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Bulky DNA adducts in white blood cells: a pooled analysis of 3600 subjects

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Abstract

Background—Bulky DNA adducts are markers of exposure to genotoxic aromatic compounds, which reflect an individual's ability to metabolically activate carcinogens and to repair DNA damage. Polycyclic aromatic hydrocarbons (PAH) represent a major class of carcinogens that are capable of forming such adducts. Factors that have been reported to be related to DNA adduct levels include smoking, diet, body mass index (BMI), genetic polymorphisms, the season of collection of biologic material, and air pollutants.

Methods—We pooled eleven studies (3,600 subjects) in which bulky DNA adducts were measured in human white blood cells with similar ^{32}P-postlabelling techniques and for which a similar set of variables was available, including individual data on age, gender, ethnicity, batch, smoking habits, BMI, season of blood collection and a limited set of gene variants.

Results—Lowest DNA adduct levels were observed in the spring (median 0.50 adducts per 10^8 nucleotides), followed by summer (0.64), autumn (0.70) and winter (0.85) (p=0.006). The same pattern emerged in multivariate analysis, but only among never smokers (p=0.02). Adduct levels were significantly lower (p=0.001) in Northern Europe (the Netherlands, Denmark) (mean 0.60, median 0.40) than in Southern Europe (Italy, Spain, France, Greece) (mean 0.79, median 0.60).

Conclusions—In this large pooled analysis, we have found only weak associations between bulky DNA adducts and exposure variables. Seasonality (with higher adducts levels in winter) and air pollution may partly explain some of the inter-area differences (North vs South Europe), but most inter-area and inter-individual variation in adduct levels still remain unexplained.

Impact—Our study describes the largest pooled analysis of bulky DNA adducts so far, showing that inter-individual variation is still largely unexplained, though seasonality appears to play a role.

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Keywords

DNA adducts; air pollution; seasonality

Background

Bulky DNA adducts are markers of exposure to genotoxic aromatic compounds and of the ability of the individual to metabolically activate carcinogens and to repair DNA damage (1). Experimental studies in animal models have highlighted the central role of DNA adduct formation in tumorigenesis (2), and key human studies have shown that carcinogenic polycyclic aromatic hydrocarbons (PAHs) represent a major class of carcinogens present in the environment, that are capable of forming DNA adducts at the same DNA bases where p53 mutations are found in lung cells of smokers (3). When unrepaired, DNA adducts can cause mutations, including mutational hot-spots in the p53 tumour suppressor gene and other genes, that may ultimately induce cancer formation (3).

Human studies have shown a dose–response relationship between occupational exposure to PAHs and the levels of DNA adducts in lymphocytes of workers (4), but at high levels of exposure saturation seems to occur. Although cigarette smoke also contains PAHs and other DNA adduct-forming compounds, studies on the association between tobacco smoking and DNA adducts in white blood cells (WBC) have yielded inconsistent results (5). In contrast, studies conducted on human lung tissue did show an association with tobacco smoke (4–7). Some studies have reported a negative correlation between DNA adduct levels and the consumption of fruit and vegetables and the intake of flavonoids (8–11), and the dose response-relationship with smoking may be affected by various dietary factors, especially in subjects with certain genetic polymorphisms in metabolic enzymes (5). Other factors that were reported to influence DNA adduct formation included: body mass index (BMI), genetic polymorphisms in genes involved in the metabolism of carcinogens, the season in which the WBC/lymphocytes were sampled, and environmental pollutants such as O_3 and particulate matter (PM) (4, 6, 12–14). A study undertaken in New York City after the events of 11 September 2001 found a direct relationship between the amount of DNA adducts in umbilical cord blood of newborn children and proximity to the World Trade Center (15), which suggests that air pollution may be a significant contributor to the formation of DNA adducts in blood.

Seasonality in DNA adduct levels has been observed and may be linked to the variability in air quality and human behaviour determining exposure between, for instance, summer and winter. The same variability with season could also be attributable to dietary habits. It is still insufficiently clear which factors contribute to the large inter-individual variation in DNA adduct levels that is observed, even when people are apparently exposed to similar doses of genotoxins.

Therefore, we have conducted a large pooled analysis in healthy individuals (~3,600 subjects) recruited in the context of case-control, cross-sectional or cohort studies, with the purpose of validating or refuting previous findings in a sufficiently powered dataset (8, 9, 11, 12, 16–29).

Methods

We have identified eleven study cohorts, investigated in 18 publications, listed in Table 1, in which bulky DNA adducts were measured by ^{32}P-postlabelling (Phillips et al. 2007), and a similar set of variables was available including individual data on age, gender, ethnicity, batch, smoking habits, BMI, season of blood collection and a limited set of gene variants.

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We contacted the principal investigators of these studies and had access to the original data sets. The study characteristics are briefly described in Table 1.

In most of the studies measurement of bulky adducts by ^{32}P-postlabelling was achieved using the nuclease P1 digestion method of enrichment, although butanol extraction was used in the study of Bak and coworkers (2006). In each investigation, subjects were enrolled after signing informed consent. Data sets were transferred to the ISI Foundation for analysis after being anonymized.

There were some differences in the mean levels of adducts among the studies, with the US study showing the highest values (23, 24). This is most likely due to inter-laboratory differences rather than to actual, exposure-related, differences in DNA adduct levels, which have been expressed in the text as RAL (relative adduct labelling) × 10^8 bases, if not specified otherwise. We addressed this problem in three ways: (a) in the main analysis data were normalized after pooling, assuming different measurement units in the different laboratories, according to the following formula:

$$RALst = (RAL - Mean_{ic})/SD_{ic}$$

where RAL = relative adduct labelling; $Mean_{ic}$ and SD_{ic} = mean and standard deviation of the group of subjects in the ith study. The rationale for using normalized values and quartiles to standardize genetic pooled analysis has been put forward previously by several authors and this approach has become common practice (4, 30–32) . Since after standardization the skewness of the distribution of RALst was still high (>2.9), we compared standardized values of adduct levels using the non parametric Kruskal-Wallis test; (b) we repeated all the statistical analyses excluding the study from the US in which DNA adduct data were on average 8-fold higher than in the other studies (see Table 2); (c) for those studies in which DNA adduct analyses were performed in different laboratories but using samples from the same populations (EPIC SPAIN and GENAIR in Spanish populations and TURIN bladder case control study, EPIC ITALY and GENAIR in Italian populations), we have applied analysis-of-variance (ANOVA) to compare the area effect with the laboratory effect.

In addition to descriptive statistics and ANOVA we have performed univariate analyses and multivariate regression models stratified by smoking habits, excluding those studies in which blood samples were not collected in all seasons (GREECE, CZECH REPUBLIC and EAST EUROPE studies). In the multivariate model we included sex, age and seasonality. To control for heterogeneity among studies we also performed multivariate regression models including the variable “study” as having a random effect. Finally, we performed a logistic regression analysis in which the response variable was 0 or 1 if the RAL value was below or above the median value respectively.

All statistical analyses were performed using SAS software (v.9.1.3).

Results

Table 2 shows the mean adduct levels, and standard deviations, for the studies that were included in the analysis. There are relatively small variations among the studies except for the US cohort that has adduct levels about a factor 8 higher than others. For this reason in the subsequent analyses we use normalized levels. No statistically significant difference in DNA adduct levels with gender and body mass index was observed (Table 3). Age showed a borderline significant association (p=0.09), although no clear trend was observed. Seasonality (i.e. the season in which blood was drawn) and smoking (with higher levels in never smokers) were significantly associated with DNA adducts, p=0.006 and 0.0003,

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respectively. Among the genetic variants that were analyzed in these studies, no statistically significant difference in DNA adduct levels with the variant genotypes was found (Table 4).

To verify if the finding on smoking is true and not an artificial effect due to the statistical correction we stratified the analysis between studies in which DNA adducts were measured in WBC and studies in which they were measured in lymphocytes and we obtained the same trend as in the global analysis.

In the stratified multivariate analysis (Table 5), we observed an effect of seasonality in nonsmokers, with the lowest levels in the spring (p=0.02), and an effect of sex, with women having higher levels, among current smokers (p=0.01). The corresponding ORs (above vs below the adduct median) were 0.74 (95% CI 0.52–1.04) for spring vs winter and 1.40 (95% CI 0.97–2.00) for women vs men. The R^2 (a measure of variance explained by the model) was very small for all models presented, always less than 0.02. Multivariate analysis for smoking showed a significant negative beta value (−0.086, p-value <0.001). Multivariate regression analysis including the variable “study” as having a random effect showed essentially similar results. Analysis of variance (ANOVA) was performed separately for the recruitment centres where subpopulations were analyzed in different laboratories or in the same laboratory at different times (simulating a batch effect) (EPIC SPAIN, TURIN Bladder Case Control Study, EPIC ITALY and GENAIR). The effect of centre was greater than the effect of batch or laboratory (F-test= 9.26, p-value<0.0001 for centre; F-test=6.65, p-value= 0.0002 for laboratory).

We also analyzed the non-standardized RAL values, adjusting for laboratory effect and cell type, across Europe. Adduct levels were 0.60 (median 0.40, SD 0.54) in Northern Europe (the Netherlands and Denmark) and 0.79 (median 0.60, SD 0.84) in Southern Europe (Italy, Spain, France and Greece), with a p-value for the difference of 0.001.

Discussion

PAHs are an important class of environmental carcinogens, capable of inducing DNA adducts after metabolic activation (33). PAHs may occur in fried and charcoal-grilled meat or in the food chain as a result of environmental pollution (34–36). As a result, human exposure to PAHs is widespread and may occur via inhalation, ingestion or via dermal contact. The latter seems less relevant for the general population, but may be of relevance in certain groups such as in occupational settings or after treatment with coal-tar ointments. These exposures are thought to contribute to cancer incidence in the general population, since the most important targets for PAH carcinogenicity include lung and possibly bladder (1) . Some evidence has also been reported for an association between dietary PAHs and colon cancer or adenomas (37, 38). Increased levels of bulky DNA adducts have been detected in the colon mucosa of colon cancer patients and in early stages of colon carcinogenesis (39, 40). More thorough understanding of factors that determine DNA adduct levels may thus contribute to improved preventive measures.

The ^{32}P-postlabelling assay is a complex procedure involving several steps (41). Although guideline protocols have been devised and tested in interlaboratory trials (42), there is no consensus on conditions for analysis or methods for quantitation. For the latter, differences between studies may reside in how DNA adduct levels are calculated from the levels of radioactivity detected on thin-layer chromatography (TLC) plates; different approaches include separate assessment of the incorporation of radioactivity into normal nucleotides, or determination of the specific activity of the [γ-^{32}P]ATP used. It is also not clear which areas of the TLC plates were included in the quantitation; this can be of some importance in cases, such as here, where DNA adduct patterns may be weak and diffuse. For the purposes of

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pooled analysis, however, interlaboratory differences can be accommodated by normalising results, as was done in the present study.

The present study is the largest pooled analysis available on bulky DNA adducts (~3,600 subjects), and shows only weak associations. The analysis restricted to studies having data for every season confirms an association with the season at the time of blood collection, as suggested in previous smaller studies. In non-smokers we found significantly lower DNA adduct levels in spring (p=0.02) than in winter, with a seasonal gradient similar to the one shown for median levels in Table 3. This may have two alternative explanations: the first is a protective effect of seasonal dietary intakes such as fresh fruit and vegetables, although this is less likely to peak in the spring, when the lowest RAL were observed. Such a protection has been suggested in previous investigations (8–11), but could not be tested directly in the current analysis, because the datasets are too heterogeneous in the way dietary data were collected. The second potential explanation is a higher level of bulky adducts in some seasons due to higher levels of exposure to pollution, particularly to particulate-bound PAHs. This can be due to seasonal differences in emissions, weather conditions and/or outdoor human activity. This hypothesis seems to be supported by some of the previous investigations (33), and is confirmed by a comparison among the areas for which we had adduct measures from different laboratories. In fact, after adjusting for the laboratory effect and cell type, mean adduct levels were 0.60 (median= 0.40; SD 0.54) in Northern Europe (the Netherlands and Denmark), and 0.79 (median= 0.60; SD 0.84) in Southern Europe (Italy, Spain, France, Greece), a trend that corresponds to the different levels of PM2.5, PM10 and NO_2 that have been observed across Europe. According to a recent comprehensive report, PM2.5 concentrations, for example, are clearly greater in cities from Southern Europe (with peaks of more than 40 mg/m3 in Turin, Italy) than in cities form Northen Europe (43).

The observation of lower adducts in smokers compared to non-smokers, is counterintuitive. A first observation can be that current smokers are low represented in our sample. Moreover, nucleotide excision repair (NER) capacity is one of the factors that could contribute to individual variation in tobacco-related biomarkers. Previous studies have shown that smokers (particularly current smokers) tended to have more proficient DNA repair capacity (DRC) than non smokers, suggesting that smokers may have an adaptive response to DNA damage induced in blood cells by chronic tobacco carcinogen exposure. In particular, higher DRC was shown in smokers in *in vitro*-induced BPDE-adduct repair (44); in oxidative damage repair (45, 46); in 4-aminobiphenyl adduct repair, also related to smoking habits (47); and in the γ-radiation repair model (48). The hypothesis that the induction of DNA damage by smoking can stimulate cellular repair activity could explain the significantly higher DNA adduct levels in non-smokers compared to smokers (p=0.0003) in our pooled-analysis.

Recently it has been demonstrated that phase II enzymes can be induced by PAHs found in cigarette smoke (49). These enzymes are involved in the process of detoxification of numerous carcinogens such as PAHs and aryl- and heterocyclic amines (50), and their induction by tobacco smoke could be an alternative explanation for the smoking effect in our study, where preferential induction of phase II enzymes can lead to more rapid clearance of PAHs prior to adduct formation. Moreover inter-individual differences exist in the levels of expression and catalytic activities of a variety of xenobiotic-metabolizing enzymes in humans and these phenomena are thought to be critical in understanding the basis of different susceptibilities of individuals to PAH action (51).

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Conclusions

In this large pooled analysis, we have reported only weak associations between bulky DNA adducts and exposure variables, namely seasonality. Most comparisons were negative, and also the R^2 of all regression models was extremely small (less than 0.02), suggesting that the part of variance explained by these models is very modest. Air pollution may partly explain some of the inter-area differences (between North and South Europe), but most inter-area and inter-individual variation in adduct levels still remains unexplained.

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Abbreviations

CI	confidence interval
PAH	polycyclic aromatic hydrocarbon
RAL	relative adduct labelling

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Table 1

Description of the studies.

Name (References)	Population	N from published work (% of men)	Cells	Smoking habits
EPIC SPAIN (ES) (16, 17)	Spain	296 (50.34)	WBC	NS 174 EX 48 SM 74
DENMARK STUDY (DK) (20)	Denmark	255 (53.7)	WBC (BE)	NS 9 EX 56 SM 185 5 missing
TURIN BLADDER CASE CONTROL STUDY (TBCC) (8, 21) Unpublished results	Italy	104 (100)	WBC	NS 29 EX 59 SM 24
EPIC ITALY (EI) (9, 22)	Italy	634 (76.3)	WBC	NS 255 EX 204 SM 171 4 missing
GENAIR (GA) (12)	West Europe	1086 (51.75)	WBC	NS 593 EX 492 1 missing
USA STUDY (US) (23,24)	USA	173 (100)	WBC	NS 32 EX 72 SM 67 2 missing
GREECE STUDY (GR) (18)	Greece	194 (30)	LYMPH.	NS 194
THE NETHERLANDS STUDY (NL) (19)	The Netherlands	41 (34.14)	LYMPH.	NS 5 SM 35 1 missing
CZECH REPUBLIC STUDY (CZ)(11, 25,26) Unpublished results	Czech Republic	360 (100)	LYMPH.	NS 330 SM 60
EAST EUROPE STUDY (EE) (27,28)	East Europe	354 (100)	LYMPH.	NS 212 SM 137 5 missing
SPAIN STUDY (SP) (29)	Spain	76 (93)	LYMPH.	NS 31 EX 45
TOTAL	**3573**			

WBC=white blood cells; lymph=lymphocytes. NS=never smokers; EX=ex-smokers; SM=current smokers; BE= butanol enrichment.

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Table 2

Studies included in the analyses.

Study	*N subjects*	*Mean RAL*	*SD RAL*
EPIC SPAIN (WBC)	296	0.83	0.66
DENMARK STUDY (WBC)	255	0.23	0.15
TBCC STUDY (WBC)	104	0.43	0.50
EPIC ITALY (WBC)	634	0.78	1.00
GENAIR (WBC)	1086	0.70	0.55
US STUDY (WBC)	173	6.85	12.56
GREECE STUDY (L)	194	1.22	0.89
NL STUDY (L)	41	1.53	0.56
CZ STUDY (L)	420	1.48	0.85
EAST EUROPE STUDY (L)	354	1.06	0.40
SPAIN STUDY (L)	76	0.23	0.58
TOTAL	3633	1.13	3.12

N=Number of subjects, mean values of RAL expressed as adducts per 10^8 nucleotides, SD=standard deviation. WBC=white blood cells (buffy coat), L=lymphocytes.

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Table 3

Median RAL values according to selected individual characteristics.

	N subjects	Median RAL (SD)	p-value
Sex (All)			
Male	2352	0.83 (3.83)	
Female	1281	0.60 (0.79)	0.65
Age (All)			
1° quartile	905	1.01 (0.83)	
2°quartile	945	0.70 (1.29)	
3° quartile	872	0.60 (3.29)	
4°quartile	909	0.50 (5.12)	0.09
Season (All)			
Spring	696	0.50 (2.70)	
Summer	599	0.64 (3.82)	
Autumn	764	0.70 (5.06)	
Winter	1232	0.85 (1.12)	**0.006**
BMI (EPIC SPAIN, GREECE, GENAIR, EPIC ITALY)			
1° quartile	532	0.69 (0.93)	
2° quartile	533	0.60 (0.75)	
3° quartile	537	0.60 (0.70)	
4° quartile	535	0.60 (0.65)	0.91
Smoking status (All)			
Never	1771	0.88 (1.40)	
Ex	1043	0.54 (5.28)	
Current	781	0.67 (1.58)	**0.0003**

Univariate analysis. P-value from Kruskal-Wallis test, based on RAL standardized values.

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Table 4

Median RAL values according to genetic data. Univariate analysis. P-value from Kruskal-Wallis test, based on RAL standardized values.

	N subjects	Median RAL (SD)	p-value
CYP1A1M1 (EPIC SPAIN, GENAIR, US STUDY)			
Wt	1216	0.70 (4.76)	
het	256	0.80 (4.74)	
mut	15	0.60 (1.04)	0.68
GSTM1 (EPIC SPAIN, NL STUDY, GENAIR, US STUDY, CZ STUDY, EAST EUROPE STUDY)			
Null	1157	0.90 (2.58)	
Present	1071	0.94 (4.93)	0.39
GSTT1 (EPIC SPAIN, GREECE, TURIN BLADDER CASE CONTROL STUDY, GENAIR, CZ STUDY, EAST EUROPE STUDY)			
Null	775	0.91 (0.66)	
Present	1527	0.80 (0.75)	0.21
MPO (TURIN BLADDER CASE CONTROL STUDY, GENAIR)			
Wt	677	0.60 (0.52)	
het	406	0.50 (0.56)	
mut	49	0.60 (0.79)	0.86
NQO1 (GREECE, GENAIR)			
wt	769	0.67 (0.66)	0.87
het	390	0.64 (0.60)	
mut	47	0.60 (0.63)	

Wt = wildtype, Het = heterozygous, Mut = homozygous variant

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Table 5

Univariate and multivariate models. DNA adducts: dependent variable (standardized values).

	Non smokers				Ex smokers				Current smokers			
Independent variable	**Parameter estimate**	**SE**	**p-value**	**model R^2**	**Parameter estimate**	**SE**	**p-value**	**model R^2**	**Parameter estimate**	**SE**	**p-value**	**model R^2**
Univariate analysis												
DNA adducts												
Sex (ref: Male)	0.064	0.066	0.33	0.001	0.011	0.076	0.88	0.000	**0.183**	**0.077**	**0.02**	**0.011**
Age (continuous)	−0.001	0.004	0.72	0.000	−0.003	0.003	0.33	0.001	0.000	0.005	0.99	0.000
Season				0.008				0.003				0.000
Spring	**−0.218**	**0.092**	**0.02**		−0.098	0.099	0.32		0.031	0.102	0.76	
Summer	−0.081	0.095	0.39		0.044	0.098	0.65		0.094	0.113	0.40	
Autumn	−0.001	0.091	0.99		0.001	0.099	0.99		0.117	0.103	0.25	
CYP1A1M1	−0.021	0.089	0.81	0.000	0.133	0.107	0.21	0.003	0.021	0.117	0.86	0.000
GSTM1	0.040	0.078	0.61	0.000	0.095	0.082	0.25	0.002	0.079	0.101	0.44	0.005
GSTT1	0.013	0.097	0.89	0.000	−0.062	0.098	0.530	0.001	0.118	0.210	0.570	0.004
MPO	0.031	0.081	0.70	0.000	−0.057	0.069	0.41	0.002	−0.075	0.197	0.71	0.010
NQO1	0.032	0.009	0.71	0.000	−0.068	0.073	0.36	0.002	-	-	-	-
Multivariate model												
DNA adducts				0.009				0.003				0.015
Sex	−0.002	0.004	0.74		−0.003	0.004	0.39		0.001	0.005	0.86	
Age (continuous)	0.062	0.066	0.35		−0.016	0.078	0.84		**0.192**	**0.078**	**0.01**	
Season												
Spring	**−0.220**	**0.092**	**0.02**		−0.096	0.099	0.33		0.011	0.102	0.91	
Summer	−0.085	0.095	0.37		0.040	0.099	0.69		0.097	0.112	0.39	
Autumn	−0.006	0.092	0.95		0.002	0.099	0.99		0.122	0.103	0.24	