3.0–10.4) for fatal coronary heart disease (CHD) and 5.8 (95% CI: 4.2–8.0) for nonfatal myocardial infarction in comparison to non-smokers [4] while in men, the RR for myocardial infarction (fatal and non-fatal) for smokers vs. non-smokers is 3.63 (95%CI: 3.03-4.35) [5]. After smoking cessation, the risks for cerebrovascular and ischemic heart disease reduce by 50% after 4.78 years (95%CI: 2.17-10.50) [6] and 4.40 years (95%CI: 3.26-5.95) [7] respectively.

Alternatives to cigarettes are being developed and marketed. These alternatives deliver nicotine but reduce the exposure to harmful chemicals and therefore have the potential to reduce the risk of smoking related diseases compared to continued smoking. As these products become available, it will be important to provide consumers accurate information about the potential risk reduction. In the absence of long term epidemiological studies, the evaluation of risk modification through the use of products substituting combustible tobacco products may not be timely enough to address the public health opportunity these new products may offer. Thus, the study of clinical risk endpoints has become an integral component of PMI’s assessment of how the reduction of toxicants in the inhaled aerosol by the consumer translates into a proxy of smoking-related disease.

Candidate endpoints of risk should be involved in biological pathways known to be affected by smoking, such as the inflammatory response or plaque formation on arterial walls [8,9]. One of the biomarkers highlighted in the CVD and smoking-related disease literature is thromboxane, which is reported as a mediator involved in the pathogenesis of cardiovascular diseases [10]. Smoking has been associated with enhanced thromboxane A2 release by platelets in healthy individuals [11] and several studies have assessed the levels of thromboxane A2 in the plasma of smokers compared to non-smokers [12,13]. As well, the excretion of the two major urinary metabolites of thromboxane A₂, namely 2,3-dinor-thromboxaneB₂ [1,14] and 11-

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ABSTRACT

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Conclusions: Urinary 11-dehydrothromboxaneB₂ levels are increased in cigarette smokers, however, more data are needed to elucidate the effects of smoking cessation on urinary 11-dehydrothromboxaneB₂ excretion.
dehydro-thromboxaneB2 (TXB2)\[^1\] have been studied. Researchers have also assessed the effect of smoking cessation [15] on urinary TXB2 levels, where the data show that as early as three days after smoking cessation (without nicotine substitution), the TXB2 levels were lowered to levels of about 75% (p-value < 0.01) of the baseline values, and after 14 days, the levels were reduced to 50% (p-value < 0.01) of the baseline values.

The aim of this research was to assess the association of smoking status and urinary levels of TXB2 by reviewing and analysing the published available literature on: a) urinary TXB2 levels in smokers vs. non-smokers and b) the influence of smoking cessation on urinary TXB2 levels.

2. Methods

Medline searches were performed through PubMed and additionally in the SCOPUS database, for publications that evaluated the relationship between smoking or smoking cessation and urinary TXB2 levels. The final search was performed on March 9th 2018. The following query was used in PubMed: (“thromboxanes”[MeSH Terms] OR “thromboxanes”[All Fields] OR “thromboxane”[All Fields]) AND (“smoking”[MeSH Terms] OR “smoking”[All Fields]) OR (“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 cessation[All Fields] OR quitting[All Fields]). In SCOPUS the following query was used: Thromboxane AND (smoking OR tobacco OR cessation OR quitting).

Retrieval of articles was limited to those written in English and considering human populations. To verify that all available publications were retrieved, the reference lists of the publications obtained through the original search were reviewed to identify any additional citation.

