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APPENDIX A6.1.4.11 OXYSTEROLS

HEALTH EFFECTS OF SMOKING CESSATION

Short Term Health Effects

Oxysterols

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1 BACKGROUND

Oxysterols (OxS) are oxidized cholesterol species that can be formed enzymatically or non-enzymatically (by autoxidation) or can be taken in from exogenous sources, e.g. diet [1-4]. They play important roles in many biological processes, including cholesterol homeostasis [5], atherosclerosis, sphingolipid metabolism, platelet aggregation, apoptosis, and protein prenylation [3, 6, 7]. OxS, in particular side-chain OxS, are postulated to regulate cholesterol homeostasis in cellular membranes [8]. However, more recently, Björkhem [9] concluded that oxysterols are not the master regulators of cholesterol homeostasis *in vivo*, as suggested previously. Because of their ability to pass cell membranes and the blood-brain barrier at a faster rate than cholesterol itself, they are also important as transport forms of cholesterol [6, 9, 10]. Furthermore, OxS are involved in the regulation of lipid metabolism and immune function cytotoxicity. These functions are mediated by specific OxS sensors including liver X receptors (LXR) and the OxS binding protein (OSBP) [11-13].

Circulating OxS levels are almost 3 orders of magnitude lower than cholesterol levels [7]. The quantitatively most important OxS in the human circulation are 27-hydroxycholesterol (27-HC, which is the most abundant OxS and can be further oxidized to 3ß-hydroxy-5-cholestenoic acid (3ß-HCA), originating primarily from the lung), 25-hydroxycholesterol (25-HC), 24(S)-hydroxycholesterol (24S-HC, originating from the brain), 4ß-hydroxycholesterol (4ß-HC) and 7α-hydroxycholesterol (7α-HC) [7]. 7α-HC, 27-HC, 3ß-HCA and 24S-HC can be further converted to bile acids [6]. Of some importance are also cholestane-3ß,5α,6ß-triol (triol) and 24,25-epoxycholesterol [6]. Enzymatically formed OxS include 24S-HC (brain), 25-HC (all cells), 27-HC (all cells except brain) and 4ß-HC (liver), whereas 7-HC, 7-ketocholesterol (7-KC) and 5α(ß),6α(ß)-epoxy-cholesterol (5,6-EC) are formed by autoxidation of cholesterol [2, 14]. OxS, in general, have been shown to exhibit cytotoxic, apoptotic and pro-inflammatory properties to cells and are thus linked to chronic diseases such atherosclerosis, neurodegenerative diseases, inflammatory bowel diseases and age-related macular degeneration [2, 15-18]. Zourrak et al. [14] stated that OxS play a role in almost all age-related diseases. A basic mechanism could be the property of OxS to influence calcium (Ca^{2+}) signal transduction in cells and thus may be involved in many pathological events including atherosclerotic lesions [19].As reviewed by Vaya et al. [20], OxS levels in cells, tissues, organs and body fluids are influenced by a number of factors including reactive oxygen and nitrogen species (ROS/RNS), lipid components, polyunsaturated fatty acids, amino acids and physiological and exogenous anti-oxidants. In addition, conditions that alter enzyme activities mediating cholesterol oxidation are also of importance for OxS levels in the organism. As a consequence, many factors can modulate the endogenous formation of OxS from cholesterol. Additionally, OxS can be taken up with food, where OxS can be formed by lipid oxidation reactions resulting from heating treatments, contact with oxygen and exposure to sunlight. Absorption of OxS from the diet may take place at different rates depending on the side chain length [2]. 27-OHC, an endogenous OxS and the most abundant circulating OxS, is correlated with cholesterol and increases progressively with age [21]. Kummerow et al. [22] reported that the blood

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levels of OxS primarily increase due to consumption of OxS present in fried foods (chicken, fish, French fries), oxidation of cholesterol *in vivo* after excessive consumption of polyunsaturated fatty acids from vegetable oils, and cigarette smoking [22]. In the same study, it was found that healthy men and women aged over 60 years had higher OxS levels compared to those aged under 60 years with women having higher levels than men in both age groups [22]. According to Björkhem [9], the reliable assessment of OxS concentrations in biological samples is complicated by artefactual formation of oxysterols from endogenous cholesterol during collection and workup of the biological samples.

