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APPENDIX A6.1.4.5 8_ISO_PGF2ALPHA HEALTH EFFECTS OF SMOKING CESSATION

Short Term Health Effects

Iso-8-Epi-Prostaglandin (PG)$F_{2\alpha}$

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1. BACKGROUND

Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defence mechanisms. Under physiological conditions, ROS are only present at moderate levels and play an important role as messengers in redox signalling. In pathological conditions, the physiological redox state of cells is disturbed and ROS can severely affect cellular signalling and function. When ROS are not neutralised by antioxidant molecules, they may damage all components of the cell, including proteins, lipids, and DNA (1). Free radicals can be generated endogenously from various sources (for example, mitochondria and oxidative burst during phagocyte activation) or derived from exogenous sources such as environmental toxins and cigarette smoke. Oxidative stress is now recognized to be a prominent feature of many acute and chronic diseases including cancer, cardiovascular disease, neurodegenerative disease, lung disease and even the normal aging process (2).

The oxidation of cellular lipids, typically referred to as lipid peroxidation, is a central feature of oxidative stress. Exposure of lipids to free radicals induces a non-enzymatic reaction cascade resulting in an increased formation of bioactive molecules named isoprostanes. Consequently, systemic isoprostane formation is significantly increased in a variety of pathological processes associated with oxidative stress Isoprostanes are increasingly recognized not only as markers of oxidative stress but also as mediators of disease progression (1).

The isoprostanes, first described in 1990 by researchers at Vanderbilt University, USA (3), (4), are a family of eicosanoids of non-enzymatic origin produced by the random oxidation of tissue phospholipids by oxygen radicals. Isoprostanes appear as artifacts in tissue and plasma samples which have undergone oxidative degradation during prolonged or improper storage. They are present in detectable amounts in all normal tissues and biological fluids, and are elevated by oxidative stress. Isoprostane 8-Epi-prostaglandin F2 alpha (Iso-8-epi-$PGF_{2\alpha}$) is a nonenzymatic, free radical-catalyzed peroxidation product of arachidonic acid that has potent biological activity, including contraction of vasculature and inhibition of aggregation induced by thromboxane A2 mimetics. It is a potent pulmonary and renal vasoconstrictor and has been implicated as a causative mediator of hepatorenal syndrome and pulmonary oxygen toxicity. Iso-8-epi-$PGF_{2\alpha}$ has been proposed as a marker of antioxidant deficiency and oxidative stress and elevated levels have been found in heavy smokers. 8-epi-$PGF_{2\alpha}$ can be measured in plasma, urine, exhaled breath condensate and induced sputum. Although the association between increased oxidative stress and disease does not necessarily imply a causative link, the fact that the increase in Iso-8-epi-$PGF_{2\alpha}$ levels is an early event in asthma, hepatic cirrhosis, scleroderma, and Alzheimer's disease suggests a causative role for oxidative stress at least in these diseases. Chronic healthy smokers have higher free and esterified F2-isoprostane plasma concentrations and urinary excretion of F2-isoprostane metabolites compared than healthy nonsmokers, indicating pro-oxidant effects of smoking in vivo (Figure 1). Since LDL oxidation may lead to atherosclerosis, these findings may provide a causative link between smoking and the development of atherosclerosis (2)

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However, there is a potential for an alternate pathway of biomarker synthesis, which draws into question the source of increases in Iso-8-epi-$PGF_{2\alpha}$; increases in Iso-8-epi $PGF_{2\alpha}$ do not necessarily reflect increases in oxidative stress; there is potential ambiguity in solely measuring Iso-8-epi $PGF_{2\alpha}$ which may be considered inadequate as an index of oxidative stress (5).

The aim of the present research was to identify published work relating to 8-epi-$PGF_{2\alpha}$ as a biomarker of oxidative stress relating to smoking conventional cigarettes, the effects of smoking and smoking cessation on the levels of the biomarker and its relationship to CVD and respiratory diseases.

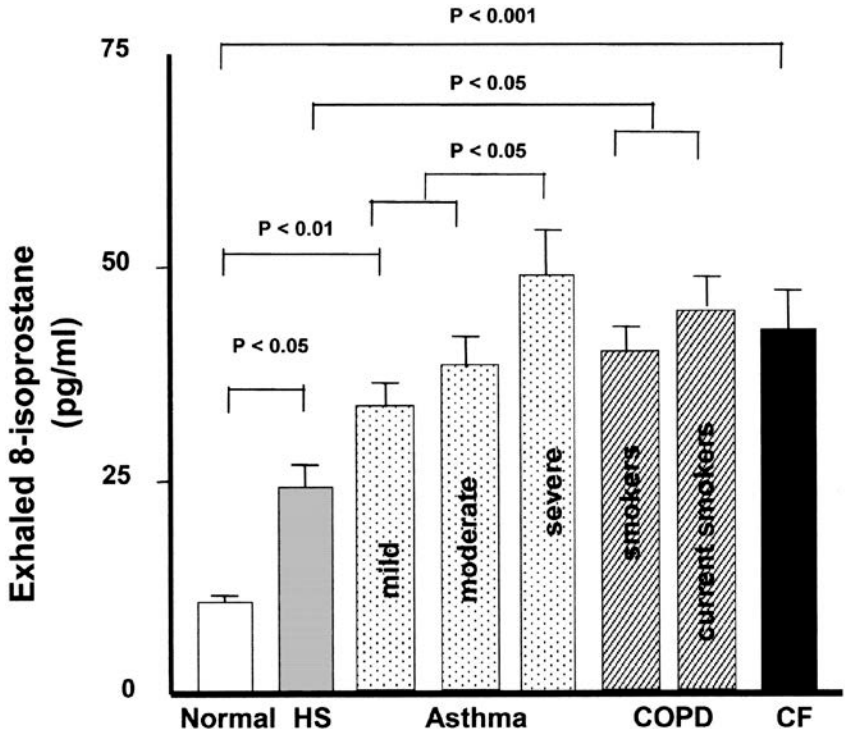


Figure 1 Exhaled breath condensate Iso-8-epi-PGF2α levels according to various respiratory status. Adapted from (2)

8-Isoprostane (15-F2t-isoprostane) concentrations in exhaled breath condensate in healthy nonsmokers (normal, n=10), healthy current smokers (HS, n=12), patients with mild (n=12), moderate (n=15), and severe (n=17) asthma, patients with chronic obstructive pulmonary disease (COPD) who were ex-smokers (n=25) or current smokers (n=15), and patients with cystic fibrosis (n=19). 8-Isoprostane concentrations were measured by an enzyme immunoassay. Values are expressed as means ± sem.

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2. METHODS

Three searches of the PubMed literature database were conducted on June 29th, 2015 for English language articles within the time frame of 2008 to the present day.

Search 1: To identify literature that linked smoking or nicotine replacement to 8-epi-$PGF_{2\alpha}$ using the search string: (8-epi-prostaglandin F2alpha OR 8-epi-PGF2)) AND (smokers OR smoking OR nicotine replacement OR cessation) AND ("2008"[Date - Publication]: "2015"[Date - Publication])))) AND English[Language]).

Search 2: To identify literature reporting an association between 8-epi-$PGF_{2\alpha}$ and cardiovascular disease using the search string: (8-epi-prostaglandin F2alpha OR 8-epi-PGF2)) AND ((cardiovascular disease) AND (association)) AND ("2008"[Date - Publication]: "2015"[Date - Publication]))) AND English[Language]).

Search 3: To identify literature reporting an association between 8-epi-$PGF_{2\alpha}$ and respiratory disease using the search string: (8-epi-prostaglandin F2alpha OR 8-epi-PGF2)) AND ((respiratory disease) AND (association)) AND ("2008"[Date - Publication]: "2015"[Date - Publication]))) AND English[Language]).

Among the identified studies a set of specific criteria was applied to determine which papers should be selected for inclusion within the report.

The following inclusion criteria were applied:

- Studies investigating the association of cigarette smoking, smoking cessation or nicotine replacement therapy (NRT) on Iso-8-epi $PGF_{2\alpha}$;
- Studies investigating the association of 8-epi-PGF2α with cardiovascular disease or respiratory disease.