2.1. Study selection

The following criteria were used for including/excluding publications from the review:

\[^{1}\] TXB2 = 11-dehydrothromboxaneB2

- a) Inclusion Criteria:
  - Case control, cross-sectional, cohort or interventional studies such as randomized controlled trials
  - Adult healthy human populations
  - Measurements of TXB2 by exposure with the following measures available: mean values by group, standard deviation (SD) or standard error (SE) (of the mean), sample size per group or with enough information to allow for the calculation of mean and SD
  - Studies published from 1970 until March 9th 2018
- b) Exclusion Criteria:
  - Review articles, case reports or editorials
  - Studies with incomplete data
  - Studies where data had been re-used in a more recent study
  - Studies including diseased populations

2.2. Data extraction

Two researchers extracted data independently, discussed any disagreements and reached consensus on all items. The following information was extracted from each study: the first author’s name, year of publication, study design and population characteristics, number of participants per group, mean, standard deviation (SD) or standard error (SE). Not all articles reported the measurements in the same units, so only publications where the values could be transformed to either pg/mg or μg/24-h were used. Transformation from median and range values was performed according to the calculations postulated by Hozo et al. [16].

2.3. Statistical analysis

Pooled means levels of urinary TXB2 by exposure group (smokers and non-smokers) were calculated by weighting the individual studies by their inverse pooled variance. To quantify the effects of smoking and smoking cessation on TXB2, pooled mean differences between smokers and non-smokers and 95% confidence intervals (95% CIs) were calculated using the fixed-effects model in Review Manager version 5.0 (Cochrane Collaboration, Oxford, UK). The degree of heterogeneity between the study results was tested by the inconsistency statistic (I\(^2\)).
| Reference            | Country     | Study Design | Study participants                                                                 | Smoking Definition | Subgroup                      | Units | Adjustment |
|----------------------|-------------|--------------|-------------------------------------------------------------------------------------|-------------------|-------------------------------|-------|------------|
| Barrow et al. [19]   | UK          | Cross sectional | 67 males aged 38–39 years                                                           | None              | All 440 ± 295.8               | pg/mg | None       |
| Uedelhoven et al. [21]| Germany    | Cross sectional | 23 healthy men and women aged 26-56 years                                               | None              | All 673.2 ± 320.5             | pg/mg | None       |
| Uyama et al. [25]    | Japan       | Cross sectional | 44 male and female healthy participants aged 31–80 years                                | 5 CPD +           | All 1063 ± 244                | pg/mg | None       |
| McAdam et al. [22]   | USA         | RCT          | 32 healthy male smokers and non-smokers aged 20–40 years                               | None              | Coxib group 284 ± 107.2       | pg/mg | None       |
| Zedler et al. [24]   | USA         | Cross sectional | 115 men and women at least 21 years                                                   | 1 CPD + for the past year | Placebo group 279 ± 103.1     | pg/mg | None       |
| Calapai et al. [10]  | Italy       | Cross sectional | 60 healthy Caucasian men and women aged 23–43 years                                   | Group B – intake below 60 mg tar/day; Group C – intake of more than 180 mg tar/day | All 1670 ± 290.7 | pg/mg | None       |
| Frost-Pineda et al. [36] | USA     | Cross sectional | 3346 adult smokers and 1051 non-smokers aged 21 or older. Men and women                | 1 CPD + for at least 1 year | All 1.34 ± 1.04               | μg/24-h | None       |
| Andreoli et al. [18] | Italy       | Cross sectional | 22 sets of healthy monozygotic twins discordant for smoking status. Men and women aged 23–46 years | Tar intake of ≥ 60 mg | All 3.02 ± 1.14               | μg/24-h | None       |
| Lowe et al. [14]     | USA         | Cross sectional | 20 smokers and 20 non-smokers aged 21 + years                                          | 20 CPD + ISO yield from pack 10 mg tar | All 1.39 ± 0.81               | μg/24-h | None       |
| Haswell et al. [21]  | Germany     | Cohort       | 265 men and women aged 23-55 years                                                    | 10-30 CPD with an ISO tar yield of 6-8 mg. | All 0.8 ± 0.35               | Ug/24-h | None       |

CPD: Cigarettes per day, RCT: randomized controlled trial.
Funnel plots were used to evaluate publication bias \[17\]. Statistical significance was assessed at $\alpha = 0.05$.

