Ubiquitous Presence of O^6-Methylguanine in Human Peripheral and Cord Blood DNA[1]

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Abstract

O^6-Methylguanine (O^6-meG) is a powerful premutagenic lesion that can arise from exposure to methylating agents. Although it has been reported to occur in human DNA, no systematic epidemiological analysis of its occurrence in populations suffering general environmental exposure is available. We report here results from a study of the presence of O^6-meG in maternal and cord blood leukocyte DNA of women not knowingly exposed to methylating agents. Using a modification of an already existing method capable of detecting the lesion at levels as low as 16 nmol/molG, the adduct was detected in 31 of 36 maternal and 30 of 36 cord samples, at levels ranging up to 192 nmol/molG. Adduct levels in maternal blood DNA were significantly higher than those in cord blood DNA ($P < 0.05$), and there was a strong correlation between adduct levels in the two tissues ($P < 0.001$). In bivariate analysis, no significant association of adduct levels in either tissue and residence air pollution, active and passive smoking status, or eating habits was found. However, intake of fruits/vegetables and of vitamin supplements showed nonstatistically significant trends toward being associated with lower adduct levels in both maternal and cord blood DNA. The same trend was observed after multivariate analysis where all the above variables were controlled for. These findings indicate that premutagenic methylation DNA damage is commonplace in individuals not known to have suffered excessive exposure to environmental methylating agents or their precursors and are compatible with an endogenous origin of this damage, possibly associated with endogenous nitrosation processes.

Introduction

Although human exposure to genotoxic methylating agents is thought to be common, little information on the extent or the origins of this exposure is available. Relatively small numbers of individuals may suffer workplace exposure to methylating agents such as dimethylsulfate (an industrial chemical) and methylbromide (an agricultural chemical). On the other hand, large population groups are exposed to powerful methylating *N*-nitroso compounds, such as NDMA[3] and NNK, which are present in significant quantities in tobacco smoke. NDMA is also found in nitrate- or nitrite-treated foods, in certain beverages, and in certain occupational settings (1). Additional and probably substantial exposure to NDMA is likely to arise from the endogenous formation of this compound in the stomach and possibly other body compartments, as a result of the reaction between nitrogenous compounds and nitrosating species (2). Finally, the observation in cells lacking the ability to repair O^6-meG (an important premutagenic DNA adduct) of increased frequency of spontaneous of mutations characteristic of this adduct (3) provides evidence that aberrant DNA methylation may occur as a continuous intracellular process, brought about by agents the identities of which are still undefined.

Biological evidence of human exposure to methylating agents comes from studies that have demonstrated the presence of methylated DNA adducts in human tissues. For example, in a number of pilot-scale studies, N^7-methylguanine, the major DNA adduct formed by methylating agents, has been detected by ^{32}P-postlabeling in the DNA of tissues (mostly lung) of individuals suffering general environmental exposure (Refs. 3–10; reviewed in Ref. 11). The adduct was usually found in the range of 0.1–1 μmol N^7-meG/mol G, levels that are high by comparison to most other DNA adducts found in human DNA. Furthermore, the frequency of adduct-positive samples was also high (usually ranging from 30 to 100%), suggesting that exposure to methylating agents is common. However, no systematic studies of the epidemiology of N^7-meG have been reported, and evidence that its occurrence in human DNA is related to tobacco smoke exposure is limited and contradictory (4, 7, 10).

A few studies have also examined the presence in human tissues of additional, biologically important methylated DNA lesions, such as the directly miscoding adducts O^6-meG and O^4-meT. In pilot-scale studies, O^4-meT was detected in human liver DNA (but not in WBC DNA) at levels <1 μmol/mol T (12). Using an HPLC/radioimmunoassay method with limit of detection of 0.5 μmol O^6-meG/mol G, Foiles *et al.* (13) found this adduct in 5 samples of human placenta of 20 examined, in the range 0.6 to 1.6 μmol/mol G, whereas in more recent investigations of similar size, utilizing more sensitive assays,

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[3] The abbreviations used are: O^6-meG, O^6-methylguanine; O^4-meT, O^4-methylthymine; NDMA, *N*-nitrosodimethylamine; MNU, *N*-methyl-*N*-nitrosourea; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; HPLC, high-performance liquid chromatography; AGT, alkylguanine-DNA alkyltransferase.

