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Maternal diet and dioxin-like activity, bulky DNA adducts and micronuclei in mother–newborns

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ABSTRACT

Maternal diet can contribute to carcinogenic exposures and also modify effects of environmental exposures on maternal and fetal genetic stability.

In this study, associations between maternal diet and the levels of dioxin-like plasma activity, bulky DNA adducts in white blood cells and micronuclei (MN) in lymphocytes from mother to newborns were examined. From 98 pregnant women living in the greater area of Copenhagen, Denmark in 2006–2007, maternal peripheral blood and umbilical cord blood were collected, together with information on health, environmental exposure and lifestyle. Maternal diet was estimated on the basis of maternal food frequency questionnaire (FFQ) completed by the end of pregnancy. Biomarkers were detected in paired blood samples through the dioxin-responsive chemical-activated luciferase expression (CALUX)® bioassay, ^{32}P-postlabelling technique and cytokinesis-block MN assay.

Maternal preference for meats with dark surface were significantly associated with higher bulky DNA adducts in both maternal (β 95%CI; 0.46 (0.08, 0.84)) and cord blood (β 95%CI; 0.46 (0.05, 0.86)) before and after adjustment for potential confounders. No other significant associations between the 18 dietary variables and the biomarkers measured in maternal and fetal samples were identified.

The present study suggests that maternal intake of meats with dark surface contributes to the bulky DNA adduct levels in maternal and umbilical cord blood. Relationship between food preparation and bulky DNA adducts appear to be captured by a FFQ while potential associations for other biomarkers might be more complex or need larger sample size.

1. Introduction

Ingestion of contaminated food is considered to be a major route of exposure to several environmental human carcinogens, e.g. polycyclic aromatic hydrocarbons (PAHs), heavy metals, and persistent organic compounds (POPs) [1–3]. Preparation of foods by roasting, pan-frying, barbecuing, and baking, may result in formation of known mutagens, including benzo[a]pyrene (B[a]P) and other PAHs, heterocyclic amines (HCAs), acrylamides and their reactive metabolites. Other groups of potent mutagens, such as nitrosamines and DNA reactive aldehydes, may be formed by endogenous processes in certain foods [4]. Especially fish and food of animal origin can be a dietary source of persistent, lipophilic and widespread environmental pollutants including dioxin-like compounds, i.e. polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzo-*p*-furans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) [5].

Abbreviations: AhR, aryl hydrocarbon; B[a]P, benzo[a]pyrene; B[a]PDE, benzo[a]pyrene diolepoxide; BMI, body mass index; BN, binucleated cells; CALUX, chemical-activated luciferase expression; CBPI, cytokinesis-block proliferation index; Co-PCBs, coplanar polychlorinated biphenyls; DNBC, Danish National Birth Cohort; DR, dioxin-responsive; ETS, environmental tobacco smoke; FFQ, food frequency questionnaire; HCAs, heterocyclic amines; HUMN, human micronucleus; LOD, limit of detection; MN, micronuclei; nt, nucleotides; PAHs, polycyclic aromatic hydrocarbons; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzo-furans; PM, particulate matter; POPs, persistent organic compounds; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ, toxic equivalency quantity; WBCs, white blood cells.

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Research on diet and cancer-related effects has so far focused mainly on adult populations; however, increased vulnerability towards in utero carcinogenic exposures has been proposed due to a high rate of cell proliferation, relatively high numbers of target cells at risk, altered ability to repair DNA damage, immaturity of metabolism, endocrine immunological systems and longer life span ahead in which to develop chronic disease as compared with adults [6,7]. Thus, studies based on cancer-related biomarkers of exposure and effect in umbilical cord blood have been initiated to investigate the effects of in utero exposures related to maternal exposures [8]. Measurements in fetal tissues have demonstrated in utero exposure, as a result of maternal exposure, to a range of diet related compounds, including PAHs, POPs, heavy metals and aflatoxins, with cancer contributory potential [9–14].

Human ex vivo placenta perfusion studies have indicated transplacental transfer of B[a]P, HCAs, aflatoxin B1 and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [14–18]. Bulky DNA adduct levels, which reflect genotoxic exposures upon individual absorption of e.g. PAHs, HCAs, metabolism and DNA interaction, have been shown to be at similar [19,20] or lower [21] levels in umbilical cord blood compared to maternal peripheral blood levels. The detectable adduct levels and the significant positive correlations between maternal and fetal adduct levels show that genotoxic exposures can cross the human placenta. Micronuclei (MN), which are formed as a result of chromosome breakage or loss, and reflect genetic instability have also been detected in umbilical cord blood, although at lower frequencies than in adults, e.g. [19,22].