### 3. Results

A flow diagram detailing the article retrieval process from the different sources used can be found in Fig. 1. For the analyses of urinary TXB2 levels and its association to smoking status, a total of 21 studies were identified where the levels of urinary TXB2 were compared in smokers vs. non-smokers. Of the 21 studies, ten were included in the meta-analyses \[10,14,18-25\]. The other 11 studies were not included due to having incomplete information \[26,27\], results given in different units \[28-31\], study results had been published in another study \[1,32\], measurements were reported from plasma concentrations \[13\] or included diseased populations \[33,34\]. Characteristics of all studies included in the analyses can be found in Table 1. The included studies reported one of two kind of measurements of urinary TXB2, namely, six reported spot urine or 24-h urine TXB2 concentration corrected for creatinine concentration \[10,19,22-24,35\] whilst four reported the total urinary excretion of TXB2 over 24-h \[14,18,36\]. For the analysis of the effect of smoking cessation on urinary TXB2 levels, three studies \[15,26,37\] were identified, but no meta-analysis could performed because the studies had different follow-up duration, ranging from 3 days to 14 days Their characteristics can be found in Table 2.

#### 3.1. Effects of smoking status on urinary TXB2 levels

Of the 21 studies assessing the levels of TXB2 in smokers vs. non-smokers, ten reported data that could be used in the meta-analyses. The pooled-mean comparison analysis showed that excretion of TXB2 over 24-h urine was higher in smokers than non-smokers (pooled mean 532.62 pg/24-h in smokers vs. 366.2 pg/24-h in non-smokers). The same was found in the analysis of excretion adjusted to creatinine (1.12 pg/mg creatinine in smokers vs. 1.04 pg/mg creatinine in non-smokers). These results show an increase of 30–45% in TXB2 levels in smokers compared with non-smokers. The meta-analysis reporting 24-h urinary excretion of TXB2 (pg/24-h urine) included three studies \[14,18,36\] and showed statistically significantly increased levels of TXB2 in smokers vs. non-smokers as seen in Table 2 (Mean Difference = 0.30 pg/mg creatinine [95% CI: 0.27–0.34], $p < 0.00001$). The inter-study heterogeneity in this meta-analysis was not statistically significant ($I^2 = 58\%$, $p = 0.09$) (Table 2). The meta-analysis of urinary TXB2 levels adjusted for creatinine excretion (pg/mg creatinine) included six studies which provided seven estimates. This analysis also found a statistically higher TXB2 level in smokers vs. non-smokers (Mean Difference = 166.45 pg/24-h [95% CI: 120.51–212.40], $p < 0.00001$). In this comparison, however, the inter-study heterogeneity was statistically significant ($I^2 = 81\%$, $p < 0.00001$) (Table 2).

Funnel plots are presented in Fig. 2 (for pg/24-h urine results) and Fig. 3 (for pg/mg creatinine). The evaluation of the funnel plots of both analyses does not point towards publication bias.

#### 3.2. Effects of smoking cessation on urinary TXB2 levels

There were three studies assessing the effects of smoking cessation on urinary TXB2 levels \[15,26,37\]. All three studies found that smoking cessation reduced urinary TXB2 excretion. Urinary TXB2 levels decreased as early as three days after cessation \[15,37\]. The study by Rangemark et al. \[37\] reported a decrease to 60% of baseline values after 3 days of smoking abstinence, while the study by Benowitz et al.
TXB2 levels were 611 ± 47 pg/mg creatinine in the period of CC smoking, 479 ± 34 pg/mg creatinine during the placebo treatment, and 496 ± 33 pg/mg creatinine in the period of transdermal nicotine application.

**Benowitz et al. [26]** US RCT 12 healthy male smokers Participants went through three different phases, cigarette smoking, nicotine chewing gum and nicotine patches as a substitution therapy, lasting 5 days.

Three days after smoking cessation without nicotine substitution, 11-dehydrothromboxane B2 levels were lowered to 75% \((P < 0.01)\) of the initial values, and after 14 days to 50% \((P < 0.01)\).