the same adduct has been found in a large proportion of human WBCs (range, 7–46 nmol/mol G) and liver (range, 110–670 nmol/mol G) DNA samples (14, 15). As in the case of N^7-meG, these studies suggested that exposure to methylating agents may be commonplace, but their limited scale did not permit the investigation of the origin of the adducts. A role for endogenously formed *N*-nitroso compounds is suggested by the observation of increased incidence of O^6-meG in DNA from the urinary bladder of individuals infected with schistosomiasis (which leads to inflammation and concomitant production of nitric oxide and related nitrosating species; Ref. 16) and by the discovery of O^6-meG in esophageal DNA of individuals in China with high dietary intake of nitrates (17). In a large-scale investigation of the epidemiology of O^6-meG, involving examination of blood leukocyte DNA of 407 individuals drawn from 17 regions worldwide, O^6-meG was found in 21 samples at levels ranging from the limit of detection of the assay used (0.08 μmol/mol G) up to 0.3 μmol/mol G (18). In this study, adducts were found more frequently among samples derived from regions with higher consumption of nitrate-treated foods and incidence of gastric cancer and from individuals with low levels of serum pepsinogen A (a marker of chronic atrophic gastritis). These observations were consistent with the hypothesis that agents formed through intragastric nitrosation contributed significantly to methylated adduct formation and implied that such adducts might have biological significance.

The suggestion that endogenous nitrosation (a process that occurs to a greater or lesser extent in everyone) may be an important source of DNA methylation implies that low levels of methylated adducts in human DNA may be commonplace, even in individuals with conditions that are not associated with high endogenous nitrosation (*i.e.*, chronic inflammation or high nitrate intake). To further test this suggestion, an assay of improved sensitivity was developed and used for the investigation of the presence of O^6-meG in maternal and cord blood leukocyte DNA obtained from a group of healthy women living in Greece, a region known not to be associated with high consumption of nitrate-containing foods.

Materials and Methods

Sample Collection and Subject Information. The cohort investigated consisted of 36 healthy pregnant women who entered a major maternity hospital located in Athens. All women entered the hospital no more than 24 h prior to delivery. During this time, they received their habitual diet (including vitamin supplements, if applicable) or standard hospital diet. None had consumed alcohol-containing beverages during this time.

Samples of venous and cord blood (10 ml) were collected at the time of birth and stored at room temperature until processing, which took place within 6 h of collection. No anaesthetic treatment had been given to any up to the time of blood collection. On the day after delivery, they underwent an interview, in the context of which a questionnaire was completed giving information on, among other matters: (*a*) exposure to products of fuel combustion (place of residence in relation to traffic density, type of home heating and cooking facilities); (*b*) dietary habits, with particular attention to consumption of fruits and vegetables and smoked or other nitrate-treated meat or fish; (*c*) active or passive smoking (habitual smoking status, number of cigarettes smoked during the last 24 h, residence with a smoker, number of cigarettes smoked in her presence during the last 24 h); (*d*) consumption of vitamin supplements; and (*e*) family history of cancer. Three of the women left the maternity clinic before this interview could be undertaken.

Total blood DNA was extracted by standard methods involving multiple proteinase K and RNase 1 treatments and phenol/chloroform extractions (19). Lymphocytes for AGT analysis were isolated using Ficoll gradient (Lymphoprep; NYCOMED Pharma, Oslo, Norway), according to the manufacturer's procedure.

Assay for O^6-meG. DNA adduct analysis was carried out using precautions to minimize the risk of cross-contamination, such as avoiding the handling of known methylated DNA samples in the same area of the laboratory and using wherever possible single-use, disposable plasticware. A modification of the competitive repair assay for O^6-meG (20) was used which had a higher sensitivity than the classical assay used in our previous studies. This assay involves the use of an excess of the repair enzyme O^6-alkylguanine-DNA alkyltransferase from *Escherichia coli* (the ada protein) to repair O^6-meG present in the DNA under examination, followed by back-titration of the remaining enzyme using a synthetic oligonucleotide containing a single residue of O^6-meG and 5′-end labeled with [^{35}S]-thiophosphate. The amount of unrepaired labeled oligonucleotide is measured by immunoprecipitation with rabbit anti-O^6-meG antiserum, and the amount of O^6-meG is finally calculated from a standard curve. This assay, which has a limit of detection of 0.5 fmol O^6-meG in up to 10 μg of DNA (thus permitting the detection 80 nmol O^6-meG/mol G) has been extensively validated in studies of animals treated with methylating agents as well as in studies of humans treated with methylating cytostatic drugs (19, 21, 22).