Bulky DNA adduct and MN levels measured in white blood cells (WBCs) and lymphocytes are considered as two of the most relevant biomarkers in respect to exposure to complex mixtures including genotoxic exposure and human cancer risk [23,24]. We have previously reported significantly elevated bulky DNA adducts and MN frequencies in newborns born by mothers who lived in areas characterized by higher air pollution from traffic as compared with those exposed to lower levels of traffic-related air pollution [19]. A significant positive correlation between dioxin-like activity in plasma and MN frequency in lymphocytes from newborns was also observed [14]. There are gaps in the existing research on predictors of these biomarkers, and it is hypothesized that maternal dietary habits may contribute to carcinogenic exposures and also modify effects of environmental exposures on maternal and fetal genetic stability.

The objective of the present study was to examine associations between maternal diet and the levels of dioxin-like activity, bulky DNA adduct and MN levels in the cross-sectional sample of mother-newborns where the previous associations were found [14,19]. Concisely, a selection of dietary variables, which included eight food groups (e.g. fish, fruits and vegetables), four macronutrients (e.g. fats), total energy, degree of meat surface darkness and consumption of supplements, fish oil, coffee and alcohol, were tested for their associations with the maternal and cord blood biomarker levels.

2. Materials and methods

2.1. Study design

A cross-sectional design as previously described was used [19]. Eligible pregnant women with singleton deliveries by planned Caesarean section at the University Hospital of Copenhagen from December 2006 to December 2007 were enrolled in the study after written informed consent. Information on maternal characteristics, e.g. health, reproductions, ethnicity, education and occupation of the parents, maternal living, working and studying environment, residence, lifestyle, pregnancy related lifestyle changes and dietary habits was obtained from a maternal questionnaire (~90 min for self-administration). The Ethics Committee of the Capital Region of Denmark and the Danish Data Protection Agency reviewed and approved the study prior to initiation (J. Nos. H-KF-01-327603; and 2007-41-0415).

A total of 98 mother–newborn pairs living in the Greater Copenhagen area, Denmark, participated in the present study. Copenhagen city has ~500,000 inhabitants, it is the most densely populated area in Denmark and located near the coast in eastern part of Zealand. In 2007 the Copenhagen annual averages of particulate matter with an aerodynamic diameter below 10 μm (PM_{10}) were 24 and 38 $\mu g/m^3$ at the urban background and urban traffic station, respectively [25]. Annual 2007 average of the $PM_{2.5}$ was 23 $\mu g/m^3$ and benzo[a]pyrene was not exceeding 1 ng/m^3 during 2007.

2.2. Dietary assessments

A detailed questionnaire was combined from existing validated questionnaires used in birth cohorts in Denmark, Norway, United Kingdom and Spain [26–29]. The food frequency questionnaire (FFQ) used in the Danish National Birth Cohort (DNBC) in 1996–2002 [30] was used for the section on diet, but several new food items, e.g. sushi, English breakfast, root fruits, pineapples and semi-skimmed milk, expected to be consumed by pregnant women living in the Greater Copenhagen area in 2006–2007, were included. The FFQ contained 431 food and drink items.

The full questionnaire provided information about maternal diet during the last month of pregnancy, supplements, pregnancy related nausea, lack of appetite and vomiting, and general dietary habits, e.g. preparations of foods, means of preparation, vegetarian food and organic food, during and before pregnancy.

Individual responses on the frequency of each food item intake were quantified into grams per day using assumptions on recipes and standard portion sizes [31]. The estimated individual daily intakes of each food item (g/day) were combined with information on energy and nutrients from the Danish food composition table, version 6 [32] for quantification of intakes of energy, macronutrients and micronutrients using the software FoodCalc, version 1.3 [33]. Related food items were aggregated into groups of food.

Blackness of meats was classified as high if the option dark (out of the three response options: light, golden and dark) was chosen more than once in response to the three photo series illustrating this topic with respect to steak, chicken and pork, respectively.

The collected information on dietary habits from five participants was incomplete and not included in the statistical analysis. Blackness of meats was classified as high if the option dark (out of the three response options: light, golden and dark) was chosen more than once in response to the three photo series illustrating this topic with respect to steak, chicken and pork, respectively.

2.3. Blood collection

Maternal peripheral blood samples (~50 mL) were drawn from 98 pregnant women by venipuncture 1–2 h before planned section between 7 and 11 a.m. The women had been fasting since the midnight before delivery. Umbilical cord blood (0.5–80.0 mL) was drawn by umbilical puncture from 96 placentas at the hospital immediately after delivery. All blood samples were collected into heparinized tubes (Vacutainer, Becton Dickinson, Oxford, UK) and paired mother–newborn samples were processed within <1–5 h of each other.

2.4. Biomarker measurements

Dioxin-like plasma activity was determined using the dioxin-responsive (DR) chemical-activated luciferase expression (CALUX)® bioassay (BioDetection System, The Netherlands) as previously described [14]. Plasma samples (~3 mL) were separated (650 g, 10 min) and stored on −20 °C until analysis. The volume of plasma for the CALUX bioassay was sufficient from 74% of the newborns. Upon *n*-hexane liquid/liquid extraction of plasma lipid, the acid-labile matrix components of extractable lipid were removed by passage through an acid silica column topped with sodium sulfate. The oxidized fat and chemically instable aryl hydrocarbon (AhR) ligands, e.g. PAHs, other combustion products, flavonoids, indole-3-carbinol or endogenous compounds were hereby removed. Cleaned lipid extracts were evaporated and redissolved in DMSO. The dioxin-like plasma activity of the extracts was determined after 24 h exposure of the DR CALUX® cells. The lipid content of the plasma was determined gravimetrically.