The special properties of OxS, for example the beneficial effects of some OxS in terms of membrane permeability and its easy passing across the blood brain barrier, open new therapeutic approaches for the pharmacological treatment of brain diseases such as Alzheimer's and other age-related disease [1, 2, 9, 14]. A general remark or caution by Poli et al. [16] is of importance when evaluating the biological properties of OxS. These authors submit that this class of compounds is always present as a mixture. OxS may interact with one another by increasing or decreasing the effects of other components in such a way as to modulate the net effect of the mixtures. Therefore, effects of OxS under realistic in vivo conditions may not always be correctly reflected by experimental studies, in which frequently single or only a few OxS were investigated.

2 METHODS

The published information for generating the present document was collected from the following eight sources:

(1) Monographs on biomarkers of effect and smoking:

- US Surgeon General Report 2010 [23]
- Life Sciences Research Office (LSRO) Report of 2007 [24]

(2) References related to OxS extracted from the two monographs (Source 1)

(3) Search 1 in the PubMed literature data base on 13.07.2015 for reviews on OxS; search string: "oxysterols[Title] Filters: Review; published in the last 5 years; Humans"

(4) Search 2 in the PubMed literature data base on 13.07.2015 for references on OxS and smoking or nicotine; search string: "(oxysterols[Title/Abstract]) AND (smokers[Title/Abstract] OR smoking[Title/Abstract] OR tobacco[Title/Abstract] OR nicotine[Title/Abstract])"

(5) Search 3 in the PubMed literature data base on 13.07.2015 for references on OxS and CVD; search string: "(((oxysterols[Title]) AND cardiovascular[Title/Abstract])) OR ((oxysterols[Title/Abstract]) AND cardiovascular[Title])"

(6) Search 4 in the PubMed literature data base on 13.07.2015 for references on OxS and respiratory disease; search string: "(((oxysterols[Title]) AND (respiratory[Title/Abstract] OR lung[Title/Abstract]))) OR ((oxysterols[Title/Abstract]) AND (respiratory[Title] OR lung[Title]))"

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(7) Search 5 in the PubMed literature data base on 13.07.2015 for references on OxS and cancer; search string: (oxysterols[Title]) AND cancer[Title]"

(8) Cited articles from Sources 2 – 7 with relevance for the topics of interest. In addition, Google Scholar was searched for articles on OxS and smoking.

Studies were selected for evaluations in the chapters 'Biomarker-disease link' and 'Smoking-biomarker link' according to the following inclusion criteria:

A: Association between OxS and cardiovascular diseases (CVD) in a human cohort was investigated

B: Association between OxS and respiratory diseases in a human cohort was investigated

C: Association between OxS and any other (smoking-related) disease in a human cohort was investigated

D: Differences in OxS levels between smokers and non-smokers were investigated and suitable data presented

E: The dose-response relationship between smoking intensity (determined for example by daily cigarette consumption, pack-years or cotinine) and OxS levels was studied and suitable data presented

F: The effect of smoking cessation on the OxS level was studied and suitable data presented

G: The effect of switching from conventional cigarettes to modified risk tobacco products (MRTPs) or nicotine products on the OxS level was investigated

R: Review article on OxS

Reviews were used for generating the chapter "Background" and, if suitable, also for chapters on the OxS link to diseases and smoking.

Studies retrieved, but not fulfilling any of the inclusion criteria A – G and R were excluded from the evaluation (assigned as '01 to 05' in Appendix 1 together with the reason for exclusion).

3 FINDINGS

From Source 1 (Monographs) [23, 24], 2 articles [25, 26] were extracted (both originating from the Surgeon General Report 2010, OxS were not mentioned in the LSRO Report).

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The distribution of articles according to the Sources 1 – 8 described in chapter 'Methods' and according the inclusion criteria A – G and R are summarized in Table 1. The individual articles according to sources and assignment to inclusion and exclusion criteria are compiled in Appendix 1.

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Table 1. Number of articles according to source and inclusion criteria.

