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ORIGINAL ARTICLE

Tobacco smoking and oxidative stress to DNA: a meta-analysis of studies using chromatographic and immunological methods

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ABSTRACT

Oxidative stress to DNA from smoking was investigated in one randomized smoking cessation study and in 36 cohort studies from excretion of urinary 8-oxo-7-hydrodeoxyguanosine (8-oxodG). Meta-analysis of the 36 cohort studies showed smoking associated with a 15.7% (95% CL 11.0:20.3, $p < 0.0001$) increased oxidative stress to DNA, in agreement with the reduction of oxidative stress to DNA found in the smoking cessation study. Meta-analysis of the 22 studies that used chromatography methodology on 1709 persons showed a significant 29.3% increase in smokers (95% CL 17.3;41.3), but meta-analysis of 14 studies on 3668 persons using ELISA methodology showed a non-significant effect of 8.7% [95% CL -1.2;18.6]. Tobacco smoke induces oxidative damage to DNA; however, this is not detected with ELISA methodology. Currently, the use of existing ELISA methodology to measure urinary excretion of 8-oxo-7-hydrodeoxyguanosine cannot be recommended.

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KEYWORDS

DNA oxidation; oxidative stress; tobacco

Introduction

In living cells reactive oxidative species (ROS) are constantly generated from biochemical reactions such as cellular metabolism, e.g. electron release from mitochondrial respiration, and external factors such as tobacco smoking, ionizing and UVA radiation. The rate of modification of DNA by oxidation can be evaluated from urinary excretion of oxidized DNA metabolites, e.g. guanine nucleosides, and an association between risk of disease and the DNA oxidation rate has been suggested [1–5].

Increasing evidence indicates that oxidative DNA damage is involved in the development of many chronic and malignant diseases [6–10]. The carcinogenic chemicals in cigarettes might increase the DNA modification by oxidation and also tobacco smoking increases the rate of cellular metabolism, which produces an abundance of ROS [11,12]. Carcinogenic factors in cigarette smoke (e.g. benzo(a)pyrene) and oxidative stress are associated with the same mutations in important tumour suppressor genes and oncogenes [3,6,13–15].

In vitro, tobacco smoke induces DNA oxidation in lung cells [16–18], human lymphoid cells [19], and human

respiratory tract epithelial cells, in agreement with similar findings in peripheral blood cells [20,21]. Since 1992 it has been reported that tobacco smoking is associated with higher oxidative DNA damage in smokers compared with non-smokers [22–25], however studies that report no effects of tobacco smoking have also emerged [26].

The oxidation of guanine in the 8-position, excreted into urine as the nucleoside 8-oxodG, is the most studied urinary biomarker in both animals and humans [3–5,27–29]; it reflects the rate of DNA oxidation in the organism [5,29,30], and is stable in urine at -20°C for 15 years or more [23]. Techniques for measuring 8-oxodG in urine are mainly based on chromatography with electrochemical or mass spectrometry detection or on immunological principles (ELISA) [31,32].

The number of studies on the influence of tobacco smoking and DNA oxidation is now considerable, mainly epidemiological, but show some discrepancy, some studies finding an effect, others reporting no effect.

We therefore conducted a systematic review and meta-analysis of the influence of tobacco smoking on DNA oxidation.

