The relationship between biomarkers of oxidative DNA damage, polycyclic aromatic hydrocarbon DNA adducts, antioxidant status and genetic susceptibility following exposure to environmental air pollution in humans

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Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; M_1dG, cyclic pyrimidopurinone N-1,N^2 malondialdehyde-2′-deoxyguanosine; PAHs, Polycyclic aromatic hydrocarbons; c-PAHs, carcinogenic PAHs; B[a]P, Benzo[a]pyrene; GST, Glutathione *S*-transferase; ROS, Reactive oxygen species; DEP, Diesel exhaust particles

Abstract

Polycyclic aromatic hydrocarbons (PAHs) appear to be significant contributors to the genotoxicity and carcinogenicity of air pollution present in the urban environment for humans. Populations exposed to environmental air pollution show increased levels of PAH DNA adducts and it has been postulated that another contributing cause of carcinogenicity by environmental air pollution may be the production of reactive oxygen species following oxidative stress leading to oxidative DNA damage. The antioxidant status as well as the genetic profile of an individual should in theory govern the amount of protection afforded against the deleterious effects associated with exposure to environmental air pollution. In this study we investigated the formation of total PAH (bulky) and B[a]P DNA adducts following exposure of individuals to environmental air pollution in three metropolitan cities and the effect on endogenously derived oxidative DNA damage. Furthermore the influence of antioxidant status (vitamin levels) and genetic susceptibility of individuals with regard to DNA damage was also investigated. There was no significant correlation for individuals between the levels of vitamin A, vitamin E, vitamin C and folate with M_1dG and 8-oxodG adducts as well as M_1dG adducts with total PAH (bulky) or B[a]P DNA adducts. The interesting find from this study was the significant negative correlation between the level of 8-oxodG adducts and the level of total PAH (bulky) and B[a]P DNA adducts implying the that the repair of oxidative DNA damage may be enhanced. This correlation was most significant for those individuals that were non smokers or those unexposed to environmental air pollution. Furthermore the significant inverse correlation between 8-oxodG and B[a]P DNA adducts was confined to individuals carrying the wild type genotype for both the GSTM1 and the

GSTT1 gene (separately and interacting). This effect was not observed for individuals carrying the null variant.

1. Introduction

There is evidence that populations exposed to urban pollution show increased levels of polycyclic aromatic hydrocarbon (PAH) DNA adducts, supporting the hypothesis that PAHs are significant contributors to the genotoxicity and carcinogenicity of air pollution in the urban environment [1], [2] and [3]. Examples include DNA samples from bus drivers in Copenhagen [4], and from populations living in regions with increased pollution in Czech Republic [5] and [6], and Poland [7]. In contrast to this, other studies have not shown a similar relationship. Thus, for example, no relationship was found between exposure to particle-bound PAHs and DNA adduct formation when two populations in Greece were compared, and exposure to environmental tobacco was proposed as a more significant determinant of DNA damage [8].The explanation of this finding may be due to the lower levels of PAHs observed in Greece compared to the Czech Republic or Poland.

Ambient air contains a highly complex mixture of components, and it has been postulated that another contributing cause of carcinogenicity may be the production of reactive oxygen species (ROS), which can oxidatively modify DNA. Thus exposure to respirable particulate matter (PM) has been shown to induce production of ROS [9] and increase levels of 8-oxo-7,8-dihydro-2'deoxyguanosine (8-oxodG) *in vitro* and *in vivo* in experimental systems (reviewed in Risom *et al.* and Sorensen *et al.* [10] and [11]). For example in a population of students whose personal exposure to respirable particulate matter ($PM_{2.5}$) was monitored, a correlation was found between 8-oxodG adducts in lymphocyte DNA, and the extent of personal exposure to $PM_{2.5}$ [12]. A significant relationship has also been observed between 8-oxodG adducts in lungs induced by diesel exhaust particles (DEP) and lung tumour incidence in mice [13].