A modification introduced in the present case involved primarily the use of labeled oligonucleotide of higher specific activity (achieved through the HPLC separation of the ^{35}S-thiophosphorylated oligonucleotide from the unlabeled starting material), as well as a few additional changes. After the 5′-end labeling of the oligonucleotide with γ-^{35}S-labeled ATP and T4 polynucleotide kinase, the radioactive oligonucleotide was recovered free of unlabeled starting material using anion exchange HPLC (monoQ HR5/5 column, gradient 0.2 to 0.8 M NaCl, pH 12, and flow 1 ml/min). The typical specific activity of the resulting material was 2200 cpm/fmol (equal to the specific activity of the γ-^{35}S-labeled ATP), 7–15-fold higher than that of the material used in the classical assay. Use of this high specific activity oligomer in the competitive repair assay as described previously (20), with AGT-pretreated (hence O^6-meG-free) DNA samples, was found to result occasionally in a weak positive signal (equivalent to up to 0.4 fmol of O^6-meG), presumably caused by nonspecific inhibitors of AGT present in the DNA. For this reason, an additional modification was introduced to control for such nonspecific AGT inhibition, based on the use the method of standard additions instead of the commonly used standard curve method for adduct quantitation. Thus, the assay was conducted as follows. Triplicate "test" mixtures consisting of 1 μg of standard, methylated calf thymus DNA, containing 0, 0.2, 0.4, or 0.6 fmol O^6-meG, each mixed with 10 μg of unknown DNA sample, plus a separate triplicate "control" mixture consisting of 1 μg of unmethylated calf thymus DNA plus 10 μg of the same unknown DNA, previously treated overnight with a large excess AGT to remove all O^6-meG and repurified by phenol extraction, were prepared. To each mixture, AGT (10 fmol) was added, and O^6-meG repair was allowed to proceed for 2 h at room temperature, followed by the addition of the ^{35}S-labeled oligonucleotide (7 fmol). After further incubation overnight, any oligonucleotide remaining unrepaired was finally immunoprecipitated, and its radioactivity was counted, as described previously (20). The inhibi-

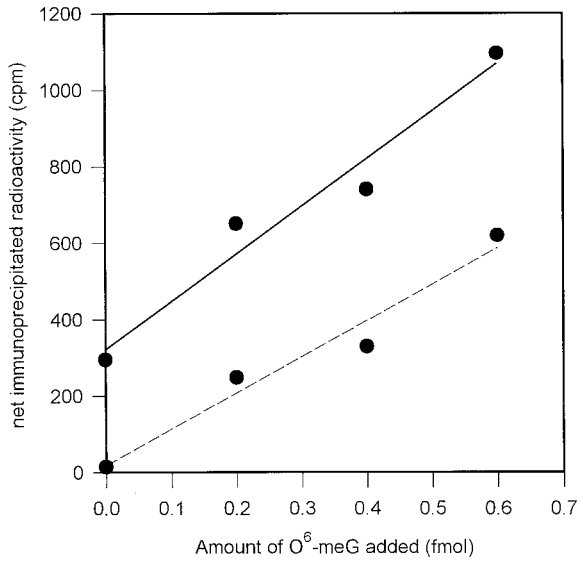

Fig. 1. Typical standard addition curves. Different amounts of standard, methylated calf thymus DNA were added to the unknown DNA and subjected to competitive repair as described. The immunoprecipitated radioactivity shown on the *vertical axis* is that obtained after subtracting the radioactivity immunoprecipitated in the presence of the same unknown DNA after complete removal of its O^6-meG (see "Materials and Methods"). The amount of O^6-meG present in the DNA was calculated by dividing the vertical intercept by the slope of the line. In the examples shown here, the amounts of O^6-meG were 42 nmol/mol G in the maternal blood sample (*solid line*) and nondetectable in the cord blood sample (*dashed line*).