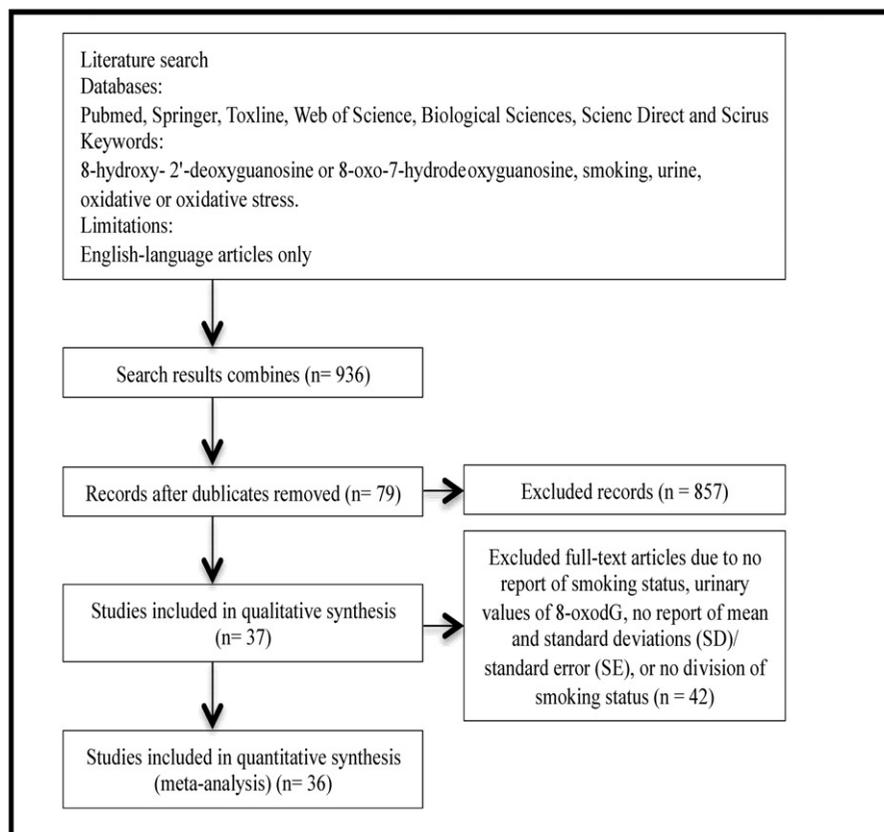


Figure 1. Literature search and elimination strategy.

Material and methods

A systematic electronic search was performed in the following databases: PubMed, Springer, Toxline, Web of Science, Biological Sciences, Science Direct, and Scirus, in 2013. The keywords were: 8-hydroxy- 2'-deoxyguanosine, 8-oxo-7-hydrodeoxyguanosine (8-oxodG), smoking, urine, oxidative or/and oxidative stress. This resulted in 936 hits. The publications were included if the studies reported tobacco smoking status, the levels of 8-oxodG in urine samples, and if the results gave the number of smokers vs. non-smokers, the mean and the variation (e.g. standard variation or range) of DNA oxidation. We found 37 such publications. The flow diagram in Figure 1 shows the elimination process towards the final included studies.

One study was a controlled smoking cessation intervention study, 36 studies reported levels in groups of smokers and non-smokers, from which we recorded whether the study found a significant difference between smokers or not. We determined the latitude of the study centres, the ethnicity of the participants from the origin of the study, and the mean and its variation in the smokers and non-smokers. If range and not variation was reported, we transformed the range to standard deviation. We performed a logistic regression of the outcome yes/no for statistical difference between smokers and non-smokers

on the variables just mentioned. Meta-analysis, as a random effect model, was performed with the meta package for R (Guido Schwarzer, 2013 Meta-Analysis with R. R package version 3.0-1. <http://CRAN.R-project.org/package=meta>). All analyses used R (R Core Team 2013. R: A language and environment for statistical computing. R Foundation for statistical computing. Vienna, Australia. URL: www.R-project.org).

Post hoc we did meta-analyses of the 22 studies using chromatographic methods and the 14 studies using ELISA methods.

The units used for reporting the 8-oxodG were different between studies; therefore, before the meta-analysis, all data, i.e. mean and standard deviation, for the individual studies were normalized to the non-smoking group that was given a value of 100. The value for the smoking group thus provides the per cent in the smoker group relative to the non-smoker group, higher or lower than 100 for positive or negative effect, and similarly for the overall estimate from the meta-analysis.

Results

The 36 cohort studies identified in the systematic review are tabulated in Tables 1 and 2. The 36 studies added up to 1915 smokers and 3462 non-smokers. The tables also

Table 1. Publications in which the urinary excretion of 8-oxodG was statistically significantly higher in smokers.