The mechanism by which these ROS are produced is uncertain. Three possibilities are that the radicals are derived (a) from inflammatory responses caused by the particles, (b) from Fenton radical mediated processes involving particle-associated transition metals, or (c) from redox cycling processes associated with metabolism of xenobiotics. If the latter was the case PAHs could be involved because of the formation of quinone metabolites of PAHs, which can induce ROS via redox cycling. Scheme 1 shows how exposure to carcinogenic PAHs (c-PAHs) such as benzo[a]pyrene (B[a]P) could lead to the generation of oxidative DNA damage as well as the formation of B[a]P DNA adducts following activation to the reactive metabolite, B[a]P diol epoxide. The catechol metabolite is regenerated by NADPH-dependent two electron reduction of the quinone metabolite resulting in redox cycling. [9], [14], [15] and [16]

Factors that may influence the extent of oxidative damage in humans include environmental factors such as exposure to tobacco smoke and passive smoke, intake of food-derived antioxidants, and genetic polymorphisms. The latter could be associated for example with DNA repair of oxidative damage products in DNA (e.g. 8-oxodG glycosylase, OGG1) or with enzymes involved in the metabolism of xenobiotics that act as an indirect source of free radicals. Genetic polymorphisms in genes involved in metabolism and detoxification such as CYP1A1, GSTM1, GSTT1 and NAT2 could potentially affect the susceptibility of an individual to the adverse effects of environmental air pollution [17] and [18]. Glutathione S-transferases (GSTs) are a class of phase II enzymes, which can detoxify PAH epoxides as well as quinones, and hydroperoxides, formed as a consequence of oxidative stress, by conjugation with gluthathione [19]. The frequency of individuals with homozygous deletions for the GSTM1 and GSTT1 genes is relatively high ranging from 20 to 50%

for the majority of populations that have been studied, showing the null genotype [20]. The effect of these polymorphisms in the current study is reported by Garte *et al.* [21]. Antioxidants have an important role in minimising the amount of oxidative DNA damage that may arise. For example a study conducted with male smokers and non smokers who received an antioxidant supplement consisting of vitamin C and E as well as β-carotene, resulted in the reduction of the level of oxidised damage in lymphocyte DNA by 40% compared to individuals given a placebo [22]. Studies *in vitro* have shown that the level of oxidative DNA damage is reduced following the exposure of HepG2 cells pretreated with vitamins E and C, to ambient air particles with complex mixtures of organic compounds adsorbed onto their surface [23]. Another *in vitro* study showed clearly that the antioxidants present in lung lining fluid provided protection against the oxidative DNA damaging effects of respirable particulate matter [24].

In this paper we will describe the results obtained for total PAH (bulky) and B[a]P DNA adducts following exposure of individuals to environmental air pollution and comparisons with endogenously derived oxidative DNA damage as well as the effect of vitamin levels and genetic susceptibility on DNA damage.

2. Materials and methods

2.1. The study population

The population under study is described in detail elsewhere [25] and [26], but in brief the exposed group was a total of 204 individuals, selected from Prague (Czech Republic) (city policemen), Kosice (Slovak Republic) (city policemen), and Sofia (Bulgaria) (city policemen and bus drivers) [26]. Unexposed controls, who were non-occupationally exposed to traffic pollutants were selected and matched for age and gender (152 individuals). Blood samples were collected from each participant for determination of DNA adduct levels, genetic susceptibility, and determination of other biomarkers [25]. A questionnaire was completed by each individual providing demographic, smoking and dietary information.

2.3. Biomarkers of DNA damage

As a measure of oxidative DNA damage, 8-oxodG was determined by liquid chromatography-tandem mass spectrometry selected reaction monitoring (LC-MS/MS SRM) in post-shift lymphocyte DNA samples from 98 exposed individuals and 105 controls from Prague and Kosice. A second measurement of oxidative stress was the determination of the malondialdehyde DNA adduct, M_1dG by an immunoslot blot assay in post-shift lymphocyte DNA from 198 exposed and 156 control individuals from all three cities.

As biomarkers of exposure to PAHs, total PAH (bulky) adducts and the specific adduct arising from B[a]P were measured in lymphocyte DNA, using ^{32}P-postlabelling and following protocols described elsewhere [27].

