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POOLED ANALYSIS OF STUDIES ON DNA ADDUCTS AND DIETARY VITAMINS

Camille Ragin[1], **Aerie Minor**[2], **Antonio Agudo**[3], **Peter Farmer**[4], **Seymour Garte**[5], **Carlos Gonzales**[3], **Ivan Kalina**[6], **Pino Matullo**[7], **Todor Popov**[8], **Domenico Palli**[9], **Marco Peluso**[9], **Fulvio Riccieri**[7], **Radim Sram**[10], **Paolo Vineis**[11,12], and **Emanuela Taioli**[1,*]

[1] SUNY Downstate Medical Center, Brooklyn, NY

[2] University of Pittsburgh Cancer Center, Pittsburgh, PA

[3]Cancer Epidemiology Research Program, Unit of Nutrition, Environment and Cancer, Catalan Institute of Oncology (ICO), Barcelona, Spain

[4]Cancer Biomarkers and Prevention Group, Biocentre, University of Leicester, UK

[5]Genetics Research Institute ONLUS, Milan, Italy

[6]Department of Medical Biology, Medical Faculty University P.J. Šafárik, Košice, Slovak Republic

[7]ISI Foundation, Turin, Italy

[8]National Center of Public Health Protection, Sofia, Bulgaria

[9]Cancer Risk Factor Branch, ISPO-Cancer Prevention and Research Institute, Florence, Italy

[10]Laboratory of Genetic Ecotoxicology, Institute of Experimental Medicine of Academy AS CR and Health Institute of Central Bohemia, Prague, Czech Republic

[11]Imperial College London, London, UK

[12]University of Turin, Turin, Italy

Abstract

Objectives—There is some evidence that dietary components that are rich in antioxidant and vitamins are inversely associated with DNA adduct levels induced by environmental carcinogens such as polycyclic aromatic hydrocarbons, although the epidemiologic data are inconsistent. This study addresses the association between vitamins, DNA adducts and smoking.

Methods—A combined analysis of individual data on the association between bulky DNA adducts and dietary vitamins were conducted. A Medline search was performed to identify studies on healthy subjects in which smoking and vitamins intake information were available, and bulky DNA adducts were measured in peripheral blood with 32P post labeling. Eight published studies met the eligibility criteria, and individual data from 7 data sets including 2,758 subjects were obtained. GSTM1 and GSTT1 were also available on all the subjects.

[*]Address for correspondence: SUNY Downstate Medical Center, 450 Clarkson Avenue, Box 43, Brooklyn, NY 11203, USA. emanuela.taioli@downstate.edu.

Results—Vitamin E was inversely significantly associated with DNA adducts after adjustment for possible confounding factors. Vitamin A and C were not independent predictors of DNA adducts. A stratified analysis showed that vitamin A had a significant inverse association with DNA adducts in ever smokers only.

Conclusions—This result is relevant to planning any future chemo-preventive interventions directed to high risk subgroups of the population, for cancer prevention.

Keywords

biomarkers; carcinogenicity; molecular epidemiology

INTRODUCTION

DNA adducts are among the most commonly measured biomarkers of genotoxicity; their presence has been linked to some of the most common cancers, such as lung and bladder cancer [1-4]. DNA adducts are a widely used measure of the biologically effective dose of genotoxic compounds bound to DNA as their target for carcinogenesis; they are generated by environmental exposure to complex mixture components such as carcinogenic polycyclic aromatic hydrocarbons (PAH)as a consequence of exposure to pollution [5], certain dietary components [6], and possibly tobacco smoking [7] although the epidemiologic data is not always consistent for the latter. Dietary patterns including habitual intake of grilled meat and fried products are usually capable of significantly increasing the amount of DNA adducts measurable in peripheral blood. [8]

There is also some evidence that dietary components that are rich in antioxidant and vitamins can obtain the opposite effect. Published data of cross-sectional analyses from large cohort studies have repeatedly shown an inverse association between fruit and/or vegetable dietary intake and DNA damage, often measured as DNA adducts in peripheral blood in healthy subjects [7,9,10]. Cross sectional studies however did not confirm the protective effect of fruits and vegetables on bulky DNA adducts derived from environmental pollution [6,11]

Given the amount of measurement error involved with dietary questionnaires, the direct measure of plasma vitamin levels in relation to DNA adducts in lymphocytes should in principle give more precise information on the role of vitamins in decreasing/preventing the formation of DNA adducts caused by other environmental exposures. However such studies are rarely performed due to the logistics associated with blood draw, sample preservation as well as the costs associated with vitamin measurements. The few studies including direct vitamin measurements in relation to DNA adducts have given conflicting results. Data from the EXPAH study [12] showed a significant inverse association between plasma levels of vitamins A, C and E and both bulky and B[a]P-like DNA adducts; Using multivariate analysis, total DNA adducts were independently and inversely associated with both vitamin A and C levels, while for B[a]P-like DNA adducts only the association with vitamin E levels persisted after adjustment for possible confounders. The associations were stronger in non-smokers than in smokers. A study conducted on a small population of bus drivers showed that vitamin E levels were positively correlated with markers of oxidative stress [13].