Source (Description)	All	Relevant	Inclusion criteria[a]								Ex-cluded
			A	B	C	D	E	F	G	R	
1 (Monographs)	2	1	1	0	0	1	0	0	0	0	1
2 (Articles extr. from 1)	2	2	2	0	0	2	0	0	0	0	0
3 (Search 1, reviews)	26	24	6	0	9	0	0	0	0	23	2
4 (Search 2, smoking)	4	3	2	0	0	3	0	0	0	0	1
5 (Search 3, CVD)	16	13	9	0	3	0	0	0	0	5	3
6 (Search 4, resp. dis.)	10	0	0	0	0	0	0	0	0	0	10
7 (Search 5, cancer other diseases)	30	22	4	0	16	0	0	0	0	10	8
8 (Cited articles in 2 – 7, Google Scholar)	14	14	0	0	4	4	0	0	0	10	0
Sum	104[b]	79	24	0	32	10	0	0	0	48	25

[a] Note that an article could fulfil several inclusion criteria

[b] Deviation from the 92 articles listed in Appendix 1 is caused by some overlap between the search results yielding identical articles

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Of the 104 articles retrieved from the various sources, 79 appeared to be suitable for the generation of this document, 25 publications were of no or very limited relevance for this purpose and were, therefore, excluded. Five reasons for exclusion are given in Appendix 1.

Most articles retrieved deal with the criteria CVD (A), other diseases (C) and smoking (D – G) or were reviews (R). OxS is of no or very limited relevance for respiratory diseases (B), as is reflected by the fact that no suitable articles were retrieved for this topic. There were 10 articles which considered the relationship between OxS and smoking (D – G). This includes retrieval hits from Google Scholar, which was performed in order to increase the number of articles for this topic. No published papers were found for the aspects of OxS and smoking dose-response relationship (E), cessation (F) or switching to MRTPs (G). However, when inspecting the full papers, a few articles were identified for the category D (dose-response relationship).

It should be noted that the classification of the retrieved articles was done after a first inspection (usually by reading the abstract). The final classification may deviate from this assignment. However, the distribution of the articles according the defined criteria was very similar to that shown in Table 1, thus, these numbers reflect rather well the knowledge and the load of evidence available for the various categories.

4 BIOMARKER-DISEASE LINK

4.1 Cardiovascular disease

A number of associations between OxS plasma levels and cardiovascular and related diseases have been reported in the last 3 decades. 7ß-HC was shown to be a suitable biomarker for the occurrence of symptomatic and asymptomatic atherosclerotic plaques in carotid and coronary arteries, however, the two types of plaques (symptomatic and asymptomatic) cannot be distinguished by this biomarker [4, 15]. Vaya et al. reported that OxS accumulate in diabetic patients [20]. Obesity and metabolic syndrome were found to be associated with changes in circulating OxS levels compared to healthy controls with females showing more consistent changes compared to males [27, 28], Lithuanian men had increased 7ß-HC levels and lower levels of anti-oxidants than Swedish men, which is thought to be an indicator for increased in vivo lipid peroxidation associated with a higher CVD risk in Lithuania compared to Sweden. Tissue and plasma concentrations of enzymatic-driven OxS are associated with advanced atherosclerosis in patients with severe peripheral artery diseases (PAD) [29]. On the other hand, van Poppel concluded from a case-control study with patients suffering from severe coronary atherosclerotic stenosis that OxS in plasma was not an additional independent risk factor for coronary atherosclerosis [30].

Compared to other biomarkers of effect linked to CVD and related diseases, the evidence for a predictive power of OxS is rather limited. In particular, OxS do not appear to have any advantages to other biomarkers for oxidative stress, inflammation or risk of atherogenic plaque formation. Nevertheless, there is growing evidence in the literature for the involvement of OxS in the pathogenesis of CVD in general and atherosclerosis in particular. Lathe et al. [31] assumed that chronic immunologic challenge and

