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Fruit and vegetable and fried food consumption and 3-(2-deoxy- β -D-erythro-pentafuranosyl)pyrimido[1,2- α]purin-10(3H)-one deoxyguanosine adduct formation

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Abstract

Diet has been shown to modulate M₁dG adduct, a biomarker of oxidative stress and lipid peroxidation. Thus, we analysed the association between diet and M₁dG in 120 controls and 67 Map Ta Phut industrial estate workers, Rayong, Thailand to evaluate the influence of fruit and vegetables, and fried and charcoal-grilled/barbecued food consumption on M₁dG.

M₁dG was decreased in controls reporting to consume 14–17 servings/week of fruit and vegetables [Mean Ratio (MR)=0.35, C.I. 0.18–0.69, p<0.05]. Conversely, a non-statistically significant M₁dG increment was detected in controls consuming 9–18 servings/week of fried food (MR=1.33, C.I. 0.88–2.00, p=0.168). No effect of charcoal-grilled/barbecued food was found. No effect of diet was observed in workers. An association with smoking was observed in controls (MR=1.88, C.I. 1.14–3.10, p<0.05), but not in workers. M₁dG can induce mutations and/or methylation changes within the promoter regions of cancer-related genes, thus promotion of healthy eating practices should be recommended.

Keywords

Fruit and vegetables; fried food; free radicals; oxidative stress; lipid peroxidation; M₁dG

Introduction

Diet is an important component of lifestyle habit and its role in the maintenance of good health and as a determinant of different chronic diseases, including cancer, has been widely analyzed [1,2]. Diet characterized by high intake of fruit and vegetables has been associated with lower cancer risk [3]. Antioxidant capacity of fruit and vegetables seems to play a relevant role in their protective effects. It is believed that agents that decrease oxidative

stress and DNA damage should also decrease the cancer risk [3,4]. However, diet can enhance cancer risk through the consumption of mutagens contained in contaminated foods and or produced by cooking at high temperature, such as heterocyclic aromatic amines (HCA) [5]. A mechanism by which food mutagens can exert their toxic effects is based on the production of reactive oxygen species (ROS) [6]. ROS are highly reactive compounds that interact with all cell constituents, including DNA and membrane, leading to oxidative DNA damage and lipid peroxidation (LPO) [7].

Malondialdehyde (MDA) is a natural product of LPO [7], which is also formed during prostaglandin-H₂ biosynthesis via cyclo-oxygenase [7]. MDA is an aldehyde capable of interacting with DNA to form exocyclic DNA adducts, including 3-(2-deoxy-β-D-erythro-pentafulanosyl)pyrimido[1,2-α]purin-10(3H)-one deoxyguanosine (M₁dG) adduct. M₁dG is also generated by deoxyribose oxidation through the formation of base propenals [8]. The relevance of M₁dG adduct in carcinogenic process is emphasized by its capability to cause base pair and frameshift mutations in reiterated sequences [9]. Physiological M₁dG level has been measured in leukocytes, breast, colon, larynx and bronchi [10–14]. Increased M₁dG amount has been found in smokers, subjects exposed to air pollution and workers of pathology wards [11,12,15,16]. M₁dG adduct has been reported to be influenced by individual susceptibility and diet [10–12,17]. For instance, fruit, salad, legumes and whole meal bread have been inversely associated with M₁dG, whereas the consumption of sunflower oil, white meat and alcohol with increased DNA damage. Finally, increased M₁dG adduct has been suggested to be related to cancer and tumour progression [12,14].

In the last years, we have conducted cross-sectional and case-control studies that have documented that the level of bulky DNA adducts, a kind of DNA damage induced from aromatic chemicals [18], is modulated by diet, mainly by fruit and vegetables [19–23]. Thus, we decided to extend our analysis to another kind of DNA damage, the M₁dG adduct, a biomarker of oxidative stress and LPO [7]. We analysed the association between Thai diet and M₁dG adduct in the leukocytes of 120 controls living in Rayong province, Thailand, and 67 Map Ta Phut industrial estate (MIE) workers located in MTP, Rayong, Thailand. Our aim was to evaluate the influence of increased consumption of fruit and vegetables and fried and grilled/barbecued food on oxidative stress and LPO related DNA damage.