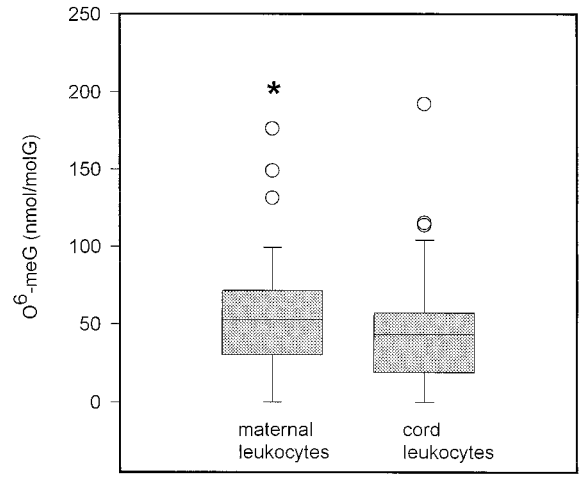

Fig. 2. O^6-meG levels in maternal and cold blood DNA. The *horizontal line* in the *box* indicates the median values, the *box boundaries* the 25th and 75th percentiles, and the *capped bars* the 10th and 90th percentiles. *, $P < 0.05$ (Student's paired *t* test).

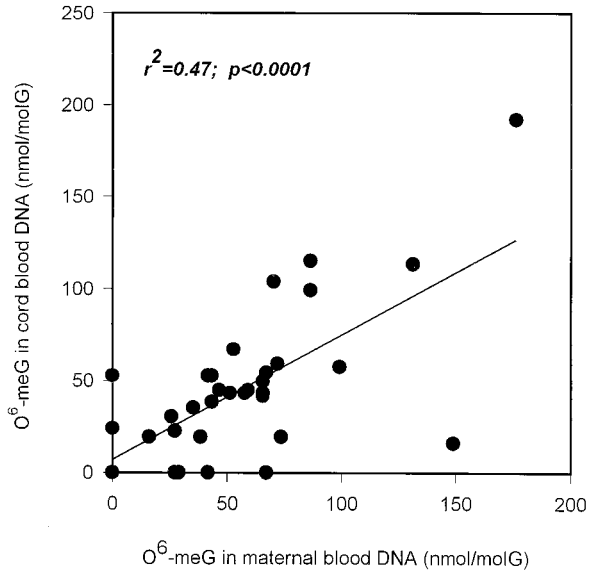

Fig. 3. Relationship between O^6-meG levels in maternal and cord blood DNA.

tion of oligonucleotide repair caused by the O^6-meG present in the unknown DNA was evaluated by subtracting the amount of radioactivity immunoprecipitated from the "control" mixture from that immunoprecipitated from the test mixture containing the unknown DNA without O^6-meG-containing standard DNA. The amount of adduct present in the unknown DNA was calculated by dividing this difference by the slope of the "test" standard addition curve. Typical standard addition curves for a pair of maternal and cord blood DNA are shown in Fig. 1. The intra- and interassay variabilities were less than 7 and 10%, respectively. The limit of sensitivity of the assay reached 0.10 fmol O^6-meG in 10 μg of DNA (16 nmol O^6-meG/mol G).

Measurement of AGT in Lymphocytes. AGT in extracts of maternal and cord blood lymphocytes was measured using ^{3}H-methylated DNA as substrate as described elsewhere (21).

Statistical Analysis. O^6-Methylguanine adduct levels and AGT levels were approximately normally distributed, as indicated by application of the Kolmogorov-Smirnov normality test, and consequently were analyzed using Student's *t* test (paired and two-sample), ANOVA, and multiple linear regression models.

Results

Among 36 pairs of maternal and cord blood DNA samples analyzed for O^6-meG, all but 5 maternal and 6 cord blood DNA samples contained detectable amounts of O^6-meG. Adduct levels in maternal DNA were in the range of 16–176 nmol/mol G and had a mean value of 56 nmol/mol G (corresponding to 0.036 fmol/μg DNA; (SD, 41 nmol/mol G), whereas those in cord blood were in the range of 16–192 nmol/mol G and had a mean value of 45 nmol/mol G (corresponding to 0.028 fmol/μg DNA; SD, 41 nmol/mol G; Fig. 2). The mean value for the adduct levels in maternal blood DNA was statistically significantly higher than that in cord blood DNA ($P = 0.047$; Student's paired *t* test). A strong correlation between adduct levels in maternal and cord blood DNA was observed ($r^2 = 0.47$; $P < 0.001$; Fig. 3).

AGT levels in lymphocyte extracts were measured in all 36 pairs of lymphocytes from maternal peripheral or cord blood (Fig. 4). The mean values were 8.32 fmol/μg DNA (SD, 2.91 fmol/μg DNA) and 7.43 fmol/μg DNA (SD, 2.2 fmol/μg DNA) for maternal and cord blood lymphocytes, respectively. Maternal lymphocytes appeared to have higher AGT activity than cord blood lymphocytes, although the difference was not statistically significant ($P = 0.089$; Student's paired *t* test). No correlation was found between AGT activities in maternal and

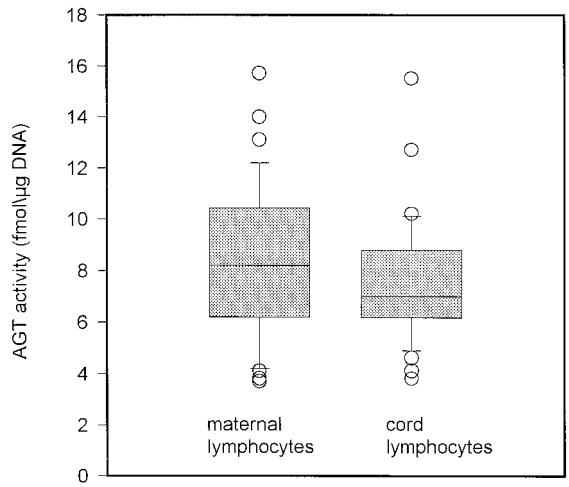

Fig. 4. AGT levels in lymphocyte extracts from maternal and cord blood lymphocytes. For explanations of the box lines, see Fig. 2.

the corresponding cord blood lymphocytes ($r^2 = 0.005$; $P = 0.634$), nor was there a correlation between AGT and adduct levels in either maternal or cord WBC DNA ($r^2 = 0.026$, $P = 0.37$; and $r^2 < 0.001$, $P = 0.93$, respectively).

The data on adduct levels were analyzed using information obtained from the questionnaire. Univariate analysis did not reveal any statistically significant effects on O^6-meG levels of the location of residence (rural, suburban, or urban; Table 1). This result was not altered when other parameters that might affect the level of residence air pollution (distance from streets with heavy traffic and type of indoor heating and cooking) were taken into consideration (results not shown). Similarly, no effect was observed for any of the other parameters examined, including habitual active or passive smoking status or corresponding recent exposures; consumption of fruits and vegetables, fruit juices, or smoked foods; or family history of cancer (Table 1). A nonstatistically significant difference toward lower adduct levels, especially in cord blood DNA, was observed in women taking vitamin supplements as compared with those not taking such supplements ($P = 0.11$, t test). This difference remained evident when a multiple linear regression model was employed utilizing adduct levels as the dependent variable and residence air pollution, exposure to active or passive smoking, consumption of fruits and vegetables, fruit juices, and vitamin supplements as independent variables (Table 2). After adjustment for the remaining model variables, those consuming vitamin supplements had on average 27 nmol O^6-meG/mol G in maternal WBC DNA ($P = 0.08$) and 26 nmol O^6-meG/mol G in cord WBC DNA less than those not taking such supplements ($P = 0.15$).

No correlations were observed between AGT levels and any of the personal parameters tested (data not shown).

Discussion

By introducing a number of modifications to an already established and well-validated assay for O^6-meG, we have been able to detect this adduct at levels corresponding to 1.6 nmol/mol G, ~5-fold lower than the limit of sensitivity of the version reported previously of the assay. Although the modified assay is significantly more complex and requires substantially larger amounts of DNA, its availability constitutes a significant development that may facilitate future studies of the epidemiology of O^6-meG.

In a previous study, we found a higher frequency of O^6-meG in the DNA of individuals drawn from regions known to be associated with high consumption of nitrate-treated foods as well as with high incidence of gastric cancer (18). In that study, none of 74 WBC DNA samples derived from Greek individuals was found to contain O^6-meG above the limit of detection of the assay used on that occasion. Greece is a region in which the prevalent diet does not include frequent consumption of nitrate-treated foods or other items likely to be rich in *N*-nitroso compounds, and additionally it has a relatively low incidence of most cancers, including gastric cancer (23, 24).