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inflammation which is known to induce the expression of cholesterol 25-hydroxylase for protection, could, in the long-term, lead to atherosclerosis or Alzheimer's disease (AD). Olkkonen et al. [32] speculated that the generation and deposition of OxS within the developing atherosclerotic plaques could modulate macrophage lipid metabolism, affecting the balance of pro-inflammatory and anti-inflammatory processes and thus determining whether the lesion remains benign or whether it develops into a hazardous, vulnerable plaque. Other researchers propose that the LXR signaling pathway, of which OxS are important activators, are involved in metabolic disorders and atherosclerosis [33, 34]. Zhou et al. [35] showed that the plasma from angina pectoris patients was cytotoxic to cultured epithelial cells and increased the influx of calcium ions (Ca^{2+}) into them. Poli et al. [16] speculated that in particular the OxS 7α-HC, 7ß-HC and 7-KC, which have been demonstrated to induce an inflammatory phenotype in human endothelial cells, could induce foam cell formation in patients suffering from atherosclerosis. Lee et al. [21] found that 27-HC stimulates the inflammatory responses via the estrogen receptor-α in macrophages and vascular endothelial cells and also promotes monocyte/macrophage infiltration into the intimal layer, leading to atherosclerosis development. On the other hand, Crosignani et al. [36] stated that in obese patients, preliminary data suggest an increase in the rate of production of 27-HC, which may be a possible mechanism to counteract the increase of atherosclerotic risk in these subjects.Taken together, the available data in the literature on the link between OxS and CVD or related diseases is limited and as yet insufficient. This is true both for the role of OxS as a predictor for these diseases as well as the possible mechanistic involvement in the pathogenesis.

4.2 Respiratory disease

No reports were found in the literature that showed an association between respiratory diseases and blood levels of OxS in humans.

As mentioned in the section 'BACKGROUND', OxS, in contrast to the precursor cholesterol, have cytotoxic, necrotic, apoptotic, pro- and anti-inflammatory effects on cells, tissues and organs and are therefore likely to be involved in the pathogenesis of a number of chronic, mostly age-related diseases including atherosclerosis, neurodegenerative diseases (e.g. Alzheimers's disease (AD), Parkinson's disease and multiple sclerosis), inflammatory bowel diseases, age-related macular degeneration, cataract, development of gallstones and osteoporosis [2, 6, 15-17, 37-40]. The OxS 24S-HC in plasma is positively associated with early stages of cognitive impairments and negatively with more advanced stages of AD [41] 27-HC antagonizes the preventive effect of 24S-HC, thus 27-OHC may be the link between hypercholesterolemia and AD and other neurodegenerative diseases [42-46]. Elevated levels of 24S-HC in cerebrospinal fluid (CSF) and simultaneously decreased levels in the blood circulation indicates a cognitive impairment disease [47]. 27-HC functions as an endogenous selective estrogen receptor modulator (SERM) linking hypercholesterolemia to osteoporosis [48]. Furthermore, OxS plasma levels are significantly elevated in patients with chronic renal failure compared to healthy controls, most likely due to oxidative stress [49]. OxS in plasma has been described as a suitable diagnostic marker for Niemann-Pick disease (a group of inherited severe metabolic

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disorders that allows the accumulation of sphingomyelins in lysomomes) [50, 51] and in inflammatory bowel disease [16].

Since OxS have been shown to attenuate cell proliferation and provoke cell death as well as modulate several immune cell subsets such macrophages, T-cells and other cell types, this class of compounds may also influence cancer development [21, 52, 53]. However, the information available so far is not sufficient for application of OxS as a suitable biomarker for cancer prediction, diagnosis or prognosis.

5 SMOKING-BIOMARKER LINK

Only limited published information is available for the association between smoking and OxS blood levels. The most probable mechanism by which smoking could elevate OxS levels is by oxidative stress and stimulation of inflammatory processes [16, 54].