**Saareks et al. [15]** Finland Cohort 60 men and women aged 20–45 years Fifteen smokers quit smoking without nicotine substitution, 15 used nicotine chewing gum and 30 used nicotine patches as a substitution therapy.

No treatment study by Saareks et al. [15] did not report actual values and the follow-up time was different in all studies, a meta-analysis could not be performed on the effect of cessation. The characteristics of the studies are found in Table 3.

### 4. Discussion

The analyses showed that smokers had statistically significantly higher levels of urinary TXB2 in the pooled analyses. The 24-h excretion of urinary TXB2 in smokers was increased by 30% compared to non-smokers, while the amount of urinary TXB2 excreted adjusted for creatinine was increased by 45%. This is an important finding as levels of urinary TXB2 are associated with poorer prognosis in cardiovascular disease [39–42].

There was substantial inter-study heterogeneity in the analysis of urinary TXB2 excreted adjusted for creatinine levels \((I^2 = 81\%)\) however as proposed by von Hippel [43], when performing meta-analysis of only a few studies, the \(I^2\) values can be biased. In any case, the results of this meta-analysis should be interpreted with caution. There were not enough studies to perform meta-analyses on the effects of smoking cessation on urinary TXB2 levels, but all publications showed that cessation reduced levels of urinary TXB2, compared to baseline. Furthermore, the increased levels of TXB2 in smokers compared to non-smokers are in line with the levels seen at baseline in the smoking cessation studies where the TXB2 levels were up to 78% higher before quitting smoking [15].

Cigarette smoking is a strong risk factor for pulmonary as well as cardiovascular diseases [44]. Smoking cessation is the recommended method for avoiding such increased risk [1–4], but quitting has been proven difficult to achieve [45]. The FDA published draft guidelines on modified risk tobacco products (MRTPs) [46], which have led to the evaluation of risk reduction through the use of clinical risk markers [14], which should, in principle, be associated with smoking as well as be influenced by smoking cessation. One of the proposed clinical risk endpoints is thromboxane A2 [24]. Thromboxane A2 is produced by activated platelets, macrophages and neutrophils, it causes platelet aggregation and is a potent vasoconstrictor [47], hence it is a marker for platelet activation [11] and it has also been associated with a number of cardiovascular disorders [18]. Measurements of systemic thromboxane A2 have been proven difficult to test because of the complexity of measuring its production in vivo, with an extremely short half-life [19], nevertheless, other ways of assessing thromboxane production in a less invasive manner include the determination of its urinary metabolites, one of which is TXB2 [48]. In actuality, TXB2 measurements are used as a prognostic tool in acute myocardial infarction patients [40], for mortality in patients with stable coronary artery disease [39,41] and as a marker of vascular inflammation and prognostic in atherosclerotic cardiovascular disease [42].

The Cochrane Collaboration (www.chochone.org) recommends meta-analyses as a statistical method to combine results of individual studies to allow researchers to make the best use of all available data and therefore increase the power of the analysis. As much as meta-analyses are a robust method, it has limitations, mainly concerning the identification of studies, inter-study heterogeneity and the availability of information [49]. For the smokers vs. non-smokers comparison there were 21 studies in the PubMed, SCOPUS and reference lists, but only ten had complete and useful information on TXB2 values that could be combined. For the effects of smoking cessation, only three studies were retrieved. One of the analyses of TXB2 levels between smokers and non-smokers (comparison in μg/24-h) showed no significant inter-study heterogeneity, thus facilitating the interpretation of results, while the second analysis (of studies reporting TXB2 levels in pg/mg creatinine) showed high inter-study heterogeneity, mainly due to the wide range of