Material and methods

Study subjects

Participants that were residents in the province of Rayong, Thailand, were contacted and recruited by the local health personnel. MIE workers employed in industrial estate complexes located in Map Ta Phut, Rayong, Thailand, were identified and recruited by the industrial health service. The study was approved by the relevant ethical committee [22,23]. The association between dietary habits and DNA damage was studied in a selected group of individuals participating to the MTP study for whom M₁dG adduct analyses were available [13].

Dietary assessment

A questionnaire aimed to collect standard demographic and life-style data, i.e. age, gender, smoking habit (last three months), residence and occupation was administered to the participants before blood sampling and after informed consent was obtained. Thai dietary information (refer to standard portion, average over the last two years) of the intake of fresh and cooked (boiled) fruit and green/yellow vegetables, and the consumption of fried and charcoal-grilled/barbecued meat (beef and pork), chicken and fish/shellfish.

M₁dG adduct standards

Calf-thymus (CT)-DNA or leukocyte DNA from a blood-donor was treated with 10 mM MDA as previously described [14]. MDA treated CT-DNA was diluted with untreated DNA to obtain decreasing levels of reference standard. Then, we decided to expose human epithelial lung carcinoma A459 cells to 100 mM H₂O₂, a known source of ROS and LPO, to evaluate the formation of M₁dG adduct not only in naked DNA but also in experimental in vitro cells, a system that should reflect the physiological conditions of in vivo organisms.

DNA extraction, DNA hydrolysis, and nuclease P1 treatment

Leukocyte DNA was extracted and purified from buffy coat samples using a method that requires digestion with ribonuclease A, ribonuclease T1 and proteinase K, extraction with saturated phenol, phenol/chloroform/isoamyl alcohol (25:24:1), chloroform/isoamyl alcohol (24:1) and ethanol precipitation [11,12]. DNA was gently dissolved in sterile distilled water and its concentration was determined using a Beckman DU 800 spectrophotometer. DNA was coded and stored at -80°C until laboratory analysis. Coded leukocyte DNA samples (1–2 µg) were digested to normal and adducted 3'-mononucleotides with micrococcal nuclease (460 mU) and spleen phosphodiesterase (34.8 mU) in 17 mM sodium succinate, 8 mM calcium chloride, pH 6.0 at 37°C for 4.5 hrs [12–14]. Normal 3'-mononucleotides were converted, by incubation with nuclease P1 (2.5 µg) in sodium acetate 40mM pH5 and ZnCl₂ 0.2mM for 30 min at 37°C, to nucleosides, which are not substrates for polynucleotide kinase (PNK) labeling. The nuclease P1 resistant, modified 3'-nucleotides were converted to 5'-³²P-labelled nucleoside 3'-5'-biphosphate adducts by T4-PNK (0.75 U/µl) catalyzed transfer of [γ-³²P]phosphate from 15–25 µCi of [γ-³²P]ATP in bicine buffer (pH 9.0) at 37°C for 30 min.

M₁dG adduct analysis

Detection of M₁dG adduct (1–2 µg ³²P-labelled nuclease P1 treated digest DNA) was carried out by multidirectional chromatography [12–14]. We used a solvent system effective for the detection of low molecular weight and high polar DNA adducts, such as M₁dG adduct: 0.35 MgCl₂ for preparatory chromatography; 2.1 M lithium formate, 3.75 M urea pH 3.75 and 0.24 M sodium phosphate, 2.4 M urea pH 6.4 for two dimensional chromatography on Macherey Nagel PEI-cellulose TLC plates. Detection and quantification of M₁dG adduct and total nucleotides was obtained by storage phosphor imaging technique employing intensifying screens from Molecular Dynamics (Sunnyvale, California, USA) for 0.20–48 hr. The screens were scanned using a Typhoon 9210 (Amersham, Buckinghamshire, U.K.). Software used to process the data was ImageQuant (Molecular Dynamics). After background subtraction, the level of M₁dG adduct was expressed such as relative adduct labelling (RAL) = screen pixel in adducted nucleotides/screen pixel in total normal nucleotides (nn). To calculate the levels of total nn, aliquots of hydrolysed DNA were appropriately diluted and reacted in the mixtures used for M₁dG adduct labelling. The obtained ³²P-labelled total nucleotides were separated on Merck plates using 280 mM (NH₄)₂SO₄, 50 mM NaH₂PO₄. Standard malondialdehyde modified CT-DNA was routinely processed in the analysis. M₁dG values were corrected across experiments based on the recovery of the internal standard. The coefficient of variation of the assay was 0.22 and the mean recovery of internal standard was 16.7%.