The following exclusion criteria were applied:

1. Studies that used the measurement of Iso-8-epi $PGF_{2\alpha}$simply for control purposes (e.g., results were adjusted for differences in 8-epi-$PGF_{2\alpha}$ between populations, but Iso-8-epi $PGF_{2\alpha}$itself was not an endpoint)
2. Studies involving dietary, pharmacological or exercise interventions in which Iso-8-epi-$PGF_{2\alpha}$ was an endpoint, but lacking a clear link between Iso-8-epi $PGF_{2\alpha}$and either a disease of relevance or smoking
3. Studies in patients with a disease not covered by the search categories (e.g., cancer, HIV infection, periodontitis) where there was no clear link between Iso-8-epi-$PGF_{2\alpha}$ and either a disease of relevance or smoking
4. Studies of passive or parental smoking
5. Studies showing a link between Iso-8-epi $PGF_{2\alpha}$and either a disease of relevance or smoking, but in a defined, narrow population (e.g., pregnant women,

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individuals with a particular haplotype, individuals with a specific disease) or tissue (e.g., retina, gums)

6. Studies reporting results from In vivo animal experiments
7. Studies in which Iso-8-epi $PGF_{2\alpha}$was measured for toxicological or forensic purposes
8. Studies with no comparison between smoking and non-smoking/switching/cessation
9. Iso-8-epi $PGF_{2\alpha}$not measured or presented as AUC

3. FINDINGS

Searches 1, 2 and 3 produced 52, 15 and 14 articles respectively. From the 81 references identified, 3 were common to 2 or 3 searches, leaving 77 unique references, of which 18 were considered for inclusion (see Table 1).

The literature searches identified 18 articles that met the inclusion and exclusion criteria: 14 smoking-related studies (smokers vs. non-smokers: (6), (7), (8), (9), (10), (11), (12), (13), (14); smokers vs. non-smokers vs. ex-smokers: (15), (16), (17), (18), (19), 3 cardiovascular disease studies [(20), (21), (22)], and 1 article about Iso-8-epi $PGF_{2\alpha}$ and respiratory diseases (23). The information from these articles was used in the preparation of this document.

The reasons for the exclusion of articles from the review are described below in Table 1, which provides an overview of the results from the three searches together with the reasons for exclusion.

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Table 1. Search results and distribution across the exclusion criteria.

Search	1	2	3	1, 2	1, 3	2, 3	Total
Number of different articles retrieved	49	12	12	2	1	1	77
Articles discarded due to:							
E1: Iso-8-epi PGF2αused only as marker of exposure	7	1		1			9
E2: Iso-8-epi PGF2αlacked link to smoking	2	3					5
E3: Disease not covered by search categories	13	1	6	1	1		22
E4: Passive or parental smoking	1						1
E5: Narrow population	7	3	5			1	16
E6: Animal		1					1
E7: Iso-8-epi PGF2α measured for forensic purposes	4						4
E8: No comparison to smoking /cessation or switch	1						1
E9: Iso-8-epi PGF2α not measured	7	3	3			1	14
Total discarded	35	9	9	2	1	1	57
Articles selected	14	3	1				18

The 77 articles retrieved by the three searches are listed in Appendix 2 - Appendix 9 with those excluded according to the above selection/exclusion criteria grouped related to their reason for exclusion (E1 – E9).

4. BIOMARKER-DISEASE LINK

The searches retrieved no literature reporting short-term or long-term links between Iso-8-epi-PGF2α and cardiovascular or respiratory diseases. All articles retrieved used Iso-8-epi PGF2α8-epi-PGF2α as a marker of oxidative stress only to try and establish a link between oxidative stress and cardiovascular or asthma conditions

4.1. Cardiovascular disease

Three articles were retrieved, reporting a link between Iso-8-epi-$PGF_{2\alpha}$ and endothelial dysfunction, coronary heart disease and heart failure.

One cross-sectional study (20) evaluated the relationship between Iso-8-epi $PGF_{2\alpha}$ and endothelial function and arterial stiffness in resistant hypertension (i.e. blood pressure that remains above goal in spite of concurrent use of three antihypertensive agents of

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different classes, one of which should be a diuretic (24)). Plasma levels of Iso-8-epi-$PGF_{2\alpha}$ were markedly higher in resistant hypertension compared to hypertensive patients (22.5 ± 11.2 vs. 17.3 ± 9.8 pg/mL; $P < 0.05$). A significant inverse correlation was observed between flow-mediated dilation, which evaluates endothelial function, and Iso-8-epi-$PGF_{2\alpha}$ ($r=-0.4$, $P= 0.001$) in resistant hypertension, but none was found for arterial stiffness, evaluated by pulse wave velocity.

The role of oxidative stress in the atherosclerotic process was confirmed in a study investigating the association between urinary Iso-8-epi $PGF_{2\alpha}$ and the presence and severity of coronary artery disease (CAD) in 100 patients scheduled for coronary angiography (21). In patients with CAD, urinary Iso-8-epi $PGF_{2\alpha}$ levels were higher than in patients without CAD (68.8 ± 5.5 pg/mL vs. 38.3 ± 3.7 pg/mL, $P<0.001$); Iso-8-epi $PGF_{2\alpha}$ was an independent predictor (odds ratio 7.1; $P = 0.007$) of extent and severity of CAD.

Relative to heart failure (22), New-York Heart Association (NYHA) class III and IV patients exhibited elevated urinary Iso-8-epi-$PGF_{2\alpha}$ levels when compared to controls and NYHA I and II patients: control 0.35 ± 0.30 ng/mg creatinine; NYHA I 0.4 ± 0.2 ng/mg creatinine (NS relative to control); NYHA II 0.6 ± 0.5 ng/mg creatinine (NS relative to control); NYHA III 1.4 ± 0.6 ng/mg creatinine ($P<0.001$ relative to NYHA I and II); NYHA IV 1.9 ± 0.9 ng/mg creatinine ($P<0.001$ relative to NYHA I and II; $P<0.01$ relative to NYHA III). The ROC curve drawn for Iso-8-epi $PGF_{2\alpha}$ allowed to differentiate NYHA class III and IV patients from NYHA class I and II patients with a sensitivity of 95.8% and specificity of 95.8% (cut off 0.8 ng/mg creatinine; area under curve 1.0; $P < 0.001$). The authors concluded that markers of oxidative damage are unlikely to play a significant role in early stages of CHF. However, they might become important in the course of CHF when their concentrations reach critical levels and that urinary Iso-8-epi $PGF_{2\alpha}$ is a reliable indicator of symptomatic CHF.

4.2. Respiratory disease

No literature was identified that provided information on the association between Iso-8-epi $PGF_{2\alpha}$ and chronic obstructive pulmonary disease.

One cross-sectional study assessed the relationship between markers of oxidative stress, antioxidant status and lung function, symptoms of asthma, atopy and bronchial hyperresponsiveness (BHR) in young adults (23). All 564 study participants (aged 22-28 years) had detectable plasma Iso-8-epi $PGF_{2\alpha}$. There was no indication for an association between oxidative stress and any of the outcomes studied. The authors concluded that the levels of oxidative stress-related biomarkers and antioxidant status in plasma may not be related to asthma in the general population in the absence of more severe symptoms or exacerbations.

5. SMOKING-BIOMARKER LINK

The link between cigarette smoking andIso-8-epi $PGF_{2\alpha}$ is moderate: there is an increase in Iso-8-epi $PGF_{2\alpha}$ with smoking history. However, there is considerable heterogeneity in Iso-8-epi $PGF_{2\alpha}$ measurement units or biological media (urine, plasma, induced sputum or exhaled breath condensate). Also, increases in Iso-8-epi $PGF_{2\alpha}$ do not necessarily

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reflect increases in oxidative stress; there is potential ambiguity in solely measuring Iso-8-epi $PGF_{2\alpha}$ which should not be considered as a marker (5).

5.1. Smoking

Table 2 summarizes the articles retrieved from the current literature search, in which smokers were compared to non-smokers with respect to Iso-8-epi $PGF_{2\alpha}$.

All articles retrieved report cross-sectional results, there is no follow-up or cohort study and changes over time cannot be assessed.

All of the articles in which smokers were compared to non-smokers showed that Iso-8-epi $PGF_{2\alpha}$ was increased in smokers compared to non-smokers. The difference between groups was not always significant, probably due to lack of power. Three studies also assessing Iso-8-epi $PGF_{2\alpha}$ in COPD showed comparable levels between smokers and COPD patients [(7), (10), (13)]. Finally, one study compared Iso-8-epi $PGF_{2\alpha}$ levels between healthy and asthmatic smokers up to 3 hours after smoking and did not find a difference between the two groups (14).