5.1 Smoking

In Table 2, three studies were listed which provide OxS data for apparently healthy smokers and non-smokers [30, 54, 55]. Only in one study were significant differences between smokers and non-smokers reported [54]. One review [22] stated that the blood levels of OxS primarily increase due to consumption of OxS in fried foods (chicken, fish, French fries), oxidation of cholesterol *in vivo* after excessive consumption of polyunsaturated fatty acids from vegetable oils, and cigarette smoking. However, no further data were provided to support the statement on cigarette smoking. In another review, Csordas et al. [56] concluded from the available evidence that the issue of whether smoking is associated with increased oxysterol levels and whether elevated levels of oxysterols contribute to adverse clinical outcomes cannot be reliably judged on the basis of the data available so far, and remains to be addressed in future population-based studies. Björkhem and Diczfalusy [6] hold the opinion that in view of the methodological problems (interference by artificial autoxidation of the abundant amounts of cholesterol), oxysterols do not appear to have clear merits in relation to other markers for oxidative modification of LDL.

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Table 2 Oxysterol (OxS) levels in smokers, non-smokers and ex-smokers derived from various populations (sorted by publication year).

Author (reference)	Population	Smokers (S)	Non-smokers (NS)	p-Values and remarks
Mol et al. [54]	Healthy volunteers, males and females, 18-70 years old, from the The Netherlands	Plasma OxS were determined with GC-MS after derivatization, nmol/L, mean±SD[a]: S (N=12): 3ß,7ß-DHC[b]: 42.2±12.8 5,6α-EC: 71.5±23.3 Triol: 36.9±16.4 7-KC: 181±81 25-HC: 22.5±3.8 Total OxS: 354±104	Plasma OxS were determined with GC-MS after derivatization, nmol/L, mean±SD[a]: NS (N=13): 3ß,7ß-DHC[b]: 38.4±12.3 5,6α-EC: 51.0±18.6 Triol: 29.3±7.3 7-KC: 121±38 25-HC: 25.6±7.9 Total OxS: 265±66	S vs NS: P<0.05 for 5,6α-EC, 7-KC and total OxS; other OxS: Not significant After 4 weeks of supplementation with vitamin E, OxS decreased in S and NS (not significant, except for 5,6-EC in S). OxS levels in diabetic patients were mostly between S and NS.

(table continues)

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Author (reference)	Population	Smokers (S)	Non-smokers (NS)	p-Values and remarks
van Poppel et al. [30]	Control subjects of a coronary stenosis study in Rotterdam (The Netherlands), males (65%) and females, age: <68, mean: 52.7 years	7 OxS were determined by a GC-FID (flame ionization detection) method, µg/100mL, mean: Current S (N=22): 1.9	7 OxS were determined by a GC-FID (flame ionization detection) method, µg/100mL, mean: Ex-S (N=39): 2.1 Never S (N=18): 1.9	S vs NS: Not sign. Ex-S vs NS: Not sign. No correlation between number of cigarettes smoked and OxS levels was found.
Yasunobu et al. [55]	Angiographically normal patients form a hospital study in Japan, males and females, mean age: 54.9 years	Four OxS were determined in plasma with a HPLC-UV method, pmol/mL, mean±SEM[c]: S (N=32): 25-HC: 7.4±2.7 27-HC: 46.4±7.3 7α-HC: 106.3±20.0 7ß-HC: 89.9±11.9	Four OxS were determined in plasma with a HPLC-UV method, pmol/mL, mean±SEM[c]: NS (N=38): 25-HC: 4.8±1.3 27-HC: 48.7±6.4 7α-HC: 101.1±16.2 7ß-HC: 104.5±12.0	S vs NS: Not significant for all OxS Also no significant differences between S and NS were found for patients with coronary stenosis

[a] SD: Standard deviation; [b] 3ß,7ß-DHC: 3ß,7ß-Dihydroxychloesterol (abbreviations for other OxS as shown in the text before); [c] SEM: Standard error of the mean;. GC=gas chromatography; MS=mass spectrometry.

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Only three studies were found which mentioned a possible dose-response relationship between OxS blood levels and the intensity of smoking. Van Poppel et al. [30] (Table 2) reported no correlation between OxS and the number of cigarettes smoked per day (CPD) and Arca et al. [57] found no significant correlation between plasma levels of oxysterols and smoking, both in a control and a familial combined hyperlipidemia (FCHL) group. Unfortunately, no information is supplied on which smoking dose parameter was used for calculating the correlation. The same is true for the study of Pordal et al. [58] investigating patients suffering from stable angina or coronary artery stenosis. The authors reported no significant correlation between plasma OxS and 'smoking' (r=0.134, P=0.243).