Mass spectrometry

DNA adducts were also detected in MDA treated CT-DNA by mass spectrometry through the following sequence of steps: (1) react DNA with NaBH₄ [24] followed by precipitation with isopropanol; (2) digest with snake venom phosphodiesterase and nuclease P1; (3) extract DNA adducts that are less polar than nn on an OASIS cartridge (Waters

Corporation); (4) tag with an isotopologue pair of benzoylhistamines (d_0 and d_4) in a phosphate-specific labeling reaction in the presence of carbodiimide [25]; (5) remove residual reagents by ion exchange solid-phase extraction; (6) resolve tagged adducts by capillary reversed-phase HPLC with collection of drops onto a MALDI plate; (7) add matrix (α -cyano-4-hydroxycinnamic acid); and (8) analyze by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Statistical analysis

All statistical analyses were performed on log transformed data to stabilize the variance and normalize the distribution of M_1dG adduct. Controls and MIE workers were grouped according to tertiles for food consumption before statistical analyses. A descriptive analysis was initially performed to explore the relationship between individual variables and M_1dG adduct. In the univariate setting, the mean levels of M_1dG adduct across the levels of each variable, i.e. age, gender, smoking habit, intake of fruit and vegetables, and fried and charcoal-grilled/barbecued food consumption, were compared by means of analysis of variance. Post hoc Dunnett tests were performed for multiple comparisons among such variable levels.

The multivariate analysis was then performed using log-normal regression models including terms for gender, age and smoking habit. The regression parameters estimated from the model are interpreted as ratios (Means Ratio) between the means of M_1dG adduct of each level of the study variables with respect to the reference level, adjusted by gender, age and smoking habit. A $p < 0.05$ (two-tailed) was considered significant for all of the tests. The data were analyzed using SPSS 13.0 (SPSS, USA).

Results

Standard M_1dG adduct by ^{32}P -DNA postlabelling

A significant increased formation of M_1dG adduct was found in MDA treated CT-DNA in respect to untreated CT-DNA ($p < 0.001$) by ^{32}P -DNA postlabelling assay. The RAL values per 10^6 were 0.32 ± 0.11 (SE), 1.6 ± 0.38 (SE) and 5.0 ± 0.6 (SE) in 1, 4 and 10 mM MDA treated CT-DNA, while a RAL of 0.06 ± 0.01 (SE) was detected in untreated DNA. A calibration curve was obtained by the analysis of decreasing level of MDA treated CT-DNA, r -squared = 0.99. A significant production of M_1dG adduct was also observed in MDA treated leukocyte DNA in respect to control leukocyte DNA ($p < 0.001$). The RAL values per 10^6 was 2.2 ± 0.6 (SE) and 0.02 ± 0.01 (SE) in MDA treated and untreated DNA. Then, our results show that the treatment with 100 mM H_2O_2 induces a significant formation of M_1dG adduct in lung carcinoma cells in respect to the unexposed cells ($p < 0.001$). The RAL values per 10^6 were 0.25 ± 0.09 (SE) and 0.07 ± 0.02 (SE) in H_2O_2 treated and untreated lung carcinoma cells.

Standard M_1dG adduct by mass spectrometry

The presence of M_1dG adduct in the 10 mM MDA treated CT-DNA, was also confirmed by us subjecting it to analysis by mass spectrometry, giving the results shown in Figure 1. As seen, 6 MDA DNA adducts apparently were identified after mass tagging by MALDI-TOF-MS in MDA treated, $NaBH_4$ -reduced CT-DNA. Relative to the accurate masses that are seen in the spectrum from one spot (Figure 1), the exact masses are as follows using the M nomenclature of Goda and Marnett [24]: M_1dG (581.166); M_1dA (583.182); M_2dG (653.187); M_3dC (667.192); M_3dA (693.219).