Another study conducted in the Czech Republic in healthy non smokers did not find any association between DNA adduct levels in lymphocytes and plasma vitamin C levels, while reported a protective effect of vitamin A on DNA adduct production under conditions of elevated exposure to carcinogenic polycyclic aromatic hydrocarbons [14-10].

There is a need to better understand whether dietary vitamins are capable of playing a significant role on genotoxicity as measured by DNA adducts, both in the general population

and in subgroups of the population exposed to particular carcinogens, such as smoking or environmental pollution. Given the reports that DNA dducts may be predictors of future cancer development [1], the present findings could have immediate applicability in chemoprevention trials towards exposed populations. For this reason, we have conducted a review and a combined analysis of individual data on the association between bulky DNA adducts and dietary vitamins.

MATERIAL AND METHODS

Study Identification and Selection

Original studies that evaluated the relationship between vitamins and DNA adducts were identified by searching the National Library of Medicine and National Institutes of Health Pubmed database up to May 31, 2009. The search strategy involved the following keyword search terms: "DNA adducts AND (fruit OR vitamins OR micronutrients)." Each of the citations and abstracts were reviewed, plus the reference lists from retrieved articles and any published review articles were reviewed in order to identify additional articles. Articles were considered eligible for inclusion in this review if they met the following inclusion criteria: 1) they were cross-sectional studies including healthy subjects; 2) smoking information is collected through a questionnaire; 3) bulky DNA adducts are measured in peripheral blood with 32P post labeling, in order to rely on a commonly used, standardized laboratory method; 4) information on vitamins intake is available, either through dietary questionnaire or direct plasma measurement; 5) sample size $\geq$ 20 subjects. These criteria were selected a priori in order to enhance the comparability across studies. The PubMed search yielded a total of 442 publications. After the list was generated, the abstracts were reviewed independently by two reviewers; 381 articles were excluded because they were studies conducted on animals; in vitro studies; did not include healthy subjects; accounted for albumin adducts and not DNA adducts; were reviews. The full texts of 61 relevant papers were reviewed. Forty-two publications measured vitamin/fruit/micronutrient intake and adduct levels, yet, included other type of adducts (i.e. benzo [a] pyrene DNA adducts), did not report data that correlated vitamin/fruit/micronutrient intake with adduct levels or did not include specific individual data on vitamins. Other types of DNA adducts were purposely excluded from the present re-analysis because they originated from exposures to specific carcinogens, while bulky DNA adducts are an overall measure of DNA damage from exposure to complex groups of carcinogens.

The final selection yielded 20 articles; four of them [15-18] were excluded because DNA adducts were not measured with 32P post labeling, two [19,20] because dietary vitamin information was missing, three [5,21,22] because the dietary information were insufficient to calculate vitamin intake.

This left 11 articles available for inclusion in the present analysis. Overlap of study subjects was evaluated by comparing sources of data described in the methods; in the case of overlap, the more inclusive publication was selected. Partial overlap was identified in 5 publications [23-25;6,9] which therefore gave origin to 2 data sets. The final number of available data sets was 8; one author never responded to our invitation to join the pooled analysis [26,27], leaving us with 7 data sets. A description of the studies selected for this analysis is presented in table 1.

Investigators from the eligible studies were contacted, invited to participate in this combined analysis, and informed of the policies for data handling, data publication and authorship. Multiple invitations were extended in an attempt to enlist the participation of all eligible studies.

Seven of the eight potential collaborators accepted to participate and sent anonymous data sets, including 2,758 subjects (Table 1). Three studies [13,14,28] sent more data than what was

published in the literature. Each data file was checked for consistency and uniformity with previously published results. Discrepancies identified were resolved with the study investigators. Policies for data publication and authorship were reviewed and accepted by study investigators. The data files received did not include personal identifiers. Each study was approved by the Institutional Review Board or appropriate ethical committee at the respective institutions, and participants provided informed consent at the time of enrollment. All variables requested were coded with a common format for this analysis.