2.3. Determination of vitamin levels

Vitamin C (ascorbic acid) was determined in plasma of the individuals using spectrophotometry method as previously described by Kiyoh and Megumi [28]. Vitamin E (alpha-tocopherol) and vitamin A were determined by using a HPLC-UV detection method described by Driskell *et al.* following n-heptane extraction from the plasma [29].

The CEDIA folate kit (Roche Diagnostics, Prague, Czech Republic) was used for the determination of folates (FA) in plasma. Sorbation was measured on the ELISA Reader Spectra (TECAN) at wavelength 415 nm (with the reference wavelength 630 nm and the limit of detection 0.6 ng/ml). According to Roche Diagnostics, the level of FA in healthy population corresponds to 2.7 – 16.1 ng/ml (6.1-36.5 nmol/L).

2.4. Cotinine analysis

Urinary cotinine levels as a marker of active and passive smoking were analyzed by radioimmunoassay [30]. Subjects with cotinine levels greater than 500 ng/mg of creatinine were considered smokers.

2.5. Statistical analysis

Due to the differences in adducts levels across the three countries (see paper 1), adducts levels were standardized, dividing values by the average of each adduct in the corresponding country. Data on 8-oxodG, M_1dG, bulky DNA adducts and B[a]P DNA adducts levels are presented as means and standard deviations (SD). Smoking status has been defined according to cotinine levels (adjusted by creatinine levels): subjects were defined as smokers when cotinine levels were greater than 500 ng cotinine/mg of creatinine, passive smokers when cotinine levels were comprised between 200 and

500 ng cotinine/mg, and non smokers when cotinine levels were lower than 200 ng cotinine/mg. Using the measurements of personal exposure obtained with the personal monitors, subjects were reclassified as exposed when personal exposure was greater than the median value of average exposure of not occupationally exposed subjects, i.e. 7.55 ng/m^3, and as non exposed when personal exposure values were lower or equal to 7.55 ng/m^3.

The correlation between oxidative DNA damage (8-oxodG and M_1dG adducts levels) and environmental exposure (bulky DNA adducts and B[a]P DNA adducts levels, vitamins and folate levels) were assessed by Pearson correlation analysis. The interaction with smoking and PAHs exposure was assessed performing the correlation analysis stratified by smoking status (current smokers, passive smokers, and non smokers), by exposure status (exposed versus unexposed both using job definition and monitor measurements) and metabolic polymorphism genes.

P values lower than 0.05 were considered as statistically significant. All the statistical analyses were performed using SAS statistical package (8.1 Version, SAS Institute Inc., Cary, NC).

3. **Results**

A total of 354 individuals were included in the present analysis. The 8-oxodG adduct levels were not measured in the DNA samples from Bulgaria, while B[a]P DNA adduct levels for the samples from the Slovak Republic could not be used for the analysis because they were below the limit of detection.

The means and Standard Deviations (SD) of vitamins and folate are shown in table 1 and table 2 shows the results of the correlation analysis between the oxidative DNA damage markers (M_1dG and 8-oxodG adducts levels) and the DNA exposure markers (B[a]P and bulky DNA adducts levels) as well as antioxidant levels (vitamin levels). The M_1dG adducts are not associated with either B[a]P adducts or bulky DNA adducts, while the correlation between 8-oxodG and B[a]P DNA adducts and bulky DNA adducts is statistically significant, p = 0.002 and 0.036, respectively. The Pearson correlation coefficient is negative, thus indicating that with increasing levels of B[a]P and bulky DNA adducts the level of 8-oxodG adducts is decreased (figure 1). The oxidative DNA damage markers do not correlate with vitamin levels. When stratified by smoking status (table 3) and c-PAHs exposure status (both monitor and job definition) (tables 4 and 5), the correlation between 8-oxodG adducts and exposure markers persists strongly in non smokers and in unexposed subjects. The group of subjects exposed to passive smoking (n = 25) is very small, therefore it is difficult to reach conclusions on the effect of passive smoking on 8-oxodG adduct levels.