Use of a more sensitive assay in the presently reported study resulted in the same adduct being detected in most of 36 pairs of maternal and cord WBC DNA samples examined, almost always at levels below the limit of detection of the previously used version of the assay. The frequent observation of O^6-meG is compatible with the hypothesis described in the "Introduction" of this report, *i.e.*, that endogenous nitrosation, a phenomenon known to occur even in individuals without excessive exposure to nitrate or other nitrosation precursors, gives rise to a ubiquitous background level of DNA methylation damage. Such a background may be increased by exposure to unusually large amounts of nitrosation precursors or to preformed *N*-nitroso compounds themselves, situations that have been epidemiologically associated with increased incidence of various types of cancer (25).

The levels of O^6-meG in peripheral WBC DNA observed in the present study are comparable with those reported by others (see "Introduction"). Adduct levels in cord blood DNA were measured for the first time in the present investigation. They were found to be lower than, and showed a strong correlation with, those in maternal blood DNA, suggesting transplacental exposure of the fetus to methylating agents circulating in the mother's blood. Methylating *N*-nitroso compounds such as NDMA, MNU, and the tobacco-specific nitrosamine NNK are active transplacental carcinogens in rodents (26–29), and they can cause DNA methylation in rodent embryonic tissues (30–33). However, information on the relative levels of DNA damage induced specifically in maternal and cord blood DNA is available only for NDMA. In a study in patas monkeys, Chhabra *et al.* (34) have shown that a single dose of NDMA gave rise to O^6-meG in cord blood leukocytes at levels ~25% of those seen in maternal WBC. Thus, the relative adduct levels observed in the present study are compatible with their being derived from NDMA.

No correlation was observed between O^6-meG and AGT levels. This is in line with similar observations in patients accumulating much large levels of O^6-meG as a result of chemotherapy with methylating drugs such as procarbazine or dacarbazine, where only in individuals with very low levels of AGT was a trend toward increased adduct accumulation observed (Refs. 19, 22, and 35; reviewed in Ref. 36). These observations suggest that AGT variation within the range usually observed in human populations may not substantially affect O^6-meG accumulation.

Studies in humans treated with methylating chemotherapeutic drugs have shown that O^6-meG in human WBCs is relatively short-lived; its half-life is approximately 22–25 h (19, 35). This implies that the adduct levels measured in the present study were probably determined primarily by recent exposure (previous 24 h). For this reason, an effort was to include among the parameters utilized in the statistical analysis factors likely to reflect recent exposure to methylating agents or their precur-

Table 1 Effects of residential air pollution, smoking, passive smoking, and diet on O^6-meG levels in maternal and cord blood DNA: bivariate analysis

	n	O^6-meG (nmol/mol G)			
		Maternal blood, mean (SD)	*P*[a]	Cord blood, mean (SD)	*P*[a]
Location residence[b]					
1	5	61 (58)	0.69[c]	39 (20)	0.49[c]
2	1	46 (−)		45 (−)	
3	4	78 (68)		77 (78)	
4	23	51 (51)		44 (30)	
Habitual smoking/smoking during last 24 h[d]					
No	28	55 (43)		47 (45)	0.45
Yes	5	60 (11)	0.83	63 (24)	
Passive smoking					
No	7	48 (30)		40 (29)	
Yes	26	58 (43)	0.61	53 (46)	0.49
Consumption of fish and meat					
<2 times/week	7	71 (54)	0.25[c]	66 (59)	0.41[c]
2–4 times/week	20	46 (27)		43 (34)	
Every day	6	70 (59)		39 (54)	
Nitrate-treated foods					
<1 time/week	28	55 (43)	0.91	47 (43)	0.9
≥1 time/week	3	58 (14)		44 (24)	
Fruits or vegetables					
Less than every day	6	37 (23)	0.29	38 (21)	0.49
Every day	25	55 (39)		50 (45)	
Vitamin supplements					
No	15	64 (43)	0.29	64 (50)	0.11
Yes	17	50 (38)		38 (35)	
Fruit juices (portions/day)					
≤1	14	59 (46)	0.88[c]	61 (54)	0.32[c]
2	10	50 (47)		33 (32)	
>2	6	56 (24)		47 (39)	
Family history of cancer					
No	27	58 (42)		50 (48)	
Yes	6	48 (34)	0.6	50 (13)	0.97

[a] *P*s from independent samples *t* test.
[b] 1, rural; 2, suburban without industrial activities; 3, suburban with industrial activities; 4, urban.
[c] *P*s from one-way ANOVA analysis.
[d] Division of subjects by habitual smoking or recent smoking resulted in identical subgroups.