Measurement of exposure

DNA adducts: total PAH-DNA (bulky) adducts were measured in white blood cells (WBC) [23-25,6,9,10] or lymphocyte [12-14,28] DNA, using 32P-postlabelling with nuclease P1 treatment for adduct enrichment as described [29]. The values measured for bulky DNA adducts were corrected based on the recovery of the internal standard after the 32P-DNA postlabelling assay. The standard was adducted-DNA from livers of mice i.p. treated with 0.5 mg benzo(a)pyrene [B(a)P] from a previous inter-laboratory trial [29]. The average level of adducts in the BaP-DNA mouse liver samples was 8.5 adducts/10^8 nucleotides by mass spectrometry and 11.4 adducts/10^8 nucleotides by 32P-postlabelling. In a reliability study conducted by some of the investigators contributing data [23-25,6,9,10], the Intra Class Correlation Coefficient was 0.98 [30].

Vitamins: the studies that measured plasma vitamins [12-14,28] used a high-performance liquid chromatographic method (HPLC) as previously described [31] for vitamin C (ascorbic acid); the method has high accuracy (mean recovery 101.2%) and within-day coefficient of variation between 3.4 and 5.7%. 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 [32]. The studies that derived vitamins from dietary questionnaires obtained dietary information on the frequency of consumption of more than 120 foods and drinks in a 12-month period prior the enrollment using a self-administered Food Frequency Questionnaire, or a Diet History Questionnaire [24,25] validated in a pilot phase in each participating country. All questionnaires were checked and coded by trained dieticians, computerized and then transformed into estimates of intake for a series of over 30 nutrients according to Food Tables specifically generated for each country participating in the study [10]. No information on dietary vitamin supplements was available from these studies.

Genotype: the presence or absence of GSTM1/ GSTT1 deletion were tested by PCR method followed by gel electrophoresis in all the available data sets. This method used the following primer sets: GSTM1 5′ AACTCCCTGAAAAGCTAAAGC 5′ GTTGGGCTCAAATATACGGTGG and GSTT1 5′ TCCTTACTGGTCCTCACATCTC 5′ TCACCGGATCATGGCCAGCA, and included a primer set for albumin as control (5′ GCCCTCTGCTAACAAGTCCTAC,5′ GCCCTAAAAAGAAAATCGCCAATC)

Statistical Analysis

The analyses were performed and presented as a whole and stratified according to two groups: one group including studies that used a questionnaire to asses vitamin levels [23-25,6,9,10], and peripheral WBC as source of DNA, another groups including studies that used plasma vitamin measurements and DNA from peripheral lymphocytes [12-14,28].

Continuous variables were presented as means ± standard deviations. The distribution of covariates was compared among cases and controls using either the parametric t-test or non-parametric Wilcoxon rank sum for continuous variables, or the chi-square test for categorical variables. Due to the high variability across studies, DNA adducts were normalized according to the following formula:

$$RAL_{st} = (RAL/Mean_{th})$$

Where RAL is the relative adduct labeling (nr of adducts × 10^8 bases); $Mean_{th}$ is the mean in the i_{th} study.

Some studies reported smoking status as ever/never smoker, others as current/ex/ never smokers. In the present analysis, ex smokers were lumped with current smokers to create the category “ever smokers” in obtain maximum comparability across studies. Sub analyses restricted to current smokers were also run.

Vitamins were categorized in quartiles according to the overall distribution in the study population, and mean values of DNA adducts were calculated for each quartile. Non parametric tests were employed to assess statistical differences in DNA adducts across quartiles of vitamins. Multiple linear regression models were used to assess the independent contribution of vitamins, smoking, age and study on DNA adduct levels.

Publication bias was tested using the Begg funnel plots and Egger's bias test [33].

RESULTS

Seven studies were included in this analysis, for a total of 2,758 White subjects. There were significant differences across studies in average age, as well as levels of vitamins and DNA adducts (table 1).

There was an inverse significant association between vitamin A and relative DNA adducts; a significant inverse association between vitamin C, age and DNA adducts was observed only in studies measuring DNA adducts in lymphocytes (Table 2). No significant association was observed between vitamin E, GSTT1 and GSTM1 deletion, either considering the genes d alone or in combination as a complete genotype, and relative DNA adducts. There was no evidence of publication bias in the association between vitamins and relative DNA adducts.