| Study | Country | Study Design | Study Participants | Treatment | Findings |
|-------|---------|--------------|---------------------|-----------|----------|
| Benowitz et al. [26] | US | RCT | 12 healthy male smokers | Participants went through three different phases, cigarette smoking, nicotine chewing gum and nicotine patches as a substitution therapy, lasting 5 days. | TXB2 levels were 611 ± 47 pg/mg creatinine in the period of CC smoking, 479 ± 34 pg/mg creatinine during the placebo treatment, and 496 ± 33 pg/mg creatinine in the period of transdermal nicotine application. Three days after smoking cessation without nicotine substitution, 11-dehydrothromboxane B2 levels were lowered to 75% \((P < 0.01)\) of the initial values, and after 14 days to 50% \((P < 0.01)\). |
| Saareks et al. [15] | Finland | Cohort | 60 men and women aged 20–45 years | Fifteen smokers quit smoking without nicotine substitution, 15 used nicotine chewing gum and 30 used nicotine patches as a substitution therapy. | No treatment study by Saareks et al. [15] did not report actual values and the follow-up time was different in all studies, a meta-analysis could not be performed on the effect of cessation. |
| Rangemark et al. [37] | Sweden | Cohort | 8 women aged 23–45 years | Baseline excretion of 11-DTX-B2 was 586 ± 41 pg/mg creatinine (mean ± SEM), which fell to about 41 pg/mg creatinine \((P < 0.01)\). |

RCT: randomized controlled trial.
values reported per individual study. Additionally, there is always the possibility that a researcher's bias could occur despite of the good design of the analyses. For this reason, two researchers performed the data extraction and discussed any discrepancies. Another potential limitation of this study is the possibility of publication bias [49], however, after evaluation of the funnel plots it does not seem that either analysis points towards publication bias. Finally, there is the possibility of confounding factors, as nine of the ten studies found in the comparison of urinary TXB2 levels in smokers vs. non-smokers were observational in design and the mean values reported were not adjusted for possible confounding variables. However, the fact that the three studies evaluating the effect of smoking cessation on urinary TXB2 levels show a marked decrease after quitting [37], suggests that there is indeed an effect of smoking in urinary TXB2 levels.

5. Conclusion

This meta-analyses show that urinary TXB2 is a clinical risk end-point that is significantly increased in cigarette smokers compared to non-smokers. The reviewed data further indicate that urinary TXB2 levels are reversible as early as three to five days after smoking cessation as TXB2 levels decrease after quitting, although longer follow-up research is needed to understand whether the level of change after cessation could impact clinical disease outcomes. These data suggest that urinary TXB2 could be a potential candidate endpoint with which to assess risk reduction of candidate MRTPs.

Transparency document

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

References

[1] G. Calapai, A.P. Caputi, C. Mannucci, E.O. Gregg, A. Pieratti, G. Aurora Russo, et al., A cross-sectional investigation of biomarkers of risk after a decade of smoking, Inhal. Toxicol. 21 (13) (2009) 1138–1143, http://dx.doi.org/10.1080/08958370902794645 PubMed PMID: 19852556.

[2] O.M. Burns, Epidemiology of smoking-induced cardiovascular disease, Prog. Cardiovasc. Dis. 46 (1) (2003) 11–29 PubMed PMID: 12902698.

[3] U.S. Department of Health and Human Services, The Health Consequences of Smoking: a Report of the Surgeon General, US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, Atlanta, GA, 2004.

[4] W.C. Willett, A. Green, M.J. Stampfer, F.E. Speizer, G.A. Colditz, B. Rosner, et al., Relative and absolute excess risks of coronary heart disease among women who smoke cigarettes, N. Engl. J. Med. 317 (21) (1987) 1303–1309, http://dx.doi.org/10.1056/NEJM198711193172102 PubMed PMID: 3683458.

[5] A.A. Gehani, A.T. Al-Hinai, M. Zubaid, W. Almahmeed, M.R. Hasani, A.H. Yusufali, et al., Metabolic and respiratory response in groups of established smokers after a decade of cigarette smoking, Basic Clin. Pharmacol. Toxicol. 104 (4) (2009) 222–238, http://dx.doi.org/10.1111/j.1742-7843.2008.00361.x PubMed PMID: 19175368.

[6] P.N. Lee, J.S. Fry, A.J. Thornton, Estimating the decline in excess risk of cerebrovascular disease following quitting smoking–a systematic review based on the negative exponential model, Regul. Toxicol. Pharmacol. 65 (1) (2014) 400–410, http://dx.doi.org/10.1016/j.yrtph.2014.12.005 PubMed PMID: 23125402.