The correlation between 8-oxodG and B[a]P DNA adducts is strongly influenced by genetic susceptibility (table 6): individuals carrying the wild type genotype for both the GSTM1 and the GSTT1 gene (separately and interacting) show a significant

strong inverse correlation between the two adducts, while this effect disappears in individuals carrying the null variant.

4. **Discussion**

A significant contribution to the genotoxicity and carcinogenicity in humans of exposure to air pollution present in the urban environment appears to be related to the content of PAHs. It is generally found that populations exposed to environmental air pollution show increased levels of PAH DNA adducts [1]. There is evidence linking the metabolism of PAHs with the generation of ROS and oxidative stress [15] and [16]. It has been postulated that another contributing cause of carcinogenicity by environmental air pollution may be the production of ROS following oxidative stress leading to oxidative DNA damage. We investigated the formation of total PAH (bulky) and B[a]P DNA adducts following exposure of individuals to environmental air pollution and the effect on endogenously derived oxidative DNA damage as well as the effect of vitamin levels on DNA damage and genetic susceptibility.

Antioxidants present in the diet in theory should provide protection against the deleterious effects of xenobiotics that have the potential or lead to the induction of oxidative stress [31] and [32]. There was no significant correlation found for individuals between the levels of vitamin A, vitamin E, vitamin C and folate with M_1dG and 8-oxodG adducts. Analogous findings were observed when the results were analysed according to the smoking status, job definition and air monitor level. A Similar observation was reported by Collins *et al.,* with no correlation being observed between 8-oxodG adducts in lymphocyte DNA and the antioxidant status in individuals from five European countries [33]. A study involving men on high fruit and vegetable diets again failed to find any correlation with level of 8-oxodG adducts excreted into the urine [34]. These findings conflict with the generally accepted consensus of opinion that an intake of fruits and vegetables by healthy individuals leads to a reduction of oxidative DNA damage [35]. A study involving coke-oven

workers who took at least one multi vitamin pill showed that the urinary excretion of 8-oxodG adducts was decreased compared to those workers that took no vitamins [36]. In a separate study the level of M_1dG adducts was found to be decreased in human colorectal mucosa DNA from men that had a diet with high levels of fruit and vegetables [37].

The level of M_1dG adducts for individuals did not show any correlation with total PAH (bulky) or B[a]P DNA adducts. Implying that exposure to PAHs does not have any significant influence on the pathways involved in the formation or removal of M_1dG adducts. The mechanism of formation of M_1dG adducts involves free radical mediated lipid peroxidation resulting in the generation of malondialdehyde that can react with DNA [38]. Another potential source of M_1dG adducts can be from malondialdehyde that is generated as a by-product of prostaglandin biosynthesis [39]. Formation of M_1dG adducts can also occur independently from lipid peroxidation by the generation of base propenals, which are formed by the hydroxyl radical mediated removal of the deoxyribose 4'-hydrogen in DNA [40]. The repair of M_1dG adducts is mediated by a nucleotide excision repair pathway [41].

The interesting finding from this study was that there was a significant negative correlation between the level of 8-oxodG adducts and the level of total PAH (bulky) and B[a]P DNA adducts. The correlation was most significant for those individuals that were non smokers or those unexposed to environmental air pollution. This finding contradicts what would be expected to occur as a consequence of the pathway involving the metabolism of PAHs by aldo-keto reductase to generate catechols and subsequent redox cycling, resulting in the generation ROS, leading to potentially increased levels of oxidative DNA damage (refer to Scheme 1) [15] and [16]. The literature contains reports of numerous studies to ascertain whether ROS production

and PAH metabolism are related and often resulting in conflicting conclusions. For example the level of 8-oxodG adducts was shown to be elevated in liver, kidney and lung following dosing of rats orally with B[a]P [42]. Dietary administration of DEP to Big Blue rats® resulted in an elevation of bulky DNA adducts in colon and liver DNA but no increase in 8-oxodG adduct levels in these tissues or in the urine [43]. In contrast inhalation of DEP by Big Blue® rats resulted in increased 8-oxodG adduct levels and in increased bulky DNA adducts in lung DNA [44]. There was no correlation found between the level of PAH DNA adducts and the level of urinary 8-oxodG adducts in non smoking Danish bus drivers and postal workers working in rural/suburban and city centre locations [4]. Also no correlation was found between the level of PAH DNA adducts and the level of 8-oxodG adducts in lymphocyte DNA obtained from individuals with and without lung cancer [45]. No associations between PAH DNA adducts and different oxidative DNA damage adducts including 8-oxodG were observed in cancerous and surrounding normal larynx tissues obtained from smoking individuals [46]. However in coke oven workers who were exposed to varying amounts of PAHs, a weak positive correlation was found between 8-oxodG adducts and PAH DNA adducts in the white blood cell DNA, but no correlation between 8-oxodG levels and urinary 1-hydroxypyrene, a metabolite used for monitoring exposure to pyrene [47]. Experiments using cell-free systems have shown that the oxidative capacity of respirable particulate matter was more associated with an aqueous extract of the particles rather than with an organic extract which contained most of the DNA adduct-yielding PAHs [48].

Alternatively a mechanism unrelated to PAH metabolism, involving inflammation may be occurring. Exposure to respirable particulate matter can result in the influx of alveolar macrophages, which in turn can generate free radicals leading to oxidative

stress [14]. Intratracheal injections of the carbonaceous (particulate) part of diesel particles resulted in increases in 8-oxodG adduct levels in the lung tissue of mice but no increase was observed when PAHs such as B[a]P were introduced into the mice. Thus highlighting the involvement of alveolar macrophages in generation of hydroxyl radicals as a consequence of phagocytosis [49]. The levels of 8-oxodG adducts may also be increased as a consequence of Fenton radical mediated processes that involve transition metals which are associated respirable particulate matter [11].

A possible reason for the decrease in levels of 8-oxodG adducts with increasing PAH DNA adducts observed in this study may be explained by the induction of increased oxidative DNA damage repair as a consequence of exposure to environmental air pollution. Evidence for this is provided by Risom *et al.* who found that there was an up-regulation of expression of the oxidative DNA damage repair enzyme DNA glycosylase OGG1 in the lung following repeated exposure of mice to diesel exhaust particles by inhalation. A single dose of diesel exhaust particles resulted in increased levels of 8-oxodG in the lung tissue but these levels returned to steady state levels following repeated dosing as consequence of increased DNA repair [50]. Furthermore Toraason *et al.* concluded that there was an induction of DNA repair mechanisms in coal-tar exposed roofers over time as determined by a statistically significant decrease in white blood cell 8-oxodG adduct levels and an increase in the urinary excretion of 8-oxodG [51]. A similar conclusion was made by Briede *et al.*, when they administered a single oral dose of B[a]P to rats and observed a decrease in the levels of 8-oxodG adducts with the formation of bulky DNA adducts in liver and lung and consequent increase in the level 8-oxodG excreted into the urine again implying the induction of DNA repair mechanisms [52].

The negative correlation between 8-oxodG and B[a]P DNA adducts was strongly positively influenced by individuals carrying the wild type genotype for both the GSTM1 and the GSTT1 gene (both separately or interacting), while this effect disappeared in individuals carrying the null variant. The general consensus of opinion is that the GSTM1 null genotype confers an enhanced risk of susceptibility to developing cancer following exposure environmental air pollution. In contrast GSTT1 null genotype does not seem to be associated with cancer risk. The increased risk of cancer development following exposure to environmental air pollution in the GSTM1 null genotype individuals may be as a consequence of increased formation of DNA adducts. Topinka *et al.* showed that placental bulky DNA adducts were higher in environmentally air pollution exposed populations with GSTM1 null genotype compared to populations with the GSTM1 present genotype [53]. A similar observation was made for non smoking students exposed to urban air pollution and environmental tobacco smoke [54]. It has been shown that never smoking women with the null GSTM1 genotype have a statistically significant greater risk of developing lung cancer from exposure to environmental tobacco smoke [55]. A similar conclusion was reached by Lan *et al.* who studied the lung cancer risk of individuals exposed to indoor coal combustion emissions [56]. The results from this study imply that the presence of detoxification enzymes such as GSTs may result in the reduction in the level of reactive PAH quinone metabolites thus lowering the level of oxidative DNA damage by subsequent redox cycling. However the formation of PAH DNA adducts may remain unaffected following metabolism via the PAH diol epoxide pathway (scheme 1).

In conclusion the contribution to the genotoxicity and carcinogenicity in humans following exposure to air pollution present in the urban environment appears not be

related just to one factor and consideration of the influence of a combination of factors such as oxidative DNA damage, PAH DNA adducts, antioxidant status and genetic susceptibility is required.

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Scheme 1 Pathways for the metabolism, the generation of ROS and formation of DNA adducts following exposure to benzo[a]pyrene

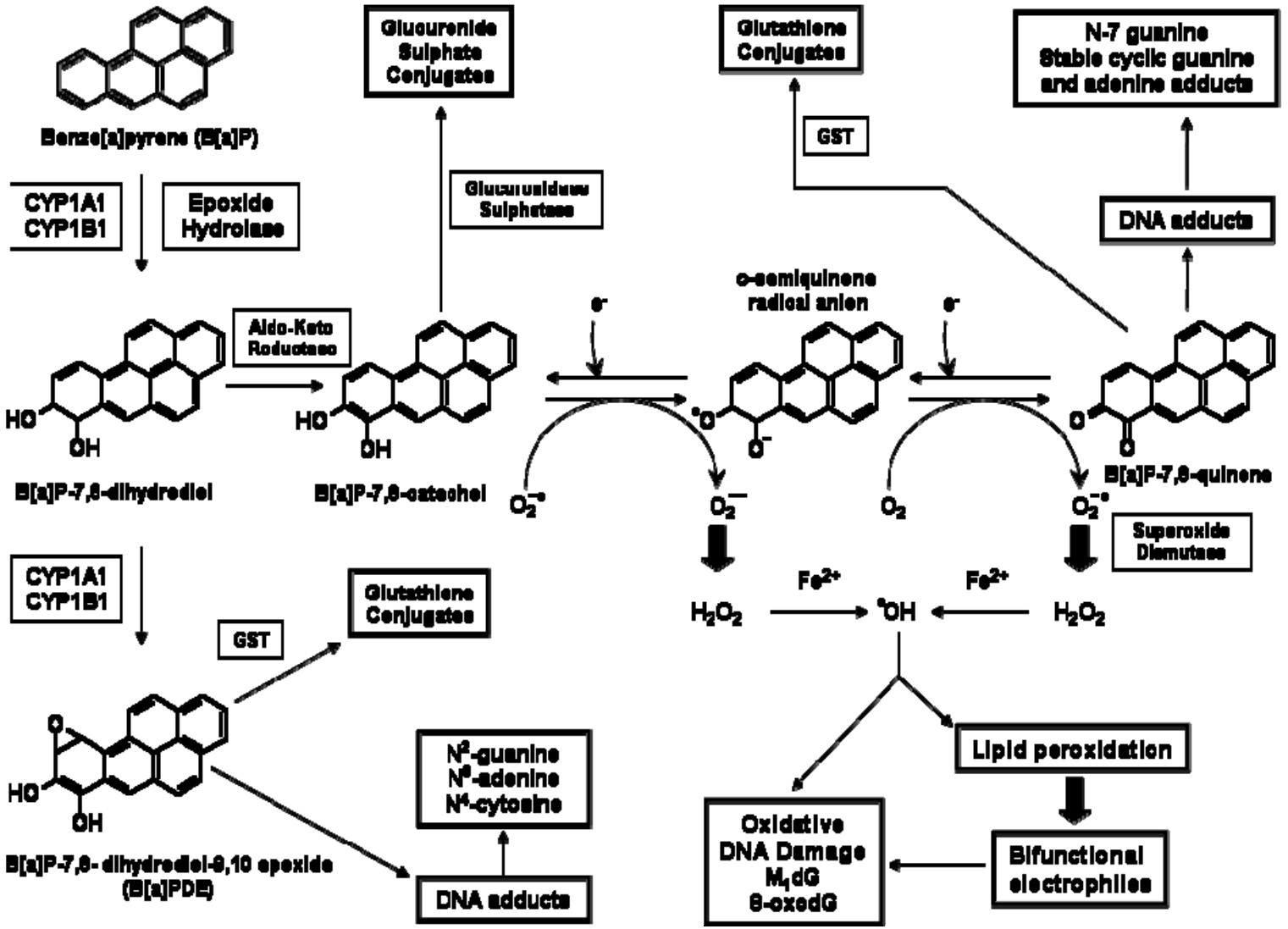
Benzo[a]pyrene (B[a]P)
CYP1A1
CYP1B1
Epoxide Hydrolase
Glucuronide Sulphate Conjugates
Glucuronidase Sulphatase
Aldo-Keto Reductase
B[a]P-7,8-dihydrodiol
B[a]P-7,8-catechol
e-
o-semiquinone radical anion
e-
Glutathione Conjugates
GST
N-7 guanine Stable cyclic guanine and adenine adducts
DNA adducts
B[a]P-7,8-quinone
Superoxide Dismutase
$O_2^{-\bullet}$
O_2
H_2O_2
Fe^{2+}
$^{\bullet}OH$
Lipid peroxidation
Bifunctional electrophiles
Oxidative DNA Damage
M_1dG
8-oxodG
CYP1A1
CYP1B1
GST
Glutathione Conjugates
B[a]P-7,8-dihydrodiol-9,10 epoxide (B[a]PDE)
N^2-guanine
N^6-adenine
N^4-cytosine
DNA adducts