The multivariate analysis suggested a significant inverse association between relative DNA adducts and quartiles of vitamin E, after adjusting for age, smoking, gender and study (table 3). An inverse association between relative DNA adducts and quartiles of vitamin A was observed only in the subset of studies measuring adducts in lymphocytes. When both vitamin A and C are included in the model, neither vitamin was significantly associated with relative DNA adducts levels (Table 4).

Data were stratified by available information on smoking status. Vitamin A showed a significant inverse association with relative DNA adducts in ever smokers only, while vitamin E showed a significant inverse association with relative DNA adducts in both ever and never smokers (Table 5).

The addition of the genotype information, either considering GSTT1 and GSTM1 alone or in combination did not modify the associations reported above. Further analyses of the association between vitamins and DNA adducts, stratified by GSTs genotypes did not yield any significant results.

DISCUSSION

This analysis assessed in a large population of healthy subjects the association between DNA adducts and vitamin levels, either directly measured in plasma or indirectly derived from food

dietary questionnaire. The current pooled analysis included seven studies and over 2,500 healthy subjects with bulky DNA adducts measured with a similar method, and represents to our knowledge the first study of this size.

Our study suggests that bulky DNA adducts are inversely associated with vitamin E after adjustment for possible confounding factors, such as age, gender, smoking and study. This result held true independently from the type of peripheral tissue used (lymphocytes versus white blood cells) or the type of vitamin measurement included (plasma measurements versus dietary questionnaires.

While the inverse association of Vitamin E and DNA adducts is observed in both never and ever smokers, vitamins A is inversely associated with DNA adducts in smokers only. These results may point to the fact that vitamins may have different mechanisms of action on DNA adducts; alternatively, vitamins may act on separate pathways of carcinogen metabolism. Unfortunately the present re-analysis could not address the effect of dietary vitamin supplements on DNA adducts, because such information was not available from the original studies.

The literature on the association between DNA adducts and dietary vitamins and the effect of smoking is scarce, therefore few examples can be reported for comparison. Among the original studies included in the present pooled analysis, one [12] indicated a significant inverse association between bulky DNA adducts and plasma vitamin A and C, but not vitamin E, with some indication that the association be stronger in non smokers than in smokers. Another study conducted in the Czech Republic [14] did not report any significant association between DNA adducts and circulating vitamin C and E levels. It is possible that the impact of vitamins on DNA damage depends on both background values as well as exposure levels. Older studies conducted in the Czech Republic indicate that vitamin intake was very low, especially in the winter and spring, and that vitamin supplements administered to workers exposed to chemical carcinogens could partly reduce chromosomal aberrations [34].

Of the three original studies that derived vitamin levels from the dietary questionnaire, two showed various degrees of inverse association between DNA adducts and vitamin A, C, E [6,9,10,23], while one of them did not [24,25]. The only study that was not included in this pooled analysis [26,27] seems to suggest an inverse association between circulating vitamin A and DNA adducts in smokers.

While no differences with smoking status in the association between vitamin E and C and DNA adducts was observed, smokers only showed a significant inverse association between vitamin A and DNA adducts. This result could partly be explained by invoking some anti-oxidant properties of vitamin A. The role of vitamin A and retinol in lung cancer risk is still controversial, although the effects may be different for nonsmokers than for smokers [35]. In the present study, no significant differences in Vitamin E and C intake with smoking status were observed, while a significant difference in vitamin A intake was present.

Another observation derived by the present analysis is that two metabolic gene polymorphisms often implicated in DNA adducts formation [7,9], GSTM1 and GSTT1, did not exhibit any role on the inverse association between vitamin intake and DNA adducts, as resulted from stratified analysis by genotype.

The 32P-post-labelling DNA test has become one of the most popular tools for detecting bulky DNA adducts, especially PAHs [1]. The technique has many advantages: it requires small amount of DNA, is highly sensitive (one adduct per 10^7–10^{10} nucleotides) and is applicable to a wide variety of DNA adduct structures, including PAHs [29]. Despite its sensitivity, the evidence that bulky DNA adducts represents mainly PAHs exposure is still limited [1].

Although one of the inclusion criteria was the method for DNA adducts detection, which therefore was the same across studies, a large inter-study variation in the levels of adducts was present. DNA utilized for adducts measurement was extracted from WBC or lymphocytes depending on the study, and this may have contributed to the observed adducts variability, since half-lives of different leukocyte subpopulations vary, being relative long for lymphocytes as compared to other cells, thus directly affecting the interpretation the time frame of exposure to which the adducts levels refer to. Althoug DNA adducts reflect past exposure, the temporal framework is highly dependent on the speed of replication of the DNA carrying the damage. In order to partially take into account this difference, stratified analyses were performed according to whether WBC or lymphocytes were used.