Bulky DNA adduct levels were detected using the ^{32}P postlabelling technique with *n*-butanol enrichment [34]. DNA was isolated from whole blood samples (0.5 mL) from all participants enrolled after March 2007. The definable adduct spots were measured by phosphorimage analysis and the B[a]P diolepoxide (B[a]PDE) adduct was used as external standard (Fig. 1). A positive and a negative control were included in all runs so as to correct for day-to-day variation in the assay. Bulky DNA adduct levels in WBCs were quantified as the average of two independent assays and expressed as adducts per 10^8 nucleotides ($n/10^8$ nt). Limit of detection (LOD) was 0.1 $n/10^8$ nt.

MN frequencies were determined using the cytokinesis-block micronucleus assay [34] with some modifications [35]. Paired mother–newborn whole blood cultures, in duplex, were initiated simultaneously on average within 6 h from collection. After 44 h cytochalasin B was added. At 72 h after phytohaemagglutinin stimulation, cells were harvested and fixed. The cell suspensions were dropped onto clean labeled slides, air-dried, and after 48 h slides were stained in freshly filtered 5% Giemsa (Sigma–Aldrich, Denmark) for 20 min (pH 6.8). All slides were coded and randomized at the end of enrolment. Evaluation of ~2000 binucleated cells per donor was carried out by the same person using a light microscope (Olympus BX 41) at 400×

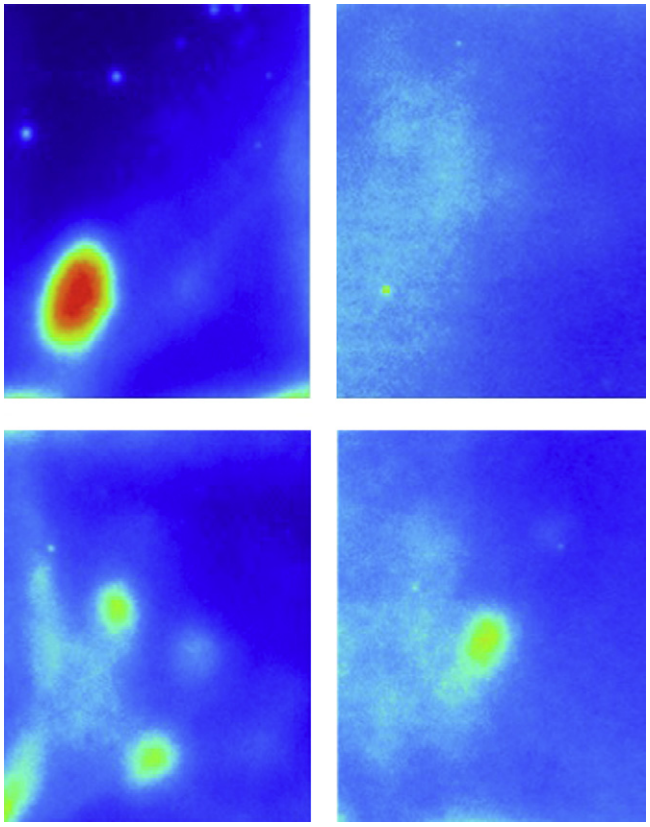

Fig. 1. Bulky DNA adduct spots measured by phosphorimage analysis. At the top, left: positive control (modification of calf thymus DNA with benzo(a)pyrene diolepoxide, i.e.TNO_3); top, right: negative control; bottom, left: maternal DNA and bottom, right: fetal DNA. Photographs by D.A. Dang, 2008.

magnification following the human micronucleus (HUMN) project's criteria for scoring [36] and the MN frequency per 1000 binucleated cells (MNBN‰) was calculated. MN slides were prepared from 98 maternal and 93 cord blood samples (three cord blood samples were lost due to logistic by the beginning of the study). Out of these, the morphology of BN cells on 76% maternal and 62% fetal slides full-filled the quality criteria and allowed for scoring of MN.

2.5. Statistical analyses

Results were presented as 5%, 25%, 50%, 75% and 95% percentiles. Non-parametric Wilcoxon Signed Rank tests (2-tailed) were used for the comparison between groups, e.g. maternal and cord blood biomarker levels. One maternal and nineteen cord blood samples with dioxin-like activities below the LOD were assigned a value equal to 0.5 × LOD prior to analyses for associations.

Linear regression models were used to examine the diet–biomarker relationships. Dietary variables were entered separately in each model. Dietary variables on continuous scale were entered as continuous variables or categorized using quartiles derived from the distribution across the entire sample to examined eventual non-linear relationships.

Both untransformed and log-transformed dioxin-like activity and bulky DNA adduct levels had skewed distributions. Robust linear regression models were therