# Effect of vitamin levels on biomarkers of exposure and oxidative damage—The EXPAH study

R.J. Sram[a], P. Farmer[b], R. Singh[b], S. Garte[c,d], I. Kalina[e], T.A. Popov[f], B. Binkova[a], C. Ragin[c], E. Taioli[c,*]

[a] Laboratory of Genetic Ecotoxicology, Institute of Experimental Medicine of Academy AS CR and Health Institute of Central Bohemia, Prague, Czech Republic
[b] Cancer Biomarkers and Prevention Group, Biocentre, University of Leicester, UK
[c] University of Pittsburgh Cancer Center, 5150 Centre Avenue, Pittsburgh, PA 15232, USA
[d] Genetics Research Institute ONLUS, Milan, Italy
[e] Department of Medical Biology, Medical Faculty University P.J. Šafárik, Košice, Slovak Republic
[f] National Center of Public Health Protection, Sofia, Bulgaria

## ARTICLE INFO

## ABSTRACT

DNA adducts are markers of carcinogen exposure and of their biological effect; they have been shown to be related to mutagenesis, and therefore they could be a predictive biomarker of human cancer. The objective of this study was to assess if there is a relationship between vitamins A, C, and E, which are known to play a significant role as free radical scavengers and antioxidant agents, and biomarkers of genotoxicity and oxidative stress.

Three hundred and fifty-six subjects from Czech Republic, Slovak Republic and Bulgaria, who completed a questionnaire on dietary information and had a measurement of plasma A, C, E vitamins, DNA adduct levels (benzo[a]pyrene (B[a]P) and bulky (DNA-Tot) DNA adducts) and oxidative damage (cyclic pyrimidopurinone N-1,N2 malondialdehyde-2 deoxyguanosine (M1dG) and 8-oxo-7,8-dihydro-2 deoxyguanosine (8-oxodG)) were analyzed.

A significant inverse correlation was observed between plasma vitamin levels and both benzo[a]pyrene (B[a]P) and bulky DNA adducts. Vitamin A was also significantly inversely correlated with M1dG, a marker of oxidative damage. The associations were stronger in non-smokers than in smokers.

Dietary intake of certain antioxidants such as vitamins is associated with reduced levels of markers of DNA damage (B[a]P and DNA-Tot) and oxidation (M1dG and 8-oxodG) measured in peripheral white blood cells. This could contribute to the protective role of such a dietary pattern on cancer risk. The protective effect of dietary vitamins is less evident in smokers.

© 2008 Elsevier B.V. All rights reserved.

## 1. Introduction

Diet may be the source of several mutagens, such as aflatoxin and heterocyclic amines, whose biological effects on DNA have been studied extensively. Other carcinogens present in diet, such as polycyclic aromatic hydrocarbons (PAH) and other aromatic compounds have been investigated less frequently. PAH may occur in fried and charcoal-grilled meat or in the food chain as a result of environmental pollution [1,2]. PAHs are an important class of carcinogens, capable of inducing the formation of DNA adducts leading to DNA damage after metabolic activation [3]. This process may modify the long-term individual cancer risk, at least with respect to lung cancer, as reported recently by a meta and pooled analysis [4]. The mea-

sure of genotoxicity in target organs such as lung, bladder and colon in relation to exposure seems to bring less convincing evidence [5].

Preliminary to the study of the effect of diet on cancer risk is the study of the biological effects of dietary constituents in healthy subjects. Data from large cohort studies have been published on the association between dietary components and DNA adducts measured in peripheral blood. An inverse association between fruit and/or vegetable intake and various measure of DNA damage in healthy subjects has been repeatedly reported in the European Prospective Investigation into Cancer and Nutrition (EPIC) study [6,7].

In a previous analysis of the present EXPAH cohort [8], the relationship between dietary factors and DNA damage was examined by assessing whether specific groups of foods known to have antioxidant properties would be inversely associated with levels of DNA adduct levels, oxidative damage to DNA and chromosomal aberrations. The results of this analysis showed modest, if any, associations between such dietary components and biomarkers of DNA damage.

* Corresponding author at: Department of Epidemiology and Biostatistics, SUNY Downstate, Brooklyn NY 11203 USA. Tel.: +718 804 7100; fax: +718 270 2533.
E-mail address: emanuela.taioli@downstate.edu (E. Taioli).

In order to better understand mechanisms through which diet affects DNA damage, it is useful to look at the effect exerted by the various micronutrients contained in diet.

Dietary vitamins and antioxidant consumption may modify the levels of DNA damage induced by both diet and smoking, the main source of PAH exposure, thus modifying individual cancer risk. The association between dietary vitamins and DNA damage/oxidation is usually investigated indirectly by calculating vitamin consumption from dietary items collected through standardized questionnaires. However, the association between vitamin levels as measured in plasma on several measures of DNA damage and oxidation has been rarely performed. A study on 109 non-smoking policemen conducted in Prague, reported a weak negative association between plasma vitamin A levels and both Total DNA adducts and B[a]P-like DNA adducts [9].

Another study from the same group on a small population of 50 bus drivers showed that vitamin E levels were positively correlated with markers of oxidative stress [10].

Vitamin C (ascorbic acid) is one of the most important natural antioxidants in living tissues [11]. Studies conducted in the early 1980s [12] showed a clear-cut ability of vitamin C to block the clastogenic action of alkylating agents. Subsequently to this finding, vitamin C was administered as a prophylactic agent in several groups of workers occupationally exposed to different mutagens and carcinogens [13–16]. Vitamin C prophylaxis significantly decreased the frequency of aberrant cells in these workers.

The present analysis investigates the role of vitamins A, C and E measured in plasma on markers of DNA damage (B[a]P and DNA-Tot) and oxidative stress (M1dG and 8-oxodG) in a healthy population collected in three geographical areas of Europe.

## 2. Methods

The population under study is described in detail elsewhere [17,18], and consisted of 204 workers selected from Prague (Czech Republic) (city policemen), Kosice (Slovak Republic) (city policemen), and Sofia (Bulgaria) (city policemen and bus drivers), and 152 subjects who were non-occupationally exposed to traffic pollutants, matched for age and gender. Blood samples were collected from each participant for determination of DNA adducts levels, genetic susceptibility, and determination of other biomarkers, such as vitamins and folates. A questionnaire was completed by each individual providing demographic, smoking and dietary information.

Smoking status was defined according to cotinine levels (adjusted by creatinine levels): smokers were subjects with cotinine levels greater than 500 ng cotinine/mg of creatinine, passive smokers were subjects with cotinine levels between 200 and 500 ng cotinine/mg, and non-smokers with cotinine levels lower than 200 ng cotinine/mg.

*Blood samples*: The blood samples were collected at the end of the working shift and processed into serum, plasma, erythrocytes and lymphocytes, and distributed to the partner laboratories using random assigned codes to assure anonymity. All the samples were kept frozen until shipment or analysis, and were transported to the laboratories in dry ice, and then stored at −80 °C until usage.

In addition to job description, personal exposure was obtained with the use of personal monitors. Subjects were classified as exposed when personal exposure to carcinogenic PAHs (c-PAHs) was greater than the median value of average exposure of non-occupationally exposed subjects, i.e. 7.55 ng/m³, and as non-exposed when personal exposure values were lower or equal to 7.55 ng/m³.

*Personal monitors*: The individual exposures of the subjects under study were determined during their working shift by the use of personal monitors, which collected PM2.5 from which the c-PAHs were extracted. Personal monitors were worn once by each subject, were applied at the beginning of the work shift, and collected at the end of it.

Carcinogenic PAHs (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene (B[a]P), dibenzo[a,h]anthracene, benzo[g,h,i]perylene andindeno[1,2,3-cd]pyrene) and phenanthrene, anthracene, fluoranthene, pyrene, coronene, were determined in the filter extracts by HPLC with fluorescence detection (NIOSH method 5506).