Table 1 Means and Standard Deviations (SD) of vitamins and folate

	Number of subjects	**Mean**	**SD**
Vitamins A	354	2.37	2.07
Vitamins E	354	18.22	9.34
Vitamins C	329	42.31	45.25
Folate	105	17.22	9.82

Table 2 Associations between oxidative DNA damage adducts and B[a]P and total PAH (bulky) DNA adducts levels plus antioxidant levels (Pearson correlation coefficients and *p* values)

	B[a]P DNA	**bulky DNA**	**Vitamins A**	**Vitamins E**	**Vitamins C**	**Folate**
M_1dG	0.05 (n.s)	-0.01 (n.s.)	-0.01 (n.s.)	-0.03 (n.s.)	-0.01 (n.s.)	-0.06 (n.s.)
8-oxodG	-0.30 (0.002)	-0.15 (0.04)	-0.03 (n.s.)	-0.01 (n.s.)	0.03 (n.s.)	-0.12 (n.s.)

Table 3A Associations between oxidative DNA damage adducts and B[a]P and total PAH (bulky) DNA adducts levels plus antioxidant levels according to smoking status

	B[a]P DNA	**bulky DNA**	**Vitamins A**	**Vitamins E**	**Vitamins C**	**Folate**
Non smokers						
M_1dG	-0.00 (n.s.)	-0.00 (n.s.)	-0.00 (n.s.)	-0.08 (n.s.)	0.03 (n.s.)	-0.09 (n.s.)
8-oxodG	-0.34 (0.003)	-0.71 (0.05)	-0.06 (n.s.)	0.01 (n.s.)	0.06 (n.s.)	-0.25 (0.03)
Smokers						
M_1dG	0.08 (n.s.)	-0.05 (n.s.)	-0.03 (n.s.)	0.06 (n.s.)	-0.02 (n.s.)	0.03 (n.s.)
8-oxodG	-0.38 (0.06)	-0.16 (n.s.)	-0.01 (n.s.)	-0.04 (n.s.)	0.01 (n.s.)	0.20 (n.s.)