5.2 Smoking cessation

Even less information is available about the effect of smoking cessation on plasma OxS levels. Van Poppel et al. [30] (Table 2) observed no significant difference in the OxS plasma concentrations between ex-smokers and never smokers.

5.3 Role in MRTP assessment

No published data are available about the effect on OxS blood levels of switching from conventional cigarettes to MRTPs or other nicotine products.

6 CONCLUSION

OxS are cholesterol oxidation products, which can be formed spontaneously and enzymatically in the human body. OxS can also be taken up exogenously (e.g. in the diet). OxS are associated with a number of diseases, in particular CVD and neurodegenerative diseases (e.g. Alzheimer's disease) and with all age-related diseases in general. Due to their biological activities (e.g. cytotoxicity, pro-inflammatory properties), OxS are most probably causally related to the generation of these diseases. Despite that, OxS have only limited value as predictive biomarkers for these pathologies and in fact, appears to be inferior to other biomarkers of oxidative stress and inflammation. Furthermore, data from the literature provide no evidence for a clear association between OxS blood levels and respiratory diseases or cancer.

There is very limited evidence that smoking is associated with an increase of OxS blood levels. No significant dose-response relationship between OxS blood concentrations and smoking intensity has as yet been observed. Also, no sufficient data are available for the effect of smoking cessation on OxS levels. In one study [30], no significant difference between ex-smokers and never smokers was observed. The effect of switching to MRTPs on the OxS levels has not yet been investigated.

Although mechanistically involved in the pathogenesis of many age-related diseases, there is no convincing evidence that OxS are suitable biomarkers of risk for these diseases in general or for evaluating MRTPs, in particular.

The evidence for suitability of OxS as biomarkers of effect in the assessment of MRTPs is summarized in Table 3.

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Table 3. Strength of evidence for the suitability of OxS as a biomarker of effect for MRTP assessment.

Property	Evidence
Biological plausibility	OxS are oxidation products of cholesterol and probably directly involved in the development of age-related diseases such as CVD, neurodegenerative diseases and possibly also cancer. Associations between OxS blood levels and these diseases have been reported. Since smoking can induce inflammation and oxidative stress, an association between smoking and elevated OxS levels is plausible. The available evidence for this relationship, however, is limited.
Dose-response	Differences between smokers and non-smokers (zero dose) were investigated in only few studies (see Table 2). Most of them showed no significant differences between smokers and non-smokers. No significant dose-response relationship between smoking intensity and OxS blood levels were reported.
Benchmark to cessation	No data are available showing that smoking cessation leads to a decrease in OxS levels.
Consistency[a]	Available data for OxS and smoking are limited. Conditions for consistency in terms of differences between smokers and non-smokers, dose-response relationship and effects of smoking cessation are not fulfilled.
Coherence[b]	In this document, almost exclusively human data from clinical and epidemiological studies were evaluated. Also not in the focus of this review, no evidence was found that results from animal and *in vitro* studies would deviate from the human data. However, due to the weak overall evidence available, coherency is not fulfilled.

[a] Consistent findings in various studies strengthens the likelihood that the finding is true

[b] Coherence of findings from epidemiological, laboratory, animal and *in vitro* studies increases the likelihood that the result is true

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7 APPENDICES

7.1 Appendix 1 References related to the included studies
7.2 Appendix 2 Inclusion criteria A
7.3 Appendix 3 Inclusion criteria B (None)
7.4 Appendix 4 Inclusion criteria C
7.5 Appendix 5 Inclusion criteria D
7.6 Appendix 6 Inclusion criteria E (None)
7.7 Appendix 7 Inclusion criteria F (None)
7.8 Appendix 8 Inclusion criteria G (None)
7.9 Appendix 9 Review articles
7.10 Appendix 10 Excluded articles

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7.1 Appendix 1 All retrieved references

Articles were assigned (included, excluded) according the following criteria

0x Excluded (not belonging to at least one category A - G or R)
A Association to CVD

B Association to respiratory diseases

C Association to other diseases

D Levels in smokers and non-smokers

E Dose-response relationship to smoking intensity

F Effect of smoking cessation

G Effect of switching to MRTPs or other nicotine products

R Review article

Articles were included, if they provide relevant information on the criteria A – G or were assigned as relevant review for the biomarker of interest. All other articles are excluded from the evaluation (assigned as 0x, x (reason for exclusion) = 1 to 5).