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Table 2. Current literature showing 8-epi-PGF2α in smokers and non-smokers

Reference	Measure	Smokers	N	Non-smokers	N	Other condition	N
Miri (2012) (6)	Plasma Iso-8-epi $PGF_{2\alpha}$ (pg/ml) mean	Estimated from graph 310	56	Estimated from graph 325	83		
Inonu (2012) (7) [a,b]	EBC Iso-8-epi $PGF_{2\alpha}$ (pg/ml) mean ± SD	41.3 ± 26	26	15.8 ± 6.9	29	COPD: 44.8 ± 40.2	25
Andreoli (2011) (8) [a]	Urinary Iso-8-epi $PGF_{2\alpha}$ (mg/24 h) mean ± SD	1.25 ± 0.60	22	0.92 ± 0.43	22		
Hoffmeyer (2009) (9)	EBC Iso-8-epi $PGF_{2\alpha}$ (pg/ml) median (range)	464.4 (223.0-661.1)	8	264.2 (82.70-713.1)	16		
Mazur (2009) (10) [b]	Iso-8-epi $PGF_{2\alpha}$in induced sputum and EBC (pg/ml)	not assessable from graphs	17	not assessable from graphs	14	COPD with exacerbation (pooled control)	
Borrill (2008) (11) [a]	EBC Iso-8-epi $PGF_{2\alpha}$ (pg/ml) mean ± SD	49.9 ± 2.9	18	8.9 ± 4.0	10		
Takeshita (2008) (12) [a]	Urinary Iso-8-epi $PGF_{2\alpha}$ (pg/mg creatinin) mean ± SD	231.9 ± 63.2	16	173.8 ± 39.8	11		
Makris (2008) (13) [b]	EBC Iso-8-epi $PGF_{2\alpha}$ (pg/ml) mean ± sd			5.6 ± 0.7	12	COPD 18.1 ± 2	18
Papaioannou (2010) (14)	EBC Iso-8-epi $PGF_{2\alpha}$ (pg/ml) mean ± sd	Healthy	10			Asthmatic	10
		Baseline 8.08 ± 3.7(				7.59 ± 4.11	
		30 min 7.59 ± 4.1				9.48 ± 3.74	
		90 min 9.18 ± 4.9				12.60 ± 5.60	
		180 min 11.05 ± 6.2(				9.68 ± 4.42	

EBC: Exhaled breath condensate
a: significant difference between smokers and nonsmokers
b: significant difference between other condition and nonsmokers

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5.2. Smoking cessation

There are only a few studies about smoking cessation effects on oxidant markers, all but one being long term cessation only. None were related to switching to a potentially less harmful product.

In the long term, the Iso-8-epi $PGF_{2\alpha}$ levels of ex-smokers (1 – 5 years quitting, when provided), measured in urine, are not different from nonsmokers' levels (Table 3). One single study with 61 smokers (21 with chronic bronchitis or COPD), 15 asthmatics and 25 asymptomatic smokers (15) showed that three months after cessation, Iso-8-epi $PGF_{2\alpha}$ levels in sputum had decreased but were still significantly higher in quitters (15 successful) than in non-smokers even though there were improvements in symptoms. The authors concluded that although symptoms improve after smoking cessation, the oxidant and protease burden in the airways continues at least for months.

Table 3. Changes in 8-epi-PGF2α following quitting.

Reference	Condition	Smoking status		N	Mean ± sd	Quitting duration
Lowe (2009) (16) [a]	Healthy Urinary Iso-8-epi $PGF_{2\alpha}$ (µg/24h)	Non-smokers		20	0.919 ± 0.638	-
		Ex-smokers		20	0.990 ± 0.423	1 – 5 years
		Light smokers		20	1.346 ± 0.681	-
		Heavy smokers		20	1.475 ± 0.724*	-
Haswell (2014) (17) [a]	Healthy Urinary Iso-8-epi $PGF_{2\alpha}$ (ng/24h)	Nonsmokers		55	166.84 ± 63.36	≥ 5 years
		Ex-smokers		52	176.27 ± 68.87	
		Smokers		120	245.72 ± 115.70	
Hakim (2012) (18)	Healthy Urinary Iso-8-epi $PGF_{2\alpha}$ (ng/mg creatinine)	Smokers	female	41	535 ± 325	
			male	34	370 ± 273	
		Ex-smokers	female	31	399 ± 258	not defined
			Male	24	308 ± 214	not defined
Sakano (2009) (19) [b]	Healthy Urinary Iso-8-epi $PGF_{2\alpha}$ (ng/mg creatinine)	Nonsmokers		226	Not assessable from graphs	not defined
		Ex-smokers		20		
		Smokers		77		

a statistically significant difference between smokers and non-smokers or ex-smokers
b statistically significant difference between smokers and non-smokers

According to these findings, smoking cessation does not result in a short-term improvement of Iso-8-epi $PGF_{2\alpha}$, However in the long term, Iso-8-epi $PGF_{2\alpha}$.levels normalize in ex-smokers after at least one year of abstinence.

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5.3. Role in MRTP assessment

No published data related to the effect on Iso-8-Epi-$PGF_{2\alpha}$ levels of switching from conventional cigarettes to modified risk tobacco products (MRTPs) or other nicotine products are available.

6. CONCLUSION

The literature search provided no evidence of a strong direct link between Iso-8-epi $PGF_{2\alpha}$ and smoking cessation. Iso-8-epi $PGF_{2\alpha}$ is regarded as the gold standard for detection of oxidative stress and also as an indicator of disease progression but "for a biomarker of exposure to be accepted as a biomarker of risk or a surrogate endpoint of disease, there should be a strong biological rational as well as compelling data from clinical and epidemiologic studies'' (25). Iso-8-epi $PGF_{2\alpha}$, as a marker of oxidative stress (and a specific marker for lipid peroxidation (26), is valuable in establishing a diagnosis or disease progression due to oxidative stress, but is apparently not suitable for the assessment of cigarette-smoking cessation in the short term, i.e. less than three months duration.

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20. de Faria AP, Fontana V, Modolo R, Barbaro NR, Sabbatini AR, Pansani IF, et al. Plasma 8-isoprostane levels are associated with endothelial dysfunction in resistant hypertension. Clin Chim Acta. 2014;433:179-83. Epub 2014/03/25. doi: 10.1016/j.cca.2014.03.009. PubMed PMID: 24657423.

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8. APPENDICES

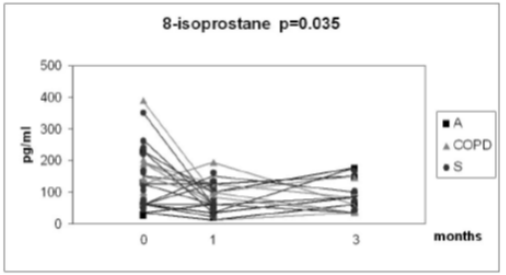
8.1 Appendix 1 Association between smoking, cessation/switching and FEV1
8.2 Appendix 2 References of included studies
8.3 Appendix 3 Excluded studies from search 1
8.4 Appendix 4 Excluded studies from search 2
8.5 Appendix 5 Excluded studies from search 3
8.6 Appendix 6 Excluded studies from searches 1 & 2
8.7 Appendix 7 Excluded studies from searches 1 & 3
8.8 Appendix 8 Excluded studies from searches 2 & 3
8.9 Appendix 9 Excluded studies from searches 1, 2 & 3

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8.1. Appendix 1 Association between smoking, cessation/switching and FEV1

Table A1. Selected studies showing association between smoking, cessation or switching and 8-epi-$PGF_{2\alpha}$ retrieved in the current search.

Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Louhelainen (2009) (15)	Finland UK	3 months	Smoking cessation program; evaluations at baseline, 1 month & 3 months after successful cessation	COPD/chronic bronchitis, asthmatic and asymptomatic smokers	asymptomatic n=25, age 41 (18–64) , M/F 11/14 COPD n=21; age 56 (41 – 72); M/F 7/14 Asthma n=15, age 42 (18–58), M/F 5/10	15 subjects succeeded quitting. sputum 8-isoprostane  Significant decline (p=0.035) in the COPD/bronchitis group. clear trend but no major or immediate decline in the oxidative stress within the first months after smoking cessation

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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Lowe (2009) (16)	UK	Not applicable	Cross-sectional	Healthy volunteers Ex-smokers: 1-5 yrs	Nonsmokers 20 Ex-smokers 20 Light-smokers 20 Heavy-smokers 20 Age 21-68 50% male	Levels of urinary PGF2a (µg/24h) Non / Ex / Light / Heavy Mean 0.919 / 0.990 / 1.346 / 1.475 ±SD 0.638 / 0.423 / 0.681 / 0.724 Median 0.739 / 0.936 / 1.241 / 1.314 min 0.422 / 0.415 / 0.581 / 0.533 max 3.315 / 2.024 / 3.093 / 3.354 Mean ratio to nonsmokers 1.079 / 1.446 / 1.607 P-value NS / NS / <0.01 Levels of urinary PGF2a (µg/mg creatinine) Non / Ex / Light / Heavy Mean 0.6115 / 0.6959 / 0.9617 / 1.0354 ±SD 0.2112 / 0.286 / 0.3686 / 0.3614 Median 0.5984 / 0.5858 / 0.8505 / 0.9183 min 0.3929 / 0.3702 / 0.5391 / 0.5836 max 1.3195 / 1.4349 / 2.0336 / 2.0013 Mean ratio to nonsmokers 1.138 / 1.573 / 1.693 P value NS / <0.001 / <0.001 Levels in ex-smokers were similar to nonsmokers, although not significantly different from smoker groups, suggesting that smoking effect may be reversible with smoking cessation