Study (n) (age group) (country of origin)	Method	Assay	Mean \pm SD	Author [Ref.]
Random subjects (83) (40–64) (DK)	Case-control study	HPLC-ED	Smokers: (n = 30) 320 \pm 99 Non-smokers: (n = 53) 213 \pm 84 pmol/kg/24 h	Loft [22]
Volunteer subjects (123) (35–65) (DK)*	Randomized control-trial with follow-up	HPLC-ED	Smokers: (n = 65) 28.7 \pm 12.6 Smoking cessation: (n = 58) 24.1 \pm 10.5 nmol/24 h	Prieme [25]
Premenopausal women (33) (23–43) (DK)	Cross-sectional study	HPLC-ED	Smokers: (n = 12) 26.7 \pm 11.7 Non-smokers: (n = 21) 18.6 \pm 7.8 nmol/24 h ⁻¹	Loft [41]
Healthy soldiers, men (90) (21–22) (CN/US)	Cross-over study	HPLC-ED	Smokers: (n = 60) 18.0 \pm 9.5 Non-smokers: (n = 30) 6.3 \pm 4.7 mol/mol creatinine/24 h	Li [42]
Healthy subjects (46) (18–71) (D E)	Longitudinal study	HPLC-ED	Smokers: (n = 23) 24.9 \pm 8.2 Non-smokers: (n = 23) 20.5 \pm 7.3 nmol/24 h	Pilger [43]
Healthy subjects (36) (18–33) (D E/SE)	Intervention study	HPLC-ED	Smokers: (n = 16) 0.805 \pm 0.845 Non-smokers: (n = 20) 0.705 \pm 0.541 nmol/kg/24 h	Helbig [44]
Healthy subjects (67) (23–54) (TW)	Case-control study	HPLC-ED	Smokers: (n = 24) 86.6 \pm 73.6 Non-smokers: (n = 43) 53.1 \pm 39.8 nmol	Kuo [45]
Healthy subjects (67) (24–45) (AT)	Case-control study	HPLC-ED	Smokers: (n = 23) 24.7 \pm 11.1 Non-smokers: (n = 44) 19.1 \pm 11.1 nmol/24 h	Pilger [46]
Random subjects (781) (18–80) (JP)	Case-control study	ELISA	Smokers: (n = 154) 12.42 \pm 7.0 Non-smokers: (n = 627) 11.0 \pm 6.6 ng/ml	Kenaya [47]
Bus drivers and office workers (178) (20–>50) (US/TW)	Case-control study	ELISA	Smokers: (n = 108) 10.6 \pm 5.8 Non-smokers: (n = 70) 5.9 \pm 3.9 μ g/g ⁻¹ creatinine/24 h	Han [48]
Taxi drivers and community men (170) (35–51) (TW)	Case-control study	ELISA	Smokers: (n = 87) 13.9 \pm 5.0 Non-smokers: (n = 83) 11.1 \pm 4.1 μ g/g creatinine/spot	Chuang [49]
Healthy subjects (63) (23–53) (ES)	Case-control study	ELISA	Smokers: (n = 22) 10.7 \pm 4.1 Non-smokers: (n = 41) 8.3 \pm 3.6 ng/mg creatinine	Campos [50]
Healthy subjects (180) (35–>35) (R)	Cross-sectional study	HPPLC	Smokers: (n = 71) 2.91 \pm 2.00 Non-smokers: (n = 109) 2.90 \pm 1.12 ng/mg creatinine/spot	Ates [51]
Healthy subjects (34) (43–80) (S)	Case-control study	HPPLC	Smokers: (n = 10) 1.30 \pm 0.38 Non-smokers: (n = 24) 1.02 \pm 0.35 mol/mol creatinine	Tagesson [52]
Healthy subjects (98) (26–87) (L)	Case-control study	HPPLC-GC-MS	Smokers: (n = 41) 2.15 \pm 0.99 Non-smokers: (n = 57) 1.49 \pm 0.52 nmol/mmol creatinine/spot	Fokinsky [53]
Healthy subjects (40) (21–66) (K)	Cross-sectional study	HPPLC-LC-MS-MS	Smokers: (n = 20) 3.51 \pm 1.11 Non-smokers: (n = 20) 2.81 \pm 0.834 ng/mg creatinine/24 h	Lowe [54]
Healthy subjects (67) (15–35) (TW)	Case-control study	SPE-LC-MS-MS	Smokers: (n = 32) 7.26 \pm 3.14 Non-smokers: (n = 35) 4.69 \pm 1.70 μ g/g creatinine	Hu [24]
Healthy volunteers, men (42) (18–60) (CN)	Case-control study	SPE-CE-AD	Smokers: (n = 21) 31.4 \pm 18.9 Non-smokers: (n = 21) 14.4 \pm 7.6 g ⁻¹ creatinine	Yao [55]

*Smoking cessation study, not included in the meta-analysis.

Table 2. Publications in which the urinary excretion of 8-oxodG was not statistically significantly different between groups.

Study (n) (age group) (country of origin)	Method	Assay	Mean \pm SD	Ref.
Office workers (323) (18–67) (JP)	Intervention study	ELISA	Smokers: (n = 97) 9.21 \pm 6.3 Non-smokers: (n = 226) 9.04 \pm 5.0 ng/mg creatinine/spot	Sakano [56]
Coke oven plants workers (217) (NA)	Case-control study	ELISA	Smokers: (n = 101) 13.5 \pm 9.9 Non-smokers: (n = 116) 14.4 \pm 15.0 ng/ml	Wu [57]
Healthy subjects, \geq 20 cigarettes per day (217) (20–69) (JP)	Cross-sectional study	ELISA	Smokers: (n = 63) 12.60 \pm 5.37 Non-smokers: (n = 154) 15.55 \pm 5.38 ng/mg creatinine/spot	Kimura [58]
Healthy subjects (677) (20–67) (JP)	Cross-sectional study	ELISA	Smokers: (n = 236) 8.59 \pm 0.26 Non-smokers: (n = 441) 8.35 \pm 0.26 ng/mg creatinine/24 h	Sakano [59]
Lung patients and control subjects (46) (18–82) (TR)	Case-control study	ELISA	Smokers: (n = 11) 79.9 \pm 165.5 Non-smokers: (n = 35) 63.8 \pm 58.3 nmol mmol ⁻¹ /spot	Karahali [60]
Urothelial carcinoma patients and control subjects (531) (7) (TW)	Case-control study	ELISA	Smokers: (n = 120) 6.41 \pm 13.0 Non-smokers: (n = 411) 6.24 \pm 5.1 ng/mg creatinine/spot	Chung [61]
Healthy subjects (291) (<25) (KR)	Cross-sectional study	ELISA	Smokers: (n = 125) 11.89 \pm 8.36 Non-smokers: (n = 166) 11.68 \pm 8.71 μ mol/mol creatinine	Park [62]
Healthy subjects (72) (60–88) (JP)	Cohort study	ELISA	Smokers: (n = 8) 11.9 \pm 3.7 Non-smokers: (n = 64) 12.1 \pm 7.1 ng/mg creatinine	Saito [63]
Inspection station workers (55) (23–29) (TW)	Case-control study (exposed group)	ELISA	Smokers: (n = 35) 15.64 \pm 13.59 Non-smokers: (n = 20) 14.05 \pm 12.71 μ g/g creatinine	Lee [64]
Women tool station workers (47) (18 \geq 30) (TW)	Case-control study (exposed group)	ELISA	Smokers: (n = 5) 10.2 \pm 4.6 Non-smokers: (n = 42) 13.6 \pm 7.3 μ g/g creatinine	Lai [65]
Filing station attendants (55) (\geq 39– \geq 55) (IT)	Cross-sectional study	HPPLC	Smokers: (n = 33) 1.41 \pm 0.46 Non-smokers: (n = 22) 1.32 \pm 0.50 μ mol/mol creatinine/spot	Lagorio [66]
Healthy women (74) (43–77) (US)	Cross-sectional study	HPPLC	Current smoker: (n = 42) 5.0 \pm 5.6 Former smokers: (n = 32) 5.9 \pm 13.2 ng/mg creatinine	Hakim [67]
Glass production workers (217) (<30–>50) (SE)	Case-control study	HPPLC	Smokers: (n = 75) 14.1 \pm 6.6 Non-smokers: (n = 142) 12.4 \pm 5.7 nmol/l/spot	Tagesson [68]
Healthy subjects (45) (32–40) (NL)	Case-control study	HPPLC-ED	Smokers: (n = 21) 1.97 \pm 2.08 Non-smokers: (n = 24) 2.40 \pm 2.21 ng/body mass index/24 h	Besaratinia [26]
Diesel exposed workers (16) (19–65) (JP)	Case-control study	HPPLC-ED	Smokers: (n = 3) 1.44 \pm 1.12 Non-smokers: (n = 13) 1.48 \pm 1.84 μ mol/mol creatinine/spot	Harri [69]
Healthy subjects (176) (19–80) (S)	Cross-sectional study	HPPLC-LC-MS	Smokers: (n = 80) 6.9 \pm 0.3 Non-smokers: (n = 96) 6.3 \pm 0.2 μ g/g creatinine/24	Harman [70]
Healthy subjects (193) (<63– \geq 63) (TW)	Cross-sectional study	SPE LC-MS-MS	Smokers: (n = 82) 4.5 \pm 5.56 Non-smokers: (n = 111) 4.3 \pm 6.95 ng/mg creatinine	Wong [71]
Healthy subjects (9) (22–49) (DE)	Intervention study	SPE-HPLC-MS-MS	Smokers: (n = 4) 1.7 \pm 0.7 Non-smokers: (n = 5) 2.4 \pm 0.7 ng/mg ⁻¹ creatinine/24 h	Renner [72]
Healthy subjects (41) (22–45) (US)	Case-control study	LCEC	Smokers: (n = 20) 4.94 \pm 3.46 Non-smokers: (n = 21) 3.70 \pm 2.62 ng/ml/24 h	Smith [73]

Table 3. The results of the random effect model meta-analysis on 36 studies on 8-oxodG and smoking, and sub-analysis of studies using chromatography versus ELISA technology.

Smokers (n)	Non-smokers (n)	No. of studies	Methodology	Mean estimate of smoking effect	95% CL
1915	3462	36	All	15.7%*	[11.0;20.3]
743	966	22	Chromatography	29.3%#*	[17.3;41.3]
1172	2496	14	ELISA	8.7% n.s.#	[-1.2;18.6]

*Indicates statistically significant.

#Statistically significant values.

n.s. indicates statistically non-significant, $p > 0.05$.

gives information on the number of participants in the studies, the methodology used (i.e. chromatography or ELISA) and the mean plus the standard deviation of the groups.

By logistic regression, the outcome of the studies, i.e. whether they reported a significant effect of smoking or not, was not predicted by the recorded characteristics (methodology, latitude of the centre of investigation, spot or 24-hour urine, ethnicity, number of persons in the trial); all the confidence limits for the respective OR reached over 1 (data not shown). In the present meta-analysis, we included all papers reporting data prior to 2013 and the studies found were almost exclusively on normal individuals. Consequently, we evaluated bias not to be of much concern and did not make confounding control in the meta-analysis.

The results of the meta-analyses of the 36 studies are given in Table 3, and in Figure 2 the overall analysis is given together with a forest plot. The 14 studies that used ELISA methodology are listed from the top according to the nominal difference found, followed by the 22 studies using chromatographic methods, listed similarly. The total number of smokers/non-smokers measured with ELISA was higher than measured by chromatographic methods, 1172/2496 versus 743/966. Table 3 shows the results of the random effect model meta-analysis in the total 36 studies and the post hoc analysis in the two different methodology groups. The overall meta-analysis showed a significant effect of smoking of 15, 7% (11.0; 20.3). The sub-analysis of the 22 studies using chromatographic methods showed significant increase of 29.3% (17.3; 41.3) and for the 14 studies with ELISA a non-significant increase of 8.7% (-1.2; 18.6). The effect estimates from the two sub-analyses according to methodology were significantly different as evaluated from the almost not overlapping confidence limits as well as with a *t*-test where the standard deviation was assessed from the span of the 95% confidence limits divided by 4, as that range represent 4 standard deviations (*t*-test: $t = 2.78$; $df = 743$, $p < 0.05$). This indicates that the findings by chromatographic- and immunologically-based methods are statistically different.

Discussion

In 1992 we reported for the first time, that tobacco smoking was associated with oxidative stress to DNA, in a random sample of the population of greater Copenhagen [22]. In a subsequent publication on extreme exercise, we found exercise to increase oxidative stress to DNA, however, an influence of tobacco smoke was not detectable [33]. In 1992 Leanderson et al. [17] also reported that *in vitro* exposure to tobacco smoke induced oxidation of DNA. We have now identified 36 studies on the relationship between oxidative stress to DNA and smoking. A total of 19 studies reported no difference while 17 studies reported a significant difference between smokers versus non-smokers, Tables 1 and 2. One study reported a significant reduction in oxidative stress to DNA after smoking cessation [25]. Our meta-analysis of the 36 studies showed a significant difference in oxidative stress to DNA between smokers and non-smokers. The overall difference found (about 15%) corresponds closely to the effect reported after smoking cessation [25]. Taken collectively, this provides firm evidence, from a total of 5377 mainly healthy persons, that tobacco smoke increases oxidative stress to DNA.

There has been considerable concern about the ELISA methodology, the problems relating to both specificity and sensitivity [34]. Our sub meta-analysis of the two methodologies, ELISA and chromatography, confirmed the problem with the ELISA methodology that it is not effective in detecting the effect of smoking. Even though the total number of smokers/non-smokers measured with ELISA was larger, 1172/2496 vs. 743/966, the ELISA could not identify the difference. By contrast, the chromatography-based methods provided a higher estimate of a 29.3% increase in smokers, indicating that the inclusion of ELISA methodology introduced a considerable bias tending to overlook the effect of tobacco smoking. Similar problems about the sensitivity and specificity of the ELISA methodology has been found in a large quality control project [32].

Since the meta-analysis of the cross-sectional epidemiological evidence and the controlled intervention study are in accordance, we conclude that tobacco

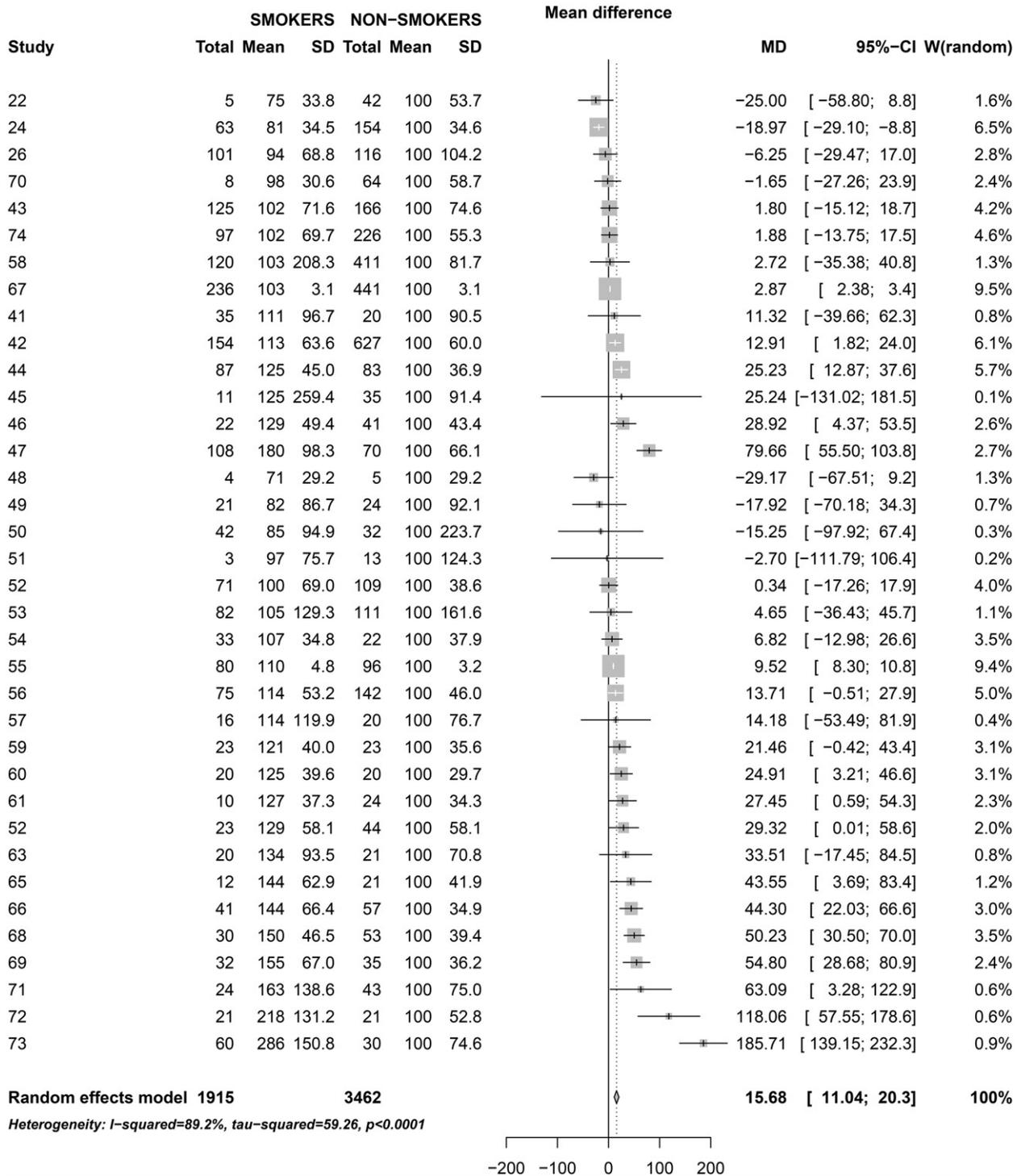


Figure 2. Meta-analysis of 36 studies on oxidative stress in smokers and non-smokers by a random effects model, showing a significant 15.3% increased DNA oxidation in smokers. Study number refers to the reference list. Data extracted from the individual publications are given left of the forest plot. Right of the forest plot is the mean difference between smokers and non-smokers, a negative value indicates that a lower average was found in smokers. Because different units was used in the 36 studies, data has been normalized by setting the non-smoker group to 100, why differences can be interpreted as per cent effect of smoking, positive or negative. Two different methodologies, chromatography and ELISA were used in the 36 studies. The studies are arranged from the top with the ELISA-based studies in increasing order of the found nominal effect of smoking, followed by the studies using chromatography arranged similarly. At the bottom the overall effect of the studies estimated by the meta-analysis is shown. Square brackets give 95% confidence limits. The forest plot gives the mean, SM and SD for each study.

smoking increases the oxidative stress to DNA in normal healthy people.

Both chromatographic- and ELISA-based studies reported positive and neutral effects of smoking on oxidative stress to DNA, but none indicated a reduction of oxidative stress to DNA. That none of 36 studies reported a negative effect strengthens the conclusion that tobacco smoke increases oxidative stress to DNA. As discussed above, the neutral finding by ELISA methodology can be explained by its high variability and lack of specificity. However, it can also be due to lack of statistical power in the individual studies, and to random effects. Apart from the bias introduced by the ELISA methodology, we did not identify bias from the factors used in the logistic regression analysis

Oxidative modification to DNA was initially reported by Kasai [35] and Floyd [36], and the 7,8-dihydro-8-oxoguanine (8oxdG) is believed to be a lesion important in disease development. The mutagenicity found both in bacterial and mammalian cells is clear [37], and the rate by which DNA is oxidized is high [38], but elaborate repair systems will remove most of the modifications. However, if replication occurs before removal/repair, the 8-oxo-deoxyguanosine can mis-pair and result in a mutation. Several groups have reported this in bacteria [34,39] and in mammalian cells [14]. As summarized by Kamiya [37], the mutagenic potential of modifications, induced by oxidation of DNA, is clear and well established, as is the mechanistic link to cancer development [7,40].

We conclude that tobacco smoking is associated with an increased oxidative stress to DNA, measured as the urinary excretion of 8-oxodeoxyguanosine. We cannot recommend the use of ELISA methodology as it is not able to detect the effect of smoking on oxidative stress to DNA.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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