M₁dG adduct and life-style and dietary variables

Standard demographic and life-style variables and the values of leukocyte M₁dG adduct per 10⁸ of 120 controls and 67 MIE workers are reported in Table I. The average level of leukocyte M₁dG adduct according to tertiles for the consumption of fruit and vegetables and fried and charcoal-grilled/barbecued food are also shown in Table I. The results of the univariate analysis show that MIE workers presented statistically significantly higher blood level of M₁dG adduct compared to the controls (5.9 ± 0.5 vs. 3.4 ± 0.3 , $p < 0.05$). No effect of age and gender was observed in the study population. A borderline significant increment of DNA damage was found in smoker controls in respect to non smoker controls (3.8 ± 0.4 vs. 2.8 ± 0.3 , $p = 0.065$). No significant effect of smoking was observed in MIE workers ($p = 0.798$).

When dietary habits were considered, the mean level of M₁dG adduct per 10⁸ normal nucleotides of Rayong controls did not differ among the three groups reporting to consume an average intake of fried and charcoal grilled barbecued food and fruit and vegetables (3.5 ± 0.5 ; 3.1 ± 0.3 and 3.4 ± 0.4 , respectively). The level of M₁dG adduct of Rayong controls with an increased intake of fruit and vegetables (14–17 servings/week) was statistically significantly lower in respect to those reporting a low consume of fruit and vegetables (2.4 ± 0.4 and 3.7 ± 0.5 , respectively, $p < 0.05$ vs. reference level). A non-statistically significant decrement was also observed in MIE workers with a high intake of fruit and vegetables in respect to the referent group (6.2 ± 0.9 and 7.4 ± 0.8 , respectively, $p = 0.979$ vs. reference level).

Conversely, a non-statistically significant enhancement of DNA damage was found in Rayong controls with a frequent intake of fried food (9–18 servings/week) in respect to those reporting to consume up to six servings per week (4.2 ± 0.6 and 2.6 ± 0.2 , respectively, $p = 0.293$ vs. reference level), but not in petrochemical workers ($p = 0.591$ vs. reference level). No significant changes associated to the charcoal-grilled/barbecued food intake were observed in Rayong controls and MIE workers ($p = 0.982$ and $p = 0.575$, respectively, vs. reference levels).

The results of the multivariate analysis are presented in Table II. When the influence of selected dietary variables on oxidative stress and LPO related DNA damage was studied, an inverse statistically significant association was found between increased intake of fruit and vegetables and M₁dG adduct. The level of DNA damage was significantly lower, after adjusting for gender, age and smoking habit, in subjects that reported to consume 14 or more servings of fruit and vegetables per week in respect to the individuals with a low intake of fruit and vegetables [Mean Ratio (MR)=0.35, C.I. 0.18–0.69, $p < 0.05$]. No effect of fruit and vegetables on M₁dG was observed in petrochemical workers (MR=0.89, C.I. 0.57–1.38).

Then, the multivariate analysis shows the presence of a non-statistically significant increment of DNA damage in control individuals consuming fried food nine or more times per week, after adjusting for gender, age and smoking, in respect to the subjects reporting a low fried food intake (MR=1.33, C.I. 0.88–2.00, $p = 0.168$ vs. reference level). Whereas, no association of M₁dG adduct with fried food was found in MIE workers (MR=1.00, C.I. 0.66–1.50). The level of M₁dG adduct was not affected from charcoal grilled/barbecued food in Rayong controls and petrochemical workers (MR=0.87, C.I. 0.55–1.37, and MR=0.99, C.I. 0.64–1.54, respectively).

When smoking habit was considered, a statistical significant association between M₁dG adduct and smoking habit was observed in Rayong control group, after adjusting for gender, age and dietary habits. The level of DNA damage was statistically significantly increased in control smokers in respect to non smoker controls (MR=1.88, C.I. 1.14–3.10, $p < 0.05$ vs.

reference level). No effect of smoking on M₁dG was found in smoker MIE workers in respect to non smoker MIE workers (MR=0.90, C.I. 0.63–1.30, p=0.864 vs. reference level).