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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Haswell (2014) (17)	Germany	Not applicable	Cross-sectional	Current, ex and never smokers Ex-smokers ≥ 5 years	Current 143, 48% male Ex 61, 51% male Never 61, 52% male Age 23 – 55	Urinary PGF2a Type III (ng/24h) Current (N=120) / Ex (N=52) / Never (N=55) Mea 245.72 / 176.27 / 166.84 ± SD 115.70 / 68.87 / 63.36 median 220.72 / 162.18 / 154.86 min 69.84 / 77.10 / 75.06 max 748.16 / 437.56 / 371.64 P-value Current vs. Ex and vs. Never <0.001; P-value Ex vs. never 0.59

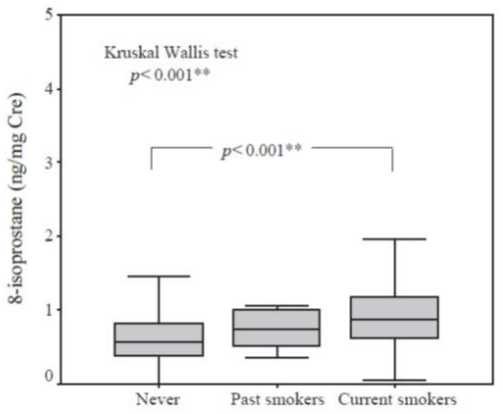
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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Hakim (2012) (18)	USA	Not applicable	Cross-sectional	Current and ex-smokers No definition of former smokers	Age Female 59.6 ± 9.1 Male 60.9 ± 8.4	Urinary 8-iso-PGF2a (ng/mg creatinine) Female / Male / p Total: 477 ± 304 (n = 72) / 344 ± 250 (n = 58) / 0.008 Former smokers: 399 ± 258 (n = 31) / 308 ± 214 (n = 24) / 0.169 Current smokers: 535 ± 325 (n = 41) / 370 ± 273 (n = 34) / 0.021 P (former vs. current): 0.059 / 0.357

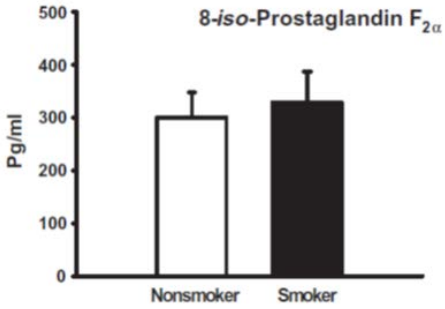
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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Sakano (2009) (19)	Japan	Not applicable	Cross-sectional	Healthy volunteers	323 subjects age <40: 187; ≥40:136 F/M 220/103 Nonsmokers 226 Ex-smokers 20 Smokers 77	Urinary 8-isoprostane 
Makris (2008) (13)	Greece	Not applicable	Cross-sectional	30 volunteers	COPD ex-smokers: n=18, age 64 ± 2, F/M 12/6 Control: n=12, age 61±3.5, F/M 9/3	EBC 8-Isoprostane levels (pg/ml): COPD mean (SE) 18.1 (2); control. 5.6 (0.7); p < 0.001

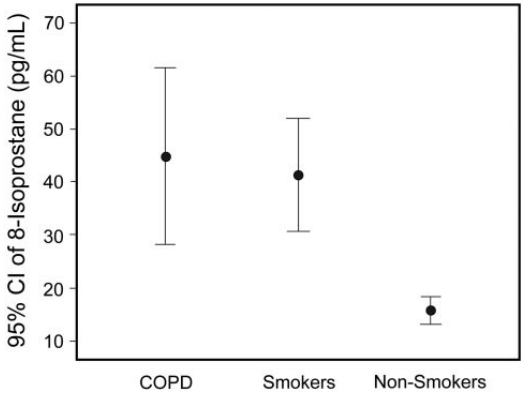
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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Miri (2012) (6)	Iran	Not applicable	Cross-sectional	Healthy male volunters, Current and nonsmokers	135 males, smokers n=56, age 41.9 ± 1.3 years nonsmokers n= 83; age 41.0 ± 1.1	No effect of smoking on plasma 8-iso-PGF2α (mean ± 2 SEM) 

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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Inonu (2012) (7)	Turkey	Not applicable	Cross-sectional	Male COPD, nonsmokers and smokers	COPD: n= 25 Smokers: n= 26 Nonsmokers: n = 29 Age 58 ± 8.9 years	8-isoprostane (pg/mL) in EBC COPD N= 25) Smokers (N = 26) Nonsmokers (N=29) 44.8 ± 40.2 41.3 ± 26 15.8 ± 6.9  ANOVA p<0.001 2x2 compar COPD-nonsmokers p < 0.005; smokers-nonsmokers p < 0.001; COPD-smokers p = 0.978

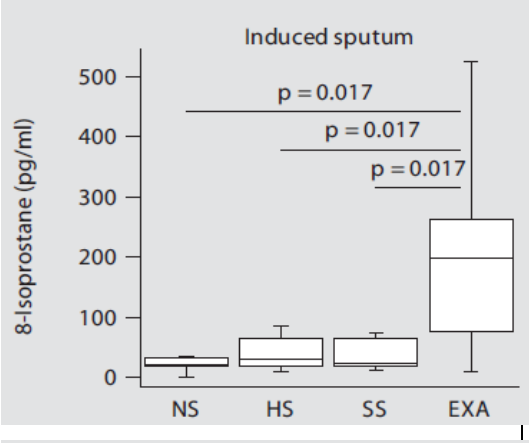
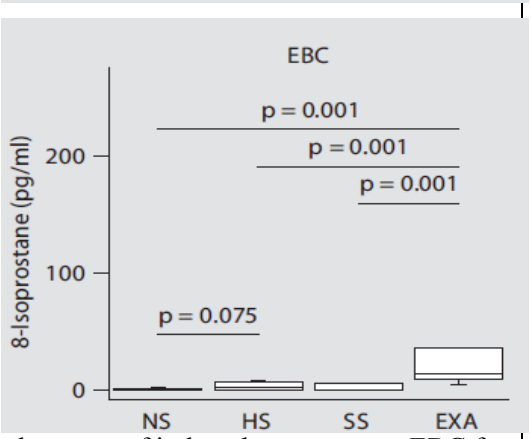
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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Andreoli (2011) (8)	Italy	Not applicable	Cross-sectional	Monozygotic twins, discordant for smoking status	26 males and 18 females Age mean 31.5 years, range 23 - 46	Urinary 8-epiPGF (mg/24 h) Nonsmokers N=22 / Smokers N = 22 Mean ± SD: 0.92 ± 0.43 / 1.25 ± 0.60 median: 0.89 / 1.17 t-test $p < 0.01$
Hoffmeyer (2009) (9)	Germany	Not applicable	Cross-sectional	Smokers & nonsmokers healthy volunters	Smokers n = 8, age 20-56 years Nonsmokers n = 16, age 18 – 60 years	8-iso-PGF2a in EBC (pg/ml) and total (pg) Smokers (N=8) / Non-smokers (N=16) / p median 464.4 / 264.2 / 0.73 range (223.0-661.1) / (82.70-713.1) median 727.2 / 488.8 / 0.43 range (334.5-1021.0) / (95.1-998.3) No difference

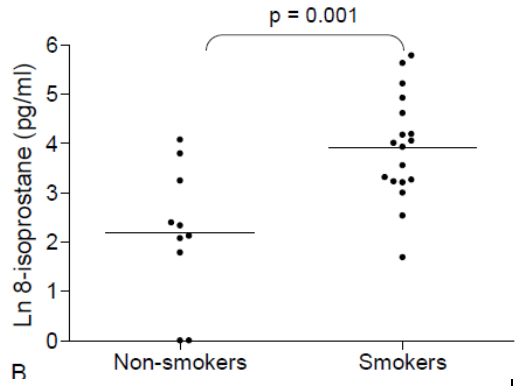
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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Mazur (2009) (10)	Finland	Not applicable	Cross-sectional	nonsmokers , healthy smokers, symptomatic smokers, CODP with exacerbations as active control	nonsmokers NS n = 14, age 57 ± 4.2, F/M 3/11 healthy smokers HS n = 17, age 43 ± 12.6, F/M 5/12 symptomatic smokers SS n = 9, age 64 ± 7.5, F/M 2/7	  advantage of induced sputum over EBC for assessing the degree of airway oxidative stress