Some of the variability could also be related to variations in some laboratory procedures and protocols; the variability was attenuated by normalizing the measures. An issue that could not be solved is how the level of measurement error of bulky adducts, which has been reported to be high, even up to 20-30%, has contributed to the observed results [1]. However, if measurement error of DNA adducts was present, it would have been equally present in all the studies, thus attenuating the strength of the associations with vitamins; this makes the observed results even more compelling.

Another possible limitation is that we have pooled studies whose main original purpose was not the study of the association between vitamins and DNA adducts, therefore there may be underlining differences in study design, population recruitment that can only partially be accounted for by adjusting for study. The comparability of the various population samples included in each study in terms of environmental exposure as well as personal behavioral risk factors is not quantifiable. In addition, the vitamin levels were derived from two different sources, plasma measurements and dietary questionnaires. Comparability between the two approaches, direct measurements of micronutrients and indirect estimates from dietary questionnaires, is not known. Although a subgroup analysis according to methods of vitamins measurement was performed, the methodological difference remains an issue.

Another issue in this analysis is publication bias, which could justify the findings if small positive studies have greater chances of being published than small negative studies. However, a formal statistical test for publication bias resulted non significant, thus making this hypothesis unlikely.

Strengths of this analysis are: the availability of individual data, which allowed for re-analysis of several exposures/DNA adducts associations, the large data seet of healthy individuals, the highly comparable set of information available on all the subjects.

In conclusion, despite the several limitations connected with the individual original studies, our pooled analysis suggests vitamin E to be the independent factor, among the other vitamins, contributing to DNA adducts, while vitamins A may modulate smoking-associated bulky DNA adducts.

Given the previously shown association between DNA adducts and cancer risk [1-4], this result is relevant to planning any future chemo-preventive interventions directed to high risk subgroups of the population, for cancer prevention purposes.

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Table 1

General characteristics of the studies selected for the review.

Author (Reference)	N of subjects	Age (years) Mean+SD	Vitamin C Mean+SD	Vitamin A Mean+SD	Vitamin E Mean+SD	DNA adducts (n × 10^8 bases) Mean+SD	GSTM1 deletion (%)	GSTT1 deletion (%)	Details on the original study	Factors associated with adducts in the original study
Palli (6, 9, 23)^	634	51.6 ±7.4	156.9±90.28	1.27±0.84	N/A	0.78±0.99	51.0	20.7	Diet analysis within a cohort, Dietary questionnaire	Inverse significant association with fresh fruit and vegetables, olive oil, antioxidants, GSTT1 null genotype.
Rossner (partly published in 13)	123	47.3±11.4	56.4 ±19.8	0.89±0.25	11.44±3.87	1.57±0.63	49.5	17.4	Environmental exposure in bus drivers, plasma vitamins	N/A
Topinka (partly published in 14)	117	35.7±8.9	86.1±16.7	1.15±0.25	13.4±3.23	1.70±1.03	52.1	16.1	Policemen exposure to air pollution, plasma vitamins	Inverse significant association with vit A
Rossnerova (partly published in 28)	61	33.6±5.7	72.3±21.2	0.97±0.23	12.0±2.91	1.17±0.39	49.0	17.0	Fertility in policemen, plasma vitamins	N/A
Sram (12)	364	34.4±9.3	48.6 ±41.8	2.37±2.07	17.93±7.67	1.06±0.41	56.2	17.8	Environmental exposure in a population sample; plasma vitamins	Inverse significant association with vit A, C, E, more evident in non smokers
Peluso (10)^	1,163	60.9±7.9	133.2±74.5	3.2±2.5	11.17±5.28	0.69±0.54	55.8	23.7	Diet analysis within a cohort, Non smokers; Dietary questionnaire	Inverse significant association with dietary fiber intake, vitamin E and alcohol
Agudo, Ibanez (24, 25)^	296	49.0±7.6	162.5±89.6	0.94±0.76	10.62±6.22	0.84±0.65	47.2	15.0	Diet analysis within a cohort, Dietary questionnaire	Significant association CYP1A1, NAT2,EPHX1; significant association meat, oils, fat, calcium, sodium, phosphorus
TOTAL	**2,758**									
p value (Kruskal Wallis)		0.0001	0.0001	0.0001	0.0001	0.0001				