[7] P.N. Lee, J.S. Fry, A.J. Thornton, Estimating the decline in excess risk of cerebro-vascular disease following quitting smoking–a systematic review based on the negative exponential model, Regul. Toxicol. Pharmacol. 65 (1) (2014) 85–95, http://dx.doi.org/10.1016/j.yrtph.2013.11.013 Epub 2013/12/03, PubMed PMID: 24291341.

[8] P.N. Lee, J.S. Fry, J.S. Hamling, Using the negative exponential distribution to quantitatively review the evidence on how rapidly the excess risk of ischaemic heart disease declines following quitting smoking, Regul. Toxicol. Pharmacol. 80 (2) (2015) 56–67, http://dx.doi.org/10.1016/j.yrtph.2015.01.016 Epub 2015/01/28 PubMed PMID: 25723866.

[9] J. Nowak, L.J. Murray, J.A. Oates, G.A. FitzGerald, Biochemical evidence of a chronic abnormality in platelet and vascular function in healthy individuals who smoke cigarettes, Circulation 76 (1) (1987) 6–14 PubMed PMID: 3297389.

[10] A Japanese cross-sectional multi-centre study of biomarkers associated with cardiovascular disease in smokers and non-smokers, Biomarkers 20 (6-7) (2015) 411–421, http://dx.doi.org/10.3109/13820691.2015.1071942 PubMed PMID: 2661146.

[11] J. Liu, Q. Liang, K. Frost-Pineda, R. Muhamed-kah, L. Rimmer, H. Roethig, et al., Relationship between biomarkers of cigarette smoke exposure and biomarkers of inflammation, oxidative stress, and platelet activation in adult cigarette smokers, Cancer Epidemiol. Biomarkers Prev. 20 (8) (2011) 1760–1769, http://dx.doi.org/10.1158/1055-9965.EPI-10-0987 PubMed PMID: 21708936.

[12] L.E. Haswell, E. Duarte, L.M. Dusse, A.P. Fernandes, A.A. Bosco, et al., Acetylsalicylic acid therapy: in vivo in chronic cigarette smokers, Circulation 94 (1) (1996) 19–23, http://dx.doi.org/10.1161/01.CIR.94.1.19 PubMed PMID: 8907252.

[13] G. Calapai, A.P. Caputi, C. Mannucci, G.A. Russo, E. Gregg, R. Puntoni, et al., Cardiovascular biomarkers in groups of established smokers after a decade of smoking, Basic Clin. Pharmacol. Toxicol. 104 (4) (2009) 222–238, http://dx.doi.org/10.1111/j.1742-7843.2008.00361.x PubMed PMID: 19175368.
treatment, Am. Heart J. 149 (5) (2005) 832–839, http://dx.doi.org/10.1016/j.ahj.2004.08.030 PubMed PMID: 15894964.

[34] A.P. DeFilippis, S.N. Rai, A. Cambon, R.J. Miles, A.S. Jaffe, A.B. Moser, et al., Fatty acids and TxA(2) generation, in the absence of platelet-COX-1 activity, Nutr. Metab. Cardiovasc. Dis. 24 (4) (2014) 428–433, http://dx.doi.org/10.1016/j.numecd.2013.08.012 PubMed PMID: 24370448; PubMed Central PMCID: PMC4409424.

[35] O. Uyama, S. Shimizu, T. Nakanishi, H. Nakahama, A. Takiguchi, Y. Hayashi, et al., Urinary 11-dehydro-thromboxane B2: a quantitative index of platelet activation in cerebral infarction, Intern. Med. 31 (6) (1992) 735–739 PubMed PMID: 1392173.

[36] K. Frost-Pineda, Q. Liang, J. Liu, L. Rimmer, Y. Jin, S. Feng, et al., Biomarkers of potential harm among adult smokers and nonsmokers in the total exposure study, Nicotine Tob. Res. (2011), http://dx.doi.org/10.1093/ntr/ntq235 Epub 2011/02/19, PubMed PMID: 21330277.