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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Borrill (2008) (11)	UK	Not applicable	Reproducibility Measures 1 week apart	Healthy smokers and never-smokers	Smokers n=18, age 46.4 ± 9.6, F/M 11/7 Never-smokers n = 10, age 44.8 ± 15.6, F/M 6/4	EBC 8-isoprostane (pg/ml) Smokers Nonsmokers t-test mean (SD) 49.9 (2.9) 8.9 (4.0) 0.001 
Takeshita (2008) (12)	Japan	Not applicable	Matched-control Cohort	27 healthy male volunteers	smokers n=16 age-matched nonsmokers n=11	Urinary level of 8-isoprostane (pg/mg creatinine), was greater in smokers (231.9±63.2) than in non-smokers (173.8±39.8; p<0.05)

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Source	Country	Period	Design	Study population	Study size (n), age (years) mean ± sd or median, range, sex	Comments
Papaioannou (2010) (14)	Greece	3 hours after smoking	Matched-control Cohort	Smokers, healthy or asthmatic	Asthmatic n = 10; age 34.3 ± 10.2; F/M 6/4 Healthy: n = 10; age 35.4 ± 14.0; F/M 6/4	EBC 8-isoprostane (pg/ml) mean (SD) Healthy Asthmatic Baseline 8.08 (3.70) 7.59 (4.11) 30 min 7.59 (4.11) 9.48 (3.74) 90 min 9.18 (4.95) **12.60 (5.60)*** 180 min 11.05 (6.20) 9.68 (4.42) * Statistically different from baseline 2-way repeated measures ANOVA revealed no difference between groups

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8.2. Appendix 2 References of included studies

Search	#	Article
1	1	Haswell LE, Papadopoulou E, Newland N, Shepperd CJ, Lowe FJ. A cross-sectional analysis of candidate biomarkers of biological effect in smokers, never-smokers and ex-smokers. Biomarkers. 2014 Aug;19(5):356-67. doi: 10.3109/1354750X.2014.912354. Epub 2014 May 22. PubMed PMID: 24854418. (17)
1	14	Hakim IA, Harris R, Garland L, Cordova CA, Mikhael DM, Sherry Chow HH. Gender difference in systemic oxidative stress and antioxidant capacity in current and former heavy smokers. Cancer Epidemiol Biomarkers Prev. 2012 Dec;21(12):2193-200. doi: 10.1158/1055-9965.EPI-12-0820. Epub 2012 Oct 2. PubMed PMID: 23033455. (18)
1	18	Miri R, Saadati H, Ardi P, Firuzi O. Alterations in oxidative stress biomarkers associated with mild hyperlipidemia and smoking. Food Chem Toxicol. 2012;50(3-4):920-6. Epub 2012/01/10. doi: 10.1016/j.fct.2011.12.031. PubMed PMID: 22227215. (6)
1	20	Inonu H, Doruk S, Sahin S, Erkorkmaz U, Celik D, Celikel S, Seyfikli Z. Oxidative stress levels in exhaled breath condensate associated with COPD and smoking. Respir Care. 2012 Mar;57(3):413-9. doi: 10.4187/respcare.01302. Epub 2011 Sep 26. PubMed PMID: 21968597. (7)
1	23	Andreoli C, Gregg EO, Puntoni R, Gobbi V, Nunziata A, Bassi A. Cross-sectional study of biomarkers of exposure and biological effect on monozygotic twins discordant for smoking. Clin Chem Lab Med. 2011 Jan;49(1):137-45. doi: 10.1515/CCLM.2011.009. Epub 2010 Nov 18. PubMed PMID: 21083439. (8)
1	29	Hoffmeyer F, Harth V, Bunger J, Bruning T, Raulf-Heimsoth M. Leukotriene B4, 8-iso-prostaglandin F2 alpha, and pH in exhaled breath condensate from asymptomatic smokers. J Physiol Pharmacol. 2009 Nov;60 Suppl 5:57-60. PubMed PMID: 20134040. (9)
1	30	Papaioannou AI, Koutsokera A, Tanou K, Kiropoulos TS, Tsilioni I, Oikonomidi S, Liadaki K, Pournaras S, Gourgoulianis KI, Kostikas K. The acute effect of smoking in healthy and asthmatic smokers. Eur J Clin Invest. 2010 Feb;40(2):103-9. doi: 10.1111/j.1365-2362.2009.02221.x. Epub 2009 Nov 12. PubMed PMID: 19912318. (14)
1	31	Lowe FJ, Gregg EO, McEwan M. Evaluation of biomarkers of exposure and potential harm in smokers, former smokers and never-smokers. Clin Chem Lab Med. 2009;47(3):311-20. doi: 10.1515/CCLM.2009.069. PubMed PMID: 19676143. (16)

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Search	#	Article
1	33	Louhelainen N, Rytilä P, Haahtela T, Kinnula VL, Djukanović R. Persistence of oxidant and protease burden in the airways after smoking cessation. BMC Pulm Med. 2009 May 27;9:25. doi: 10.1186/1471-2466-9-25. PubMed PMID: 19473482; PubMed Central PMCID: PMC2697135. (15)
1	37	Mazur W, Stark H, Sovijärvi A, Myllärniemi M, Kinnula VL. Comparison of 8-isoprostane and interleukin-8 in induced sputum and exhaled breath condensate from asymptomatic and symptomatic smokers. Respiration. 2009;78(2):209-16. doi: 10.1159/000206010. Epub 2009 Mar 2. PubMed PMID: 19252398. (10)
1	38	Sakano N, Takahashi N, Wang DH, Sauriasari R, Takemoto K, Kanbara S, Sato Y, Takigawa T, Takaki J, Ogino K. Plasma 3-nitrotyrosine, urinary 8-isoprostane and 8-OHdG among healthy Japanese people. Free Radic Res. 2009 Feb;43(2):183-92. doi: 10.1080/10715760802663124. PubMed PMID: 19204871. (19)
1	45	Borrill ZL, Roy K, Vessey RS, Woodcock AA, Singh D. Non-invasive biomarkers and pulmonary function in smokers. Int J Chron Obstruct Pulmon Dis. 2008;3(1):171-83. PubMed PMID: 18488441; PubMed Central PMCID: PMC2528202. (11)
1	46	Takeshita Y, Katsuki Y, Katsuda Y, Kai H, Saito Y, Arima K, Sasaki K, Imaizumi T. Vitamin C reversed malfunction of peripheral blood-derived mononuclear cells in smokers through antioxidant properties. Circ J. 2008 Apr;72(4):654-9. PubMed PMID: 18362440. (12)
1	52	Makris D, Paraskakis E, Korakas P, Karagiannakis E, Sourvinos G, Siafakas NM, Tzanakis N. Exhaled breath condensate 8-isoprostane, clinical parameters,radiological indices and airway inflammation in COPD. Respiration.2008;75(2):138-44. Epub 2007 Jul 19. PubMed PMID: 17641539. (13)
2	3	de Faria AP, Fontana V, Modolo R, Barbaro NR, Sabbatini AR, Pansani IF, Ferreira-Melo SE, Moreno H. Plasma 8-isoprostane levels are associated with endothelial dysfunction in resistant hypertension. Clin Chim Acta. 2014 Jun 10;433:179-83. doi: 10.1016/j.cca.2014.03.009. Epub 2014 Mar 19. PubMed PMID: 24657423. (20)
2	13	Basarici I, Altekin RE, Demir I, Yilmaz H. Urinary 8-isoprostane levels can indicate the presence, severity and extent of angiographic coronary artery disease. Acta Cardiol. 2008 Aug;63(4):415-22. PubMed PMID: 18795577. (21)

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Search	#	Article
2	14	Radovanovic S, Krotin M, Simic DV, Mimic-Oka J, Savic-Radojevic A, Pljesa-Ercegovac M, Matic M, Ninkovic N, Ivanovic B, Simic T. Markers of oxidative damage in chronic heart failure: role in disease progression. Redox Rep. 2008;13(3):109-16. doi: 10.1179/135100008X259204. PubMed PMID: 18544228. (22)
3	13	García-Larsen V, Chinn S, Rodrigo R, Amigo H, Bustos P, Rona RJ. Relationship between oxidative stress-related biomarkers and antioxidant status with asthma and atopy in young adults: a population-based study. Clin Exp Allergy. 2009 Mar;39(3):379-86. doi: 10.1111/j.1365-2222.2008.03163.x. PubMed PMID: 19187326. (23)

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8.3. Appendix 3 Excluded studies from search 1

To identify literature that linked smoking or nicotine replacement to 8-epi-$PGF_{2\alpha}$ using the search string: (8-epi-prostaglandin F2alpha OR 8-epi-PGF2)) AND (smokers OR smoking OR nicotine replacement OR cessation) AND ("2008"[Date - Publication]: "2015"[Date - Publication]))) AND English[Language]).