Discussion

Traditional Thai diet is currently attracting considerable interest because Thai vegetables commonly used for flavour and/or condiment have been shown to have antitumor and antimutagenic properties [26–28]. Antioxidative property of culinary herbs and spices used in Thai cooking such as lemon grass, kaffir lime, mints, and basil have been reported [29]. Thai fruits are also a good source of carotenoids and flavonoides [30]. However, during the past three decades, the food intake pattern of Thai population has changed considerably. Thai staples and side dishes are being replaced by diets containing higher proportion of fats and animal meat. The findings from two national surveys in 1991 and 1996 indicated that the problem of overweight and other risk factors for chronic diseases, including cancer, have increased significantly in Thailand [31].

In the present study, we analyzed the association between Thai diet and leukocyte M₁dG adduct, a biomarker of oxidative stress and LPO. A significant inverse association between increased intake of fruit and vegetables, the main dietary source of micronutrients with antioxidant property such as β -carotene, vitamin C and E, and the level of M₁dG adduct was found in controls.

Conversely, no association of diet with M₁dG was found in MIE workers. It is reasonable to assume that the antioxidant properties of a diet rich in fruit and vegetables was contrasted by the genotoxic effects of heavy air pollution exposure. Indeed, MIE workers can be exposed to complex mixtures of air pollutants, including nitrogen dioxide, propylene, ethylene, benzene, toluene, polycyclic aromatic hydrocarbons (PAH) and transition metals, characteristic of petrochemical installations and energy plants located in MTP [13].

A survey of 47 intervention studies of the ingestion of antioxidant containing foods or tablets of antioxidants indicates that about one-third of the selected studies reported a protective effect in terms of lower oxidative DNA damage [3]. Consumption of food rich in antioxidants has been associated with lower inflammation and oxidative stress [32]. Decreased M₁dG adduct has been found in colon-rectum mucosa of subjects reporting an increased intake of fruit and vegetables [10]. One of the possible mechanism by which the protective effect of diet rich in fruit and vegetables takes form, can be based on the antioxidant properties of fruit constituents. Vitamin A, C and E and phenolic compounds are effective free radical scavengers and protect against ROS generation [3]. Betacarotene has anti-oxidant properties towards carbon centered radicals, an important LPO intermediates [33]. Fruit constituents can increase the rates of DNA repair [1]. However, since we did not measure plasma antioxidant levels, we cannot demonstrate that the effects of these foods are really due to their antioxidant properties. Thereby, the hypothesis that higher intake of food containing antioxidants is associated to reduced level of leukocyte M₁dG adduct needs to be addressed from further investigations.

The relationship between fruit and vegetables and DNA damage has been already examined by our group in the Italian cohort of the European Prospective Investigation into Cancer and nutrition (EPIC) study and a significant inverse association between DNA adducts and fruit and vegetables was found [19,20]. In particular, the plasmatic concentrations of antioxidant vitamins, e.g. retinol, α - and γ -tocopherol, were inversely associated with DNA damage. A significant inverse relationship between DNA adducts and fruit and vegetables has been also found by us in a case-control study on bladder cancer [21]. Interestingly, in this study, the

association between case-control status and the level of DNA damage was stronger in individuals who were low vegetable consumers.

The next result shows a non-statistically significant increment of M₁dG adduct in frequent consumers of fried food among controls. Our finding is in keeping with a previous study showing that a significant increment of DNA damage was associated with fried food consumption in subjects resident in Czech Republic, Slovak Republic and Bulgaria [34]. Higher level of M₁dG adduct in frequent consumers of fried food can be due to the exposure to ROS produced during the metabolism of food mutagens, such as HCA, that arise from cooking food at high temperatures [6]. 1,3-Butadiene, benzene, acrolein, formaldehyde and other related compounds have also been detected in cooking oils heated at high temperature [35]. Some food mutagen derived metabolites, such as derived quinones, are reactive intermediated with the ability to redox-cycle, which is a reaction that generate ROS. Formaldehyde can induce oxidative stress and enhanced ROS production by different ways, including by the activation of oxidases and the inhibition of scavenger systems [15]. Furthermore, thermal stressing of cooking oils rich in linoleic acid (ω -6 PUFA, C18:2), such as soybean and sunflower oils, has been shown generating ROS and aldehydes [36].