01 No usable data or information on OxS were presented

02 Clinical study with patients, focus on diagnosis, therapy and prognosis

03 Population investigated is not relevant for the purpose of this document

(e.g. children, pregnant women, ill persons)

04 The topic investigated is not relevant for smoking or a smoking-related disease

05 Animal or in vitro studies with no obvious relation to humans

Sources for published articles to be used for generation of the document

Source 1: Two monographs on biomarkers of effect and smoking [1, 2]

Source 2: Articles derived from monographs: 2 References [3, 4]

Source 3: Articles from PubMed Search 1 (Reviews on OxS): 26 References [5-30]

Source 4: Articles derived from PubMed Search 2 (smoking-related studies on OxS: 4 References [31-34]

Source 5: Articles derived from PubMed Search 3 (association between OxS and cardiovascular diseases): 16 References [23, 35-49]

Source 6: Articles derived from PubMed Search 4 (association between OxS and respiratory diseases): 10 References [50-59]

Source 7: Articles derived from PubMed Search 4 (association between OxS and other diseases, including cancer): 30 References [8, 9, 12, 13, 21, 23, 26, 40, 43, 60-80]

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Source 8: Articles derived from citations in publications from Sources 2 – 7; additionally, Google Scholar was searched for 'smoking' AND 'oxysterols' (4 relevant hits): 14 References [61, 62, 81-92]

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7.2 Appendix 2 Inclusion criteria A

(2) US Department of Health and Human Services. (2010) *How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease*

A Report of the Surgeon General. **A, D**

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(34) van Poppel, G., van de Vijver, L. P., Kosmeyer-Schuil, T., Johanns, E. S., Kardinaal, A. F., van de Bovenkamp, P., Kruyssen, D. A., and Kok, F. J. (1997) Plasma oxysterols and angiographically determined coronary atherosclerosis: a case control study. *Biomarkers*, *2*, 373-378. doi: 310.1080/135475097231472. **D, A**

(35) Aviram, M. (2000) Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. *Free Radic Res.*, *33*, S85-97. **A**

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7.3 Appendix 3 Inclusion criteria B

No articles were identified with relevance to this inclusion criteria.

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(38) Lee, W. R., Ishikawa, T., and Umetani, M. (2014) The interaction between metabolism, cancer and cardiovascular disease, connected by 27-hydroxycholesterol. *Clin Lipidol*, *9*, 617-624. **A, C, R**

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(43) Vejux, A., and Lizard, G. (2009) Cytotoxic effects of oxysterols associated with human diseases: Induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis. *Mol Aspects Med.*, *30*, 153-170. doi: 110.1016/j.mam.2009.1002.1006. Epub 2009 Feb 1025. **C**

(60) Biasi, F., Chiarpotto, E., Sottero, B., Maina, M., Mascia, C., Guina, T., Gamba, P., Gargiulo, S., Testa, G., Leonarduzzi, G., and Poli, G. (2013) Evidence of cell damage induced by major components of a diet-compatible mixture of oxysterols in human colon cancer CaCo-2 cell line. *Biochimie.*, *95*, 632-640. doi: 610.1016/j.biochi.2012.1010.1011. Epub 2012 Oct 1022. **C**

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(62) Bjorkhem, I., Heverin, M., Leoni, V., Meaney, S., and Diczfalusy, U. (2006) Oxysterols and Alzheimer's disease. *Acta Neurol Scand Suppl*, *185*, 43-49. **C, R**

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(71) Klinke, G., Rohrbach, M., Giugliani, R., Burda, P., Baumgartner, M. R., Tran, C., Gautschi, M., Mathis, D., and Hersberger, M. (2015) LC-MS/MS based assay and reference intervals in children and adolescents for oxysterols elevated in Niemann-Pick diseases. *Clin Biochem.*, *48*, 596-602. doi: 510.1016/j.clinbiochem.2015.1003.1007. Epub 2015 Mar 1025. **C**