E1. $PGF_{2\alpha}$ used only as control/biomarker of exposure

7 Vazzana N, Ganci A, Cefalù AB, Lattanzio S, Noto D, Santoro N, Saggini R, Puccetti L, Averna M, Davì G. Enhanced lipid peroxidation and platelet activation as potential contributors to increased cardiovascular risk in the low-HDL phenotype. J Am Heart Assoc. 2013 Apr 4;2(2):e000063. doi: 10.1161/JAHA.113.000063. PubMed PMID: 23557750; PubMed Central PMCID: PMC3647282.

9 Goldoni M, Corradi M, Mozzoni P, Folesani G, Alinovi R, Pinelli S, Andreoli R, Pigini D, Tillo R, Filetti A, Garavelli C, Mutti A. Concentration of exhaled breath condensate biomarkers after fractionated collection based on exhaled CO2 signal. J Breath Res. 2013 Mar;7(1):017101. doi: 10.1088/1752-7155/7/1/017101. Epub 2013 Feb 27. PubMed PMID: 23445573.

21 García-Rio F, Romero D, Lores V, Casitas R, Hernanz A, Galera R, Alvarez-Sala R, Torres I. Dynamic hyperinflation, arterial blood oxygen, and airway oxidative stress in stable patients with COPD. Chest. 2011 Oct;140(4):961-9. doi: 10.1378/chest.10-2788. Epub 2011 Mar 24. PubMed PMID: 21436248.

24 Sauriasari R, Sakano N, Wang DH, Takaki J, Takemoto K, Wang B, Sugiyama H, Sato Y, Takigawa T, Takahashi N, Kanbara S, Hitomi Y, Nakamura H, Ogino K. C-reactive protein is associated with cigarette smoking-induced hyperfiltration and proteinuria in an apparently healthy population. Hypertens Res. 2010 Nov;33(11):1129-36. doi: 10.1038/hr.2010.154. Epub 2010 Aug 12. PubMed PMID: 20703235.

27 Kanazawa H, Kodama T, Asai K, Matsumura S, Hirata K. Increased levels of N(epsilon)-(carboxymethyl)lysine in epithelial lining fluid from peripheral airways in patients with chronic obstructive pulmonary disease: a pilot study. Clin Sci (Lond). 2010 May 6;119(3):143-9. doi: 10.1042/CS20100096. PubMed PMID: 20236091.

28 Warner JH, Liang Q, Sarkar M, Mendes PE, Roethig HJ. Adaptive regression modeling of biomarkers of potential harm in a population of U.S. adult cigarette smokers and nonsmokers. BMC Med Res Methodol. 2010 Mar 16;10:19. doi: 10.1186/1471-2288-10-19. PubMed PMID: 20233412; PubMed Central PMCID: PMC2846953.

34 Kane B, Borrill Z, Southworth T, Woodcock A, Singh D. Reduced exhaled breath condensate pH in asthmatic smokers using inhaled corticosteroids. Respirology. 2009 Apr;14(3):419-23. PubMed PMID: 19353777.

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E2. $PGF_{2\alpha}$ lacked link to disease or smoking

35 Helbig D, Wagner A, Glei M, Basu S, Schubert R, Jahreis G. Blackcurrant seed press residue increases tocopherol concentrations in serum and stool whilst biomarkers in stool and urine indicate increased oxidative stress in human subjects. Br J Nutr. 2009 Aug;102(4):554-62. doi: 10.1017/S0007114509220812. Epub 2009 Mar 23. PubMed PMID: 19302719.

49 Rodrigo R, Prat H, Passalacqua W, Araya J, Bächler JP. Decrease in oxidative stress through supplementation of vitamins C and E is associated with a reduction in blood pressure in patients with essential hypertension. Clin Sci (Lond). 2008 May;114(10):625-34. PubMed PMID: 17999638.

E3. Disease not covered by search categories

3 Deng Q, Dai X, Guo H, Huang S, Kuang D, Feng J, Wang T, Zhang W, Huang K, Hu D, Deng H, Zhang X, Wu T. Polycyclic aromatic hydrocarbons-associated microRNAs and their interactions with the environment: influences on oxidative DNA damage and lipid peroxidation in coke oven workers. Environ Sci Technol. 2014 Apr 1;48(7):4120-8. doi: 10.1021/es4055516. Epub 2014 Mar 12. PubMed PMID: 24555497.

4 Bertazzi PA, Cantone L, Pignatelli P, Angelici L, Bollati V, Bonzini M, Carugno M, Mannucci PM, Violi F. Does enhancement of oxidative stress markers mediate health effects of ambient air particles? Antioxid Redox Signal. 2014 Jul 1;21(1):46-51. doi: 10.1089/ars.2013.5694. Epub 2014 Mar 27. PubMed PMID: 24350583.

6 Kuang D, Zhang W, Deng Q, Zhang X, Huang K, Guan L, Hu D, Wu T, Guo H. Dose-response relationships of polycyclic aromatic hydrocarbons exposure and oxidative damage to DNA and lipid in coke oven workers. Environ Sci Technol. 2013 Jul 2;47(13):7446-56. doi: 10.1021/es401639x. Epub 2013 Jun 21. PubMed PMID: 23745771.

8 Asombang AW, Kayamba V, Mwanza-Lisulo M, Colditz G, Mudenda V, Yarasheski K, Chott R, Rubin DC, Gyawali CP, Sinkala E, Mwanamakondo S, Anderson-Spearie C, Kelly P. Gastric cancer in Zambian adults: a prospective case-control study that assessed dietary intake and antioxidant status by using urinary isoprostane excretion. Am J Clin Nutr. 2013 May;97(5):1029-35. doi: 10.3945/ajcn.112.051284. Epub 2013 Mar 27. PubMed PMID: 23535107; PubMed Central PMCID: PMC3628375.

12 Paik JK, Kim M, Kwak JH, Lee EK, Lee SH, Lee JH. Increased arterial stiffness in subjects with impaired fasting glucose. J Diabetes Complications. 2013 May-Jun;27(3):224-8. doi: 10.1016/j.jdiacomp.2012.10.012. Epub 2012 Nov 22. PubMed PMID: 23182995.

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25 Piotrowski WJ, Kurmanowska Z, Antczak A, Marczak J, Ciebiada M, Górski P. Exhaled 8-isoprostane in sarcoidosis: relation to superoxide anion production by bronchoalveolar lavage cells. Inflamm Res. 2010 Dec;59(12):1027-32. doi: 10.1007/s00011-010-0222-4. Epub 2010 Jun 3. PubMed PMID: 20521080; PubMed Central PMCID: PMC2978317.

32 Singer RE, Moss K, Beck JD, Offenbacher S. Association of systemic oxidative stress with suppressed serum IgG to commensal oral biofilm and modulation by periodontal infection. Antioxid Redox Signal. 2009 Dec;11(12):2973-83. doi: 10.1089/ARS.2009.2591. PubMed PMID: 19496702; PubMed Central PMCID: PMC2861534.

36 Su H, Gornitsky M, Velly AM, Yu H, Benarroch M, Schipper HM. Salivary DNA, lipid, and protein oxidation in nonsmokers with periodontal disease. Free Radic Biol Med. 2009 Apr 1;46(7):914-21. PubMed PMID: 19280702.

39 Tanou K, Koutsokera A, Kiropoulos TS, Maniati M, Papaioannou AI, Georga K, Zarogiannis S, Gourgoulianis KI, Kostikas K. Inflammatory and oxidative stress biomarkers in allergic rhinitis: the effect of smoking. Clin Exp Allergy. 2009 Mar;39(3):345-53. doi: 10.1111/j.1365-2222.2008.03149.x. PubMed PMID: 19187324.

40 Chan HP, Tran V, Lewis C, Thomas PS. Elevated levels of oxidative stress markers in exhaled breath condensate. J Thorac Oncol. 2009 Feb;4(2):172-8. doi: 10.1097/JTO.0b013e3181949eb9. PubMed PMID: 19179892.

42 Dalaveris E, Kerenidi T, Katsabeki-Katsafli A, Kiropoulos T, Tanou K, Gourgoulianis KI, Kostikas K. VEGF, TNF-alpha and 8-isoprostane levels in exhaled breath condensate and serum of patients with lung cancer. Lung Cancer. 2009 May;64(2):219-25. doi: 10.1016/j.lungcan.2008.08.015. Epub 2008 Oct 9. PubMed PMID: 18845357.

48 Li Y, Chongsuvivatwong V, Geater A, Liu A. Exhaled breath condensate cytokine level as a diagnostic tool for obstructive sleep apnea syndrome. Sleep Med. 2009 Jan;10(1):95-103. doi: 10.1016/j.sleep.2007.11.013. Epub 2008 Jan 22. PubMed PMID: 18207457.