^denotes studies were white blood cells were used for DNA adducts testing

Table 2

Association between DNA adducts and personal data-univariate analysis

VARIABLE	Vitamin intake from questionnaire, DNA adducts in WBC (Refs. [6],9,10,23-25)	p-value (Kruskal-Wallis test)	Plasma vitamins, DNA adducts in lymphocytes (Refs 12-14,28)	p-value (Kruskal-Wallis test)
	Relative RAL (mean± SD)		**Relative RAL (mean± SD)**	
Gender		*0.08*		*n.a.*
Females Males	1.05± 1.03 0.96± 0.88		-- 0.99±0.43	
Age (years)		*0.38*		*0.008*
≤ 39.8 39.9-51.4 51.5-60 >60	1.22±1.76 0.99±1.00 1.01±0.96 0.98±0.81		0.97±0.45 0.99±0.39 1.13±0.39 1.05±0.29	
Vitamin A		*0.03*		*0.001*
≤ 0.86 0.87 -1.28 1.29 -2.37 > 2.37	1.03 ± 1.15 0.97 ± 1.12 1.04 ± 0.95 0.98 ± 0.77		1.11± 0.37 0.97± 0.37 0.95± 0.58 0.84 ±0.36	
Vitamin E		*0.10*		*0.28*
≤ 8.3 8.4 - 11.3 11.4 - 14.9 > 14.9	1.06 ± 0.87 1.04 ± 0.75 0.94 ± 0.73 0.92 ± 0.68		1.19±0.92 0.98±0.33 1.00±0.39 0.95±0.36	
Vitamin C		*0.58*		*0.0001*
< 63 63-95.4 95.5-147.4 >147.4	1.00±1.03 1.04±0.91 0.99±0.94 0.99±0.99		1.02±0.49 0.91±0.67 0.95±0.39 0.78±0.21	
Smoking		*0.08*		*0.0001*
Never Ever	1.03±0.95 0.98±0.97		0.94±0.46 1.09±0.36	
GSTT1		*0.47*		*0.4*
Present Null	1.00±0.96 0.99±0.84		1.03±0.46 0.98±0.39	
GSTM1		*0.59*		*0.26*
Present Null	1.02±0.95 0.99±0.93		1.05±0.55 1.00±0.43	
GSTT1&GSTM1		*0.56*		*0.42*
Both present GSTT1 null GTM1 null Both null	1.02±0.99 1.02±0.81 0.99±0.95 0.97±0.88		1.06±0.55 1.01±0.43 1.02±0.38 0.91±0.31	

Table 3

Correlation coefficients between standardized DNA adducts and each vitamin according to study design - Multivariate analysis

Dependent variable: relative DNA adducts*

	Study refs.[6,9,10,23–25]		Study refs. [12–14,28]	
Independent variable	Parameter Estimate (SE)	P value	Parameter Estimate (SE)	P value
Vit A (quartiles)	−0.008 (0.019)	0.7	−0.082 (0.015)	0.001
Vit C (quartiles)	−0.013 (0.021)	0.6	−0.036 (0.023)	0.1
Vit E (quartiles)	−0.049 (0.018)	0.007	−0.055 (0.018)	0.002

* Data adjusted for study, age (quartiles), smoking (never/ever), gender

Table 4

Association between Standardized DNA adducts and vitamin A and C - Multivariate analysis
Dependent variable: relative DNA adducts*

Combined analysis- vit. A, C in the same model (n=2457)			
Independent variable	**Parameter Estimate**	**(SE)**	**P value**
Vit A (quartiles)	−0.02	(0.02)	0.2
Vit C (quartiles)	−0.02	(0.02)	0.3

*Data adjusted for study, age (quartiles), smoking (never/ever), gender, GSTT1 GSTM1

Table 5

Association between standardized DNA adducts and each vitamin according to smoking status - Dependent variable: relative DNA adducts*

Independent variable	Never smokers				Smokers			
	N	Parameter estimate	S. E.	p value	N	Parameter estimate	S. E.	p value
Vit A (quartiles)	1337	0.0005	0.02	0.1	1149	−0.06	0.03	0.02
Vit C (quartiles)	1326	0.004	0.03	0.9	1131	−0.05	0.03	0.1
Vit E (quartiles)	1097	−0.05	0.02	0.01	789	−0.04	0.02	0.05

*Data adjusted for study, Age (quartiles), gender, GSTT1, GSTM1