[37] C. Rangemark, G. Ciabattoni, A. Wennmalm, Excretion of thromboxane metabolites in healthy women after cessation of smoking, Arterioscler. Thromb. 13 (6) (1993) 777–782 PubMed PMID: 8499397.

[38] G. Schwarzer, J.R. Carpenter, G. Rucker, Fixed effect and random effects meta-analysis, Meta-Anal. R. XII (2015) 252.

[39] P.A. McCullough, A. Vasudevan, M. Sathyamoorthy, J.M. Schussler, C.E. Velasco, L.R. Lopez, et al., Urinary 11-dehydro-thromboxane B2 and mortality in patients with stable coronary artery disease, Am. J. Cardiol. 119 (7) (2017) 972–977, http://dx.doi.org/10.1016/j.amjcard.2016.12.004 PubMed PMID: 28139223.

[40] W. Szczeklik, E. Stodolakiewicz, M. Rzeszutko, M. Tomala, A. Chrustowicz, K. Zmudka, et al., Urinary 11-dehydro-thromboxane B2 as a predictor of acute myocardial infarction: results of leukotrienes and thromboxane in myocardial infarction (LITMI) study, J. Am. Heart Assoc. 5 (8) (2016), http://dx.doi.org/10.1161/JAHA.116.003702 PubMed PMID: 27481134; PubMed Central PMCID: PMC5015290.

[41] A. Vasudevan, K.M. Teeson, J. Bennett-Firmin, T. Bottiglieri, I.R. Lopez, M. Peterson, et al., Prognostic value of urinary 11-dehydro-thromboxane B2 for mortality: A cohort study of stable coronary artery disease patients treated with aspirin, Catheter. Cardiovasc. Inter. (2017) 1–6, http://dx.doi.org/10.1002/ccd.27437 PubMed PMID: 29193683.

[42] N. Wang, K.C. Vendrov, B.P. Simmons, R.N. Schuck, G.A. Stouffer, C.R. Lee, Urinary 11-dehydro-thromboxane B2 levels are associated with vascular inflammation and prognosis in atherosclerotic cardiovascular disease, Prostaglandins Other Lipid Mediat. 134 (2018) 24–31, http://dx.doi.org/10.1016/j.prostaglandins.2017.11.003 PubMed PMID: 29155368; PubMed Central PMCID: PMC5038341.

[43] P.T. Von Hippel, The heterogeneity statistic I^2 can be biased in small meta-analyses, BMC Med. Res. Methodol. 15 (35) (2015).

[44] R. Doll, R. Peto, Mortality in relation to smoking: 20 years' observations on male British doctors, Br. Med. J. 2 (6051) (1976) 1525–1536 PubMed PMID: 1009386; PubMed Central PMCID:PMC1690096.

[45] J.O. Prochaska, C.C. DiClemente, Stages and processes of self-change of smoking: toward an integrative model of change, J. Consult. Clin. Psychol. 51 (3) (1983) 390–395 PubMed PMID: 6865699.

[46] FDA (Food and Drug Administration), Guidance for Industry—Modified Risk Tobacco Product Applications - Draft Guidance, (2012).

[47] M. Hamberg, J. Svensson, B. Samuelsson, Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides, Proc. Natl. Acad. Sci. U. S. A. 72 (8) (1975) 2994–2998 PubMed PMID: 1059088; PubMed Central PMCID: PMC542905.

[48] J.A. Lawson, C. Patrono, G. Ciabattoni, G.A. Fitzgerald, Long-lived enzymatic metabolites of thromboxane B2 in the human circulation, Anal. Biochem. 155 (1) (1986) 198–205 PubMed PMID: 3087234.

[49] E. Walker, A.V. Hernandez, M.W. Kattan, Meta-analysis: its strengths and limitations, Cleve Clin. J. Med. 75 (6) (2008) 431–439 PubMed PMID: 18595551.