50 Wentholt IM, Kulik W, Michels RP, Hoekstra JB, DeVries JH. Glucose fluctuations and activation of oxidative stress in patients with type 1 diabetes. Diabetologia. 2008 Jan;51(1):183-90. Epub 2007 Nov 10. PubMed PMID: 17994218.

E4. Studies of passive or parental smoking

5 Bono R, Bellisario V, Romanazzi V, Pirro V, Piccioni P, Pazzi M, Bugiani M, Vincenti M. Oxidative stress in adolescent passive smokers living in urban and rural environments. Int J Hyg Environ Health. 2014 Mar;217(2-3):287-93. doi: 10.1016/j.ijheh.2013.06.008. Epub 2013 Jul 8. PubMed PMID: 23890683.

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E5. Studies in narrow population (e.g. pregnant women)

10 Wannhoff A, Bölck B, Kübler AC, Bloch W, Reuther T. Oxidative and nitrosative stress and apoptosis in oral mucosa cells after ex vivo exposure to lead and benzo[a]pyrene. Toxicol In Vitro. 2013 Mar;27(2):915-21. doi: 10.1016/j.tiv.2013.01.007. Epub 2013 Jan 11. PubMed PMID: 23318731.

13 Kim M, Paik JK, Kang R, Kim SY, Lee SH, Lee JH. Increased oxidative stress in normal-weight postmenopausal women with metabolic syndrome compared with metabolically healthy overweight/obese individuals. Metabolism. 2013 Apr;62(4):554-60. doi: 10.1016/j.metabol.2012.10.006. Epub 2012 Nov 9. PubMed PMID: 23142163.

15 Park S, Kim M, Paik JK, Jang YJ, Lee SH, Lee JH. Oxidative stress is associated with C-reactive protein in nondiabetic postmenopausal women, independent of obesity and insulin resistance. Clin Endocrinol (Oxf). 2013 Jul;79(1):65-70. doi: 10.1111/j.1365-2265.2012.04512.x. Epub 2013 Mar 25. PubMed PMID: 22816656.

16 Dragani A, Falco A, Santilli F, Basili S, Rolandi G, Cerasa L, Lattanzio S, Ciabattoni G, Patrono C, Davì G. Oxidative stress and platelet activation in subjects with moderate hyperhomocysteinaemia due to MTHFR 677 C→T polymorphism. Thromb Haemost. 2012 Sep;108(3):533-42. doi: 10.1160/TH11-12-0899. Epub 2012 Jul 10. PubMed PMID: 22782530.

17 Hoffmeyer F, Raulf-Heimsoth M, Lehnert M, Kendzia B, Bernard S, Berresheim H, Düser M, Henry J, Weiss T, Koch HM, Pesch B, Brüning T; Weldox Group. Impact of different welding techniques on biological effect markers in exhaled breath condensate of 58 mild steel welders. J Toxicol Environ Health A. 2012;75(8-10):525-32. doi: 10.1080/15287394.2012.675303. PubMed PMID: 22686312.

43 Signore C, Aros S, Morrow JD, Troendle J, Conley MR, Flanigan EY, Cassorla F, Mills JL. Markers of oxidative stress and systemic vasoconstriction in pregnant women drinking > or =48 g of alcohol per day. Alcohol Clin Exp Res. 2008 Nov;32(11):1893-8. doi: 10.1111/j.1530-0277.2008.00773.x. Epub 2008 Aug 18. PubMed PMID: 18715278; PubMed Central PMCID: PMC2615682.

47 Nuernberg AM, Boyce PD, Cavallari JM, Fang SC, Eisen EA, Christiani DC. Urinary 8-isoprostane and 8-OHdG concentrations in boilermakers with welding exposure. J Occup Environ Med. 2008 Feb;50(2):182-9. doi: 10.1097/JOM.0b013e31815cf6cc. PubMed PMID: 18301175.

E8. Studies with no comparison to smoking and cessation/switching

11 Berg K, Langaas M, Ericsson M, Pleym H, Basu S, Nordrum IS, Vitale N, Haaverstad R. Acetylsalicylic acid treatment until surgery reduces oxidative stress and inflammation in patients undergoing coronary artery bypass grafting. Eur J

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Cardiothorac Surg. 2013 Jun;43(6):1154-63. doi: 10.1093/ejcts/ezs591. Epub 2012 Dec 2. PubMed PMID: 23209276.

19 Muzembo BA, Narongpon D, Ngatu NR, Eitoku M, Hirota R, Suganuma N. Assessment of lifestyle effect on oxidative stress biomarkers in free-living elderly in rural Japan. Geriatr Gerontol Int. 2012 Jul;12(3):547-54. doi: 10.1111/j.1447-0594.2011.00793.x. Epub 2011 Dec 28. PubMed PMID: 22212822.

41 Kodama T, Kanazawa H, Tochino Y, Kyoh S, Asai K, Hirata K. A technological advance comparing epithelial lining fluid from different regions of the lung in smokers. Respir Med. 2009 Jan;103(1):35-40. doi: 10.1016/j.rmed.2008.09.004. Epub 2008 Oct 18. PubMed PMID: 18930648.

51 Dal Negro RW, Visconti M, Micheletto C, Tognella S. Changes in blood ROS, e-NO, and some pro-inflammatory mediators in bronchial secretions following erdosteine or placebo: a controlled study in current smokers with mild COPD. Pulm Pharmacol Ther. 2008;21(2):304-8. Epub 2007 Aug 14. PubMed PMID: 17889580.

E9. $PGF_{2\alpha}$ not measured or presented as AUC

22 Campos C, Guzmán R, López-Fernández E, Casado Á. Urinary biomarkers of oxidative/nitrosative stress in healthy smokers. Inhal Toxicol. 2011 Feb;23(3):148-56. doi: 10.3109/08958378.2011.554460. PubMed PMID: 21391783.

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8.4. Appendix 4 Excluded studies from search 2

Search 2: To identify literature reporting an association between 8-epi-$PGF_{2\alpha}$ and cardiovascular disease using the search string: (8-epi-prostaglandin F2alpha OR 8-epi-PGF2)) AND ((cardiovascular disease) AND (association)) AND ("2008"[Date - Publication]: "2015"[Date - Publication]))) AND English[Language]).

E1. $PGF_{2\alpha}$ used only as control/biomarker of exposure

8 Suyama Y, Matsuda C, Isomura M, Hamano T, Karino K, Yamasaki M, Yamaguchi S, Shiwaku K, Masuda J, Nabika T. Effects of six functional SNPs on the urinary 8-isoprostane level in a general Japanese population; Shimane COHRE Study. Dis Markers. 2011;30(6):291-8. doi: 10.3233/DMA-2011-0787. PubMed PMID: 21725157; PubMed Central PMCID: PMC3825119.

E2. $PGF_{2\alpha}$ lacked link to disease or smoking

7 Peairs AD, Rankin JW, Lee YW. Effects of acute ingestion of different fats on oxidative stress and inflammation in overweight and obese adults. Nutr J. 2011 Nov 7;10:122. doi: 10.1186/1475-2891-10-122. PubMed PMID: 22059644; PubMed Central PMCID: PMC3225315.

12 Tissot van Patot MC, Serkova NJ, Haschke M, Kominsky DJ, Roach RC, Christians U, Henthorn TK, Honigman B. Enhanced leukocyte HIF-1alpha and HIF-1 DNA binding in humans after rapid ascent to 4300 m. Free Radic Biol Med. 2009 Jun 1;46(11):1551-7. doi: 10.1016/j.freeradbiomed.2009.03.009. Epub 2009 Mar 19. PubMed PMID: 19303436.

15 Hirooka Y, Kimura Y, Sagara Y, Ito K, Sunagawa K. Effects of valsartan or amlodipine on endothelial function and oxidative stress after one year follow-up in patients with essential hypertension. Clin Exp Hypertens. 2008 Apr;30(3):267-76. doi: 10.1080/10641960802071000. PubMed PMID: 18425706.

E3. Disease not covered by search categories

4 Ono M, Takebe N, Oda T, Nakagawa R, Matsui M, Sasai T, Nagasawa K, Honma H, Kajiwara T, Taneichi H, Takahashi Y, Takahashi K, Satoh J. Association of coronary artery calcification with MDA-LDL-C/LDL-C and urinary 8-isoprostane in Japanese patients with type 2 diabetes. Intern Med. 2014;53(5):391-6. PubMed PMID: 24583425.

E5. Studies in narrow population (e.g. pregnant women)

6 Vazzana N, Guagnano MT, Cuccurullo C, Ferrante E, Lattanzio S, Liani R, Romano M, Davì G. Endogenous secretory RAGE in obese women: association with platelet activation and oxidative stress. J Clin Endocrinol Metab. 2012

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Sep;97(9):E1726-30. doi: 10.1210/jc.2012-1473. Epub 2012 Jul 3. PubMed PMID: 22761461.