The baseline level of M₁dG adduct of fried food consumers was lower in comparison to the other dietary groups, emphasising the possibility that fried food intake play a relevant role in influencing the background level of M₁dG adduct in Rayong controls.

Charcoal-grilled/barbecued food can contained high level of PAH and becomes, in this manner, an important source of dietary carcinogens [5]. Consumption of charcoal-grilled meat has been documented to increase the levels of PAH related DNA adducts [37]. However, there was no effect of frequent consumption of charcoal-grilled/barbecued food on the level of M₁dG adduct in the present study. The association between charcoal-grilled/barbecued food and M₁dG adduct level may have been confounded by air pollution exposure, smoking habit or other factors. A limitation of this study is that we have not collected information on other life-style factors, such as alcohol, tea and coffee consumption, that could be involved in the M₁dG adduct formation. For example, the production of M₁dG adduct has been associated with increased alcohol consumption in the laryngeal and colon mucosa [10,11]. Green tea has been also shown to decrease the levels of DNA damage by inducing cell grow arrest and apoptosis in heavy smokers [38].

Tobacco smoke contains a variety of carcinogens and ROS derived from tobacco pyrolysis products that can induce M₁dG adduct, therefore, we have studied the association between smoking and M₁dG adduct. A significant difference between the level of M₁dG adduct of smokers and non smokers was found among controls, but not in petrochemical workers. The association of M₁dG adduct with tobacco smoking has been already examined in different studies and increased amount of M₁dG adduct has been found in leukocytes, bronchial and laryngeal mucosa of smokers [11,12,15]. A recent study has indicated that cigarette smoking causes the elevations of urinary F₂-isoprostanes, an indicator of oxidative stress [39]. However, discrepant results were detected in colon-rectum and breast mucosa, for example [10,14].

Conclusions

Our study shows that M₁dG was significantly decreased in controls reporting to consume more than 14 servings of fruit and vegetables per week, whereas a non-statistically significant increment of the level of M₁dG was detected in controls consuming fried food more than nine times per week. Increased M₁dG can affect the nuclei of undifferentiated, proliferating stem cells inducing mutations and/or methylation changes within the promoter

regions of cancer related genes. Thus, appropriate dietary modification and promotion of healthy eating practices should be recommended.

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Abbreviations

CT	calf-thymus
HCA	heterocyclic aromatic amines
LPO	lipid peroxidation
MALDI-TOF-MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MDA	malondialdehyde
M₁dG	3-(2-deoxy-β-D-erythro-penta-furanosyl)pyrimido[1,2-α]purin-10(3H)-one deoxyguanosine
MR	Means Ratio
MIE	Map Ta Phut industrial estate
MR	Mean Ratio
MTP	Map Ta Phut
nn	normal nucleotides
PAH	polycyclic aromatic hydrocarbons
PNK	polynucleotide kinase
RAL	relative adduct labelling
ROS	reactive oxygen species

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Figure 1. Detection of malondialdehyde (MDA) DNA adducts after mass tagging by MALDI-TOF-MS in MDA-treated, NaBH₄-reduced calf thymus DNA. The exact masses are as follows: M₁dG (581.166); M₁dA (583.182); M₂dG (653.187); M₃dC (667.192); M₃dA (693.219).

Table 1

Characteristics of the study population and mean levels $\pm$ SE of malondialdehyde-2-deoxyguanosine adduct per 10⁸ normal nucleotides in Rayong controls and Map Ta Phut industrial estate (MIE) workers according to study variables.