As a measure of oxidative DNA damage, 8-oxodG was determined in post-shift lymphocyte DNA samples from 98 exposed individuals and 105 controls from Prague and Kosice [19]. A second measurement of oxidative stress was malondialdehyde DNA adduct (M1dG) in post-shift lymphocyte DNA from 198 exposed and 156 control individuals from all three cities.

8-OxodG levels were determined by liquid chromatography–tandem mass spectrometry multiple reaction monitoring (LC–MS/MS MRM) technique, using

**Table 1**
General characteristics of the population and average values of vitamins as measured in plasma.

| Variable | N subjects | Mean | S.D. | Range |
|---|---|---|---|---|
| Age (years) | 356 | 34.1 | 9.0 | 19–65 |
| c-PAHs exposure (ng/m³) | 347 | 22.0 | 26.5 | 3.05–263.3 |
| Vitamin C (μmol/l) | 329 | 48.6 | 41.84 | 0.2–282.86 |
| Vitamin A (μmol/l) | 354 | 2.37 | 2.07 | 0.05–9.80 |
| Vitamin E (μmol/l) | 354 | 18.22 | 9.34 | 2.8–118.55 |

immunoaffinity or HPLC purification of 8-oxo-dG [19]. This technique was also recommended by the ESCODD guidelines [20]. M1dG DNA adduct was measured by immunoslot blot procedure described in detail elsewhere [21].

As biomarkers of exposure to c-PAHs, total PAH-DNA (bulky) adducts and the specific adduct arising from B[a]P (B[a]P-like DNA adduct) were measured in lymphocyte DNA, using ³²P-postlabelling and following protocols described elsewhere [22].

Vitamin C (ascorbic acid) was determined in plasma using a HPLC as previously described [23]. 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 [24]. The CEDIA folate kit (Roche Diagnostics, Prague, Czech Republic) was used for the determination of folates in plasma. Chromosome aberrations were measured by fluorescence in situ hybridization (FISH) [25,26].

## 3. Statistical analysis

Data were transformed when appropriate to obtain normality of the distribution of each variable. The correlations between vitamin levels and biomarkers of oxidative DNA damage (8-oxodG and M1dG adducts levels) and environmental exposure (bulky DNA adducts and B[a]P DNA adducts levels) were assessed by Pearson correlation analysis. Multiple linear regression models were used to assess the independent contribution of exposure, age, vitamin levels and smoking to the levels of each biomarker of exposure or oxidative damage. F values and corresponding p values are reported.

A p-value lower than 0.05 was considered as statistically significant. All the statistical analyses were performed using SAS statistical package (8.1 Version, SAS Institute Inc., Cary, NC).

## 4. Results

The average age of the population was $34.1 \pm 9.0$ years; 39.1% of the subjects were current smokers, the average c-PAHs exposure measured with personal monitors was $22.0 \pm 26.5$ ng/m³, with the highest findings in Bulgaria, followed by the subjects recruited in Slovakia, and then by the subjects from the Czech Republic.

The mean values of plasma vitamins are reported in Table 1. There was a high variability in values across subjects, as shown by the wide ranges of values.

The correlation between vitamin levels and the various biomarkers of genotoxicity/oxidative damage is reported in Table 2 and Figs. 1 and 2. There was a significant inverse correlation between the B[a]P-like DNA adduct and vitamin C (Fig. 1a), vitamin A (Fig. 2a), and vitamin E levels (Table 2). The correlation was stronger in non-smokers than in current smokers. Total DNA adducts were also statistically inversely associated with vitamin C (Fig. 1b), vitamin A (Fig. 2b), vitamin E levels (Table 2); for vitamin E, the association was stronger in non-smokers. A weak positive association between vitamin A and 8-oxodG was observed (Table 2). Vitamin A levels were significantly inversely associated with M1dG adducts (Table 2, Fig. 2c). There was no significant correlation between chromosomal aberrations by FISH and vitamin levels. The analysis of subjects exposed to passive smoking seems to follow the direction of the analysis conducted in non-smokers, although the reduced number of subjects does not allow a detailed analysis of this subgroup.

The multivariate analysis of the determinants on DNA adduct levels is presented in Table 3. Total DNA adducts remain sig-