9 Ostrow V, Wu S, Aguilar A, Bonner R Jr, Suarez E, De Luca F. Association between oxidative stress and masked hypertension in a multi-ethnic population of obese children and adolescents. J Pediatr. 2011 Apr;158(4):628-633.e1. doi: 10.1016/j.jpeds.2010.09.081. PubMed PMID: 21075381.

10 Gaskins AJ, Rovner AJ, Mumford SL, Yeung E, Browne RW, Trevisan M, Perkins NJ, Wactawski-Wende J, Schisterman EF; BioCycle Study Group. Adherence to a Mediterranean diet and plasma concentrations of lipid peroxidation in premenopausal women. Am J Clin Nutr. 2010 Dec;92(6):1461-7. doi: 10.3945/ajcn.110.000026. Epub 2010 Oct 13. PubMed PMID: 20943796; PubMed Central PMCID: PMC2980970.

E6. Studies using in vivo animal models

1 Sasser JM, Cunningham MW Jr, Baylis C. Serelaxin reduces oxidative stress and asymmetric dimethylarginine in angiotensin II-induced hypertension. Am J Physiol Renal Physiol. 2014 Dec 15;307(12):F1355-62. doi: 10.1152/ajprenal.00407.2014. Epub 2014 Oct 8. PubMed PMID: 25298524; PubMed Central PMCID: PMC4269693.

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8.5. Appendix 5 Excluded studies from search 3

Search 3: To identify literature reporting an association between 8-epi-$PGF_{2\alpha}$ and respiratory disease using the search string: (8-epi-prostaglandin F2alpha OR 8-epi-PGF2)) AND ((respiratory disease) AND (association)) AND ("2008"[Date - Publication]: "2015"[Date - Publication]))) AND English[Language]).

E3. Disease not covered by search categories

4 Fila L, Grandcourtová A, Chládek J, Musil J. Oxidative stress in cystic fibrosis patients with Burkholderia cenocepacia airway colonization: relation of 8-isoprostane concentration in exhaled breath condensate to lung function decline. Folia Microbiol (Praha). 2014 May;59(3):217-22. doi: 10.1007/s12223-013-0285-z. Epub 2013 Oct 17. PubMed PMID: 24132725.

6 Del Ben M, Fabiani M, Loffredo L, Polimeni L, Carnevale R, Baratta F, Brunori M, Albanese F, Augelletti T, Violi F, Angelico F. Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea and the effect of continuous positive airway pressure treatment. BMC Pulm Med. 2012 Jul 23;12:36. doi: 10.1186/1471-2466-12-36. PubMed PMID: 22824065; PubMed Central PMCID: PMC3414800.

8 Font-Ribera L, Kogevinas M, Zock JP, Gómez FP, Barreiro E, Nieuwenhuijsen MJ, Fernandez P, Lourencetti C, Pérez-Olabarría M, Bustamante M, Marcos R, Grimalt JO, Villanueva CM. Short-term changes in respiratory biomarkers after swimming in a chlorinated pool. Environ Health Perspect. 2010 Nov;118(11):1538-44. doi: 10.1289/ehp.1001961. PubMed PMID: 20833607; PubMed Central PMCID: PMC2974690.

9 Rodríguez-Trigo G, Zock JP, Pozo-Rodríguez F, Gómez FP, Monyarch G, Bouso L, Coll MD, Verea H, Antó JM, Fuster C, Barberà JA; SEPAR-Prestige Study Group. Health changes in fishermen 2 years after clean-up of the Prestige oil spill. Ann Intern Med. 2010 Oct 19;153(8):489-98. doi: 10.7326/0003-4819-153-8-201010190-00279. Epub 2010 Aug 23. PubMed PMID: 20733177.

11 Ogawa F, Shimizu K, Hara T, Muroi E, Komura K, Takenaka M, Hasegawa M, Fujimoto M, Takehara K, Sato S. Autoantibody against one of the antioxidant repair enzymes, methionine sulfoxide reductase A, in systemic sclerosis: association with pulmonary fibrosis and vascular damage. Arch Dermatol Res. 2010 Jan;302(1):27-35. doi: 10.1007/s00403-009-0996-9. Epub 2009 Oct 21. PubMed PMID: 19844733.

12 Hara T, Ogawa F, Yanaba K, Iwata Y, Muroi E, Komura K, Takenaka M, Shimizu K, Hasegawa M, Fujimoto M, Sato S. Elevated serum concentrations of polymorphonuclear neutrophilic leukocyte elastase in systemic sclerosis: association with pulmonary fibrosis. J Rheumatol. 2009 Jan;36(1):99-105. doi: 10.3899/jrheum.080269. PubMed PMID: 19208531.

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E5. Studies in narrow population (e.g. pregnant women)

1 Rosa MJ, Yan B, Chillrud SN, Acosta LM, Divjan A, Jacobson JS, Miller RL, Goldstein IF, Perzanowski MS. Domestic airborne black carbon levels and 8-isoprostane in exhaled breath condensate among children in New York City. Environ Res. 2014 Nov;135:105-10. doi: 10.1016/j.envres.2014.09.003. Epub 2014 Sep 28. PubMed PMID: 25262082; PubMed Central PMCID: PMC4346209.

3 Wan GH, Yan DC, Tseng HY, Tung TH, Lin SJ, Lin YW. Cysteinyl leukotriene levels correlate with 8-isoprostane levels in exhaled breath condensates of atopic and healthy children. Pediatr Res. 2013 Nov;74(5):584-91. doi: 10.1038/pr.2013.142. Epub 2013 Sep 2. PubMed PMID: 24153334.

5 Ali Z, Ulrik CS. Obesity and asthma: a coincidence or a causal relationship? A systematic review. Respir Med. 2013 Sep;107(9):1287-300. doi: 10.1016/j.rmed.2013.03.019. Epub 2013 May 1. Review. PubMed PMID: 23642708.

7 Brown SD, Baxter KM, Stephenson ST, Esper AM, Brown LA, Fitzpatrick AM. Airway TGF-β1 and oxidant stress in children with severe asthma: association with airflow limitation. J Allergy Clin Immunol. 2012 Feb;129(2):388-96, 396.e1-8. doi: 10.1016/j.jaci.2011.11.037. Epub 2011 Dec 28. PubMed PMID: 22206775; PubMed Central PMCID: PMC3268912.

10 Holguin F, Fitzpatrick A. Obesity, asthma, and oxidative stress. J Appl Physiol (1985). 2010 Mar;108(3):754-9. doi: 10.1152/japplphysiol.00702.2009. Epub 2009 Nov 19. Review. PubMed PMID: 19926826.

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8.6. Appendix 6 Excluded studies from searches 1 & 2

E1. $PGF_{2\alpha}$ used only as control/biomarker of exposure

2 Kim M, Jung S, Kim SY, Lee SH, Lee JH. Prehypertension-associated elevation in circulating lysophosphatidlycholines, Lp-PLA2 activity, and oxidative stress. PLoS One. 2014 May 6;9(5):e96735. doi: 10.1371/journal.pone.0096735. eCollection 2014. PubMed PMID: 24800806; PubMed Central PMCID: PMC4011750.

E3. Disease not covered by search categories

26 Yager S, Forlenza MJ, Miller GE. Depression and oxidative damage to lipids. Psychoneuroendocrinology. 2010 Oct;35(9):1356-62. doi: 10.1016/j.psyneuen.2010.03.010. Epub 2010 Apr 22. PubMed PMID: 20417039.

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8.7. Appendix 7 Excluded studies from searches 1 & 3

E3. Disease not covered by search categories

44 Antonopoulou S, Loukides S, Papatheodorou G, Roussos C, Alchanatis M. Airway inflammation in obstructive sleep apnea: is leptin the missing link? Respir Med. 2008 Oct;102(10):1399-405. doi: 10.1016/j.rmed.2008.04.021. Epub 2008 Jul 7. PubMed PMID: 18606530.

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8.8. Appendix 8 Excluded studies from searches 2 & 3

E5. Studies in narrow population (e.g. pregnant women)

5 Cangemi R, Calvieri C, Bucci T, Carnevale R, Casciaro M, Rossi E, Calabrese CM, Taliani G, Grieco S, Falcone M, Palange P, Bertazzoni G, Celestini A, Pignatelli P, Violi F; SIXTUS study group. Is NOX2 upregulation implicated in myocardial injury in patients with pneumonia? Antioxid Redox Signal. 2014 Jun 20;20(18):2949-54. doi: 10.1089/ars.2013.5766. Epub 2014 Mar 14. PubMed PMID: 24328853; PubMed Central PMCID: PMC4038979.

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8.9. Appendix 9 Excluded studies from searches 1, 2 & 3

None

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