	Rayong controls			MIE workers		
	n ^a	Mean $\pm$ SE	p-value	n ^a	Mean $\pm$ SE	p-value
Gender						
Male ^b	91	3.3 $\pm$ 0.3		58	7.2 $\pm$ 0.5	
Female	29	3.3 $\pm$ 0.5	0.324	9	6.9 $\pm$ 1.7	0.277
Age						
≤ 31 ^b	31	3.1 $\pm$ 0.4		30	7.4 $\pm$ 0.8	
32–38	37	3.2 $\pm$ 0.4	0.883	24	6.7 $\pm$ 0.4	0.957
≥ 39	52	3.5 $\pm$ 0.5	0.666	13	7.3 $\pm$ 1.8	0.851
Smoking habit						
Non smokers ^b	53	2.8 $\pm$ 0.3		17	7.7 $\pm$ 1.2	
Former smokers	3	3.6 $\pm$ 1.1	0.085	4	5.9 $\pm$ 1.3	0.745
Current smokers	64	3.8 $\pm$ 0.4	0.065	46	7.1 $\pm$ 0.6	0.798
Fried food						
Low intake (0–6 servings/week) ^b	44	2.6 $\pm$ 0.2		26	7.5 $\pm$ 0.7	
Average intake (6–8 servings/week)	38	3.5 $\pm$ 0.5	0.874	18	7.0 $\pm$ 0.1	0.603
High intake (9–18 servings/week)	36	4.2 $\pm$ 0.6	0.293	22	6.9 $\pm$ 1.1	0.591
Charcoal grilled/barbecued food						
Low intake (0–2 servings/week) ^b	48	3.5 $\pm$ 0.5		26	7.3 $\pm$ 0.1	
Average intake (3–5 servings/week)	46	3.1 $\pm$ 0.3	0.728	25	6.4 $\pm$ 0.7	0.971
High intake (6–12 servings/week)	25	3.6 $\pm$ 0.7	0.982	16	8.2 $\pm$ 0.8	0.575
Fruit and vegetables						
Low intake (0–8 servings/week) ^b	48	3.7 $\pm$ 0.5		27	7.4 $\pm$ 0.8	
Average intake (9–13 servings/week)	48	3.4 $\pm$ 0.4	0.403	29	7.3 $\pm$ 0.9	0.902
High intake (14–17 servings/week)	23	2.4 $\pm$ 0.4	<0.05	10	6.2 $\pm$ 0.9	0.979
MIE workers vs. Rayong controls ^b	120	3.4 $\pm$ 0.3		67	5.9 $\pm$ 0.5	<0.05

^a Some figures do not add up to the total because of missing values.

^b Reference level.

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Table II

Means ratio of malondialdehyde-2-deoxyguanosine adduct according to tertiles for fruit and vegetables, and fried and charcoal grilled/barbecued food in Rayong controls and Map Ta Phut industrial estate (MIE) workers. In the multivariate regression model, the effect of each variable (means ratio) is the ratio between the mean adducts of each levels of study variables with respect to the reference level, adjusted by gender, age and smoking habit.

	Rayong controls		MIE workers	
	Means Ratio	95% C.I. ^a	Means Ratio	95% C.I. ^a
Gender				
Male ^b	1	1		
Female	1.46	0.79–2.79	0.88	0.55–1.43
Age				
Per unit	1.01	0.98–1.04	1.01	0.99–1.04
Smoking habit				
Non smokers ^b	1	1		
Former smokers	2.10	0.58–7.68	0.72	0.36–1.44
Current smokers	1.88 ^c	1.14–3.10	0.90	0.63–1.30
Fried food				
Low intake (0–6 servings/week) ^b	1	1		
Average intake (6–8 servings/week)	1.11	0.74–1.68	0.78	0.52–1.16
High intake (9–18 servings/week)	1.33	0.88–2.00	1.00	0.66–1.50
Charcoal grilled/barbecued food				
Low intake (0–2 servings/week) ^b	1	1		
Average intake (3–5 servings/week)	0.91	0.62–1.33	0.88	0.61–1.29
High intake (6–12 servings/week)	0.87	0.55–1.37	0.99	0.64–1.54
Fruit and vegetables				
Low intake (0–8 servings/week) ^b	1	1		
Average intake (9–13 servings/week)	0.74	0.43–1.30	1.32	0.87–2.01
High intake (14–17 servings/week)	0.35 ^c	0.18–0.69	0.89	0.57–1.38

^aReference level.

^bConfidence Interval.

^cp-value < 0.05.