Table 2 Effects of residential air pollution, cigarette smoking exposure, and diet on O^6-meG levels in maternal and cord blood DNA: Multiple linear regression model

	Maternal blood adducts (nmol/mol G)			Cord blood adducts (nmol/mol G)		
	β coefficients	SE	*P*	β coefficients	SE	*P*
Residence air pollution 1, low . . . 5, high	−1.8	5.4	0.74	−6.5	6.5	0.33
Passive smoking No, 0/Yes, 1	−0.7	16.1	0.96	8.8	19.5	0.66
Active smoking No, 0/Yes, 1	27.4	34.3	0.44	2.4	41.5	0.96
Consumption of fruits or vegetables less than every day, 1/every day, 2	32.6	23	0.18	24.1	27.9	0.4
Consumption of fruit juices (portions/day) ≤1:1/2:2/≥2:3	−1.3	9.7	0.89	−5.1	11.7	0.67
Consumption of vitamin supplements No, 0/Yes, 1	−26.5	14.3	0.08	−26.3	17.3	0.15

sors. Given that the study subjects were pregnant women who had spent the last 24 h prior to sample donation in the same maternity hospital, suffering similar ambient and dietary exposures during this time, the most important additional factors to control for had to do with recent smoking exposure. This was done by including in our analysis information on habitual active or passive smoking, as well as on cigarettes smoked by or in the presence of each subject during the previous 24 h. However, neither these nor any of the other questionnaire parameters examined showed a statistically significant correlation with adduct levels.

The only parameter that showed a consistent and nearly statistically significant trend toward being associated with lower adduct levels, in both maternal and cord WBC DNA, was intake of vitamin supplements (Tables 1 and 2). In all cases the supplements consisted of vitamin C alone or in combination with other vitamins. Vitamin C is known to be a potent inhibitor of nitrosation reactions *in vitro* and *in vivo* (37), especially at high doses such as those associated with the intake of vitamin supplements (38). Thus, the observed association is compatible with, and provides plausibility to, the suggestion that methylation damage may arise via endogenous generation of methylating *N*-nitroso compounds. It is notable that maternal consumption of vitamin supplements has been reported recently in

two studies to be associated with a protective effect against childhood brain tumours (39, 40). In the latter study, maternal consumption of vitamin supplements appeared to reduce childhood brain cancer risks associated with consumption of cured meats during pregnancy, an observation compatible with their acting to block endogenous nitrosation reactions. A similar protective effect of vitamin supplements against cured meat-associated brain cancer risks has also been reported in a study of adult brain cancer in women (41). Finally, vitamin supplement consumption has also been reported to reduce the risk of brain cancer in men (42). These epidemiological observations, in combination with our observation of a possible protective effect of vitamin supplements against the induction of the precarcinogenic DNA adduct O^6-meG in both maternal and cord blood DNA, provides some support for the hypothesis that endogenous nitrosation processes may play a role in the etiology of adult or childhood brain carcinogenesis.

The identity of the methylating agent(s) responsible for the induction of the observed adducts is unknown. The well-known ability of S_N 1-type methylating agents to induce relatively high levels of *O*-methylation makes it likely that the agent in question belongs to this chemical category. Animal studies have shown that NDMA is, on a per orally administered dose basis, by far the most efficient agent capable of generating O^6-meG in blood cell DNA when compared with other known methylating agents (*e.g.*, MNU and NNK; Refs. 21 and 43).[4] Hence, given the known endogenous formation of NDMA in the human body, as indicated by its presence in gastric juice and its excretion in urine (44, 45), this nitrosamine seems a plausible candidate for giving rise to the observed adducts. This possibility is also compatible with the available information, already mentioned, on the relative ability of NDMA to methylate maternal and cord blood leukocyte DNA of patas monkeys (34).

In conclusion, our demonstration of the presence of the precarcinogenic adduct O^6-meG in maternal and cord leukocyte DNA of a large proportion of women not thought to have suffered high exposure to known methylating agents or *N*-nitroso compounds or their precursors indicates that DNA methylation constitutes a significant genotoxic burden for the general population. Furthermore, the observation of a possible protective effect of intake of vitamin C supplements provides limited support for the hypothesis that endogenous nitrosation processes may contribute significantly to this burden.

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