(Pearson correlation coefficients and *p* values)

Table 3B Pearson correlation coefficients and *p* values, according to smoking status

	B[a]P DNA	**bulky DNA**	**Vitamins A**	**Vitamins E**	**Vitamins C**	**Folate**
Non smokers						
M_1dG	0.02 (n.s.)	-0.03 (n.s.)	-0.01 (n.s.)	-0.07 (n.s.)	0.02 (n.s.)	-0.11 (n.s.)
8-oxodG	-0.33 (0.004)	-0.17 (0.07)	-0.09 (n.s.)	0.01 (n.s.)	0.07 (n.s.)	-0.26 (0.02)
Passive smokers						
M_1dG	0.12 (n.s.)	0.11 (n.s.)	0.01 (n.s.)	-0.24 (n.s.)	0.18 (n.s.)	-
8-oxodG	-	-0.17 (n.s.)	0.06 (n.s.)	0.26 (n.s.)	0.38 (n.s.)	-
Smokers						
M_1dG	0.08 (n.s.)	-0.05 (n.s.)	-0.03 (n.s.)	0.06 (n.s.)	-0.02 (n.s.)	0.03 (n.s.)
8-oxodG	-0.38 (0.06)	-0.16 (n.s.)	-0.01 (n.s.)	-0.04 (n.s.)	0.01 (n.s.)	0.20 (n.s.)

Table 4 Associations between oxidative DNA damage adducts and B[a]P and total PAH (bulky) DNA adducts levels plus antioxidant levels according job definition (Pearson correlation coefficients and *p* values)

	B[a]P DNA	bulky DNA	Vitamins A	Vitamins E	Vitamins C	Folate
Unexposed						
M_1dG	-0.06 (n.s.)	-0.08 (n.s.)	0.06 (n.s.)	0.00 (n.s.)	0.12 (n.s.)	0.01 (n.s.)
8-oxodG	-0.36 (0.010)	-0.28 (0.004)	-0.16 (n.s.)	0.02 (n.s.)	0.15 (n.s.)	-0.19 (n.s.)
Exposed						
M_1dG	0.10 (n.s.)	0.01 (n.s.)	-0.05 (n.s.)	-0.05 (n.s.)	-0.09 (n.s.)	-0.10 (n.s.)
8-oxodG	-0.22 (n.s.)	-0.12 (n.s.)	0.15 (n.s.)	-0.05 (n.s.)	-0.09 (n.s.)	-0.05 (n.s.)

Table 5 Associations between oxidative DNA damage adducts and B[a]P and total PAH (bulky) DNA adducts levels plus antioxidant levels according to monitor definition of exposure to PAHs (Pearson correlation coefficients and *p* values)

	B[a]P DNA	bulky DNA	Vit A	Vit E	Vit C	Folate
Unexposed						
M_1dG	-0.15 (n.s.)	-0.13 (n.s.)	-0.14 (n.s.)	-0.08 (n.s.)	0.05 (n.s.)	-0.17 (n.s.)
8-oxodG	-0.28 (0.02)	-0.16 (n.s.)	-0.09 (n.s.)	-0.02 (n.s.)	0.11 (n.s.)	-0.20 (n.s.)
Exposed						
M_1dG	0.12 (n.s.)	0.02 (n.s.)	0.03 (n.s.)	-0.02 (n.s.)	-0.04 (n.s.)	0.15 (n.s.)
8-oxodG	-0.32 (0.05)	-0.15 (n.s.)	-0.03 (n.s.)	-0.02 (n.s.)	-0.07 (n.s.)	0.11 (n.s.)

Table 6 Correlation between 8-oxodG and B[a]P DNA adducts according to GSTM1 and GSTT1 polymorphisms.

	N	**Pearson Coefficient**	***p***
GSTM1 present	42	-0.46	0.002
GSTM1 null	60	-0.21	n.s.
GSTT1 present	83	-0.29	0.009
GSTT1 null	19	0.38	n.s.
GSTM1 present GSTT1 present	33	-0.40	0.02
GSTM1 present GSTT1 null	9	-0.68	0.04
GSTM1 null GSTT1 present	50	-0.23	n.s.
GSTM1 null GSTT1 null	10	-0.24	n.s.

Figure 1 Correlation between 8-oxodG and B[a]P DNA adducts levels for the whole sample set.