(72) Leoni, V. (2009) Oxysterols as markers of neurological disease--a review. *Scand J Clin Lab Invest*, *69*, 22-25. doi: 10.1080/00365510802651858. **C, R**

(77) Vaya, J., and Schipper, H. M. (2007) Oxysterols, cholesterol homeostasis, and Alzheimer disease. *J Neurochem.*, *102*, 1727-1737. Epub 2007 Jun 1715. **C**

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(83) Bjorkhem, I. (2006) Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. *J Intern Med.*, *260*, 493-508. **R, C**

(85) Bjorkhem, I., and Diczfalusy, U. (2002) Oxysterols: friends, foes, or just fellow passengers? *Arterioscler Thromb Vasc Biol.*, *22*, 734-742. **R, C**

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7.5 Appendix 5 Inclusion criteria D

(2) US Department of Health and Human Services. (2010) *How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease*

A Report of the Surgeon General. **A, D**

(3) Mahfouz, M. M., Hulea, S. A., and Kummerow, F. A. (1995) Cigarette smoke increases cholesterol oxidation and lipid peroxidation of human low-density lipoprotein and decreases its binding to the hepatic receptor in vitro. *J Environ Pathol Toxicol Oncol*, *14*, 181-192. **A, D**

(4) Panagiotakos, D. B., Pitsavos, C., Chrysohoou, C., Skoumas, J., Masoura, C., Toutouzas, P., and Stefanadis, C. (2004) Effect of exposure to secondhand smoke on markers of inflammation: the ATTICA study. *Am J Med.*, *116*, 145-150. **A, D**

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(34) van Poppel, G., van de Vijver, L. P., Kosmeyer-Schuil, T., Johanns, E. S., Kardinaal, A. F., van de Bovenkamp, P., Kruyssen, D. A., and Kok, F. J. (1997) Plasma oxysterols and angiographically determined coronary atherosclerosis: a case control study. *Biomarkers*, *2*, 373-378. doi: 310.1080/135475097231472. **D, A**

(81) Arca, M., Natoli, S., Micheletta, F., Riggi, S., Di Angelantonio, E., Montali, A., Antonini, T. M., Antonini, R., Diczfalusy, U., and Iuliano, L. (2007) Increased plasma levels of oxysterols, in vivo markers of oxidative stress, in patients with familial combined hyperlipidemia: reduction during atorvastatin and fenofibrate therapy. *Free Radical Biology and Medicine*, *42*, 698-705. **D**

(84) Bjorkhem, I. (2009) Are side-chain oxidized oxysterols regulators also in vivo? *J Lipid Res.*, *50*, S213-218. doi: 210.1194/jlr.R800025-JLR800200. Epub 802008 Oct 800023. **D**

(87) Csordas, A., Wick, G., Laufer, G., and Bernhard, D. (2008) An evaluation of the clinical evidence on the role of inflammation and oxidative stress in smoking-mediated cardiovascular disease. *Biomarker insights*, *3*, 127. **D**

(90) Pordal, A.-H., Hajmiresmail, S. J., Assadpoor-Piranfar, M., Hedayati, M., and Ajami, M. (2015) Plasma oxysterol level in patients with coronary artery stenosis and its changes in response to the treatment with atorvastatin. *Medical Journal of The Islamic Republic of Iran*, *29*, 192-190. **D**

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(92) Yasunobu, Y., Hayashi, K., Shingu, T., Yamagata, T., Kajiyama, G., and Kambe, M. (2001) Coronary atherosclerosis and oxidative stress as reflected by autoantibodies against oxidized low-density lipoprotein and oxysterols. *Atherosclerosis*, *155*, 445-453. **D**

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7.6 Appendix 6 Inclusion criteria E

No articles were identified with relevance to this inclusion criteria.

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7.7 Appendix 7 Inclusion criteria F

No articles were identified with relevance to this inclusion criteria.

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7.8 Appendix 8 Inclusion criteria G

No articles were identified with relevance to this inclusion criteria.

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7.9 Appendix 9 Review articles

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7.10 Appendix 10 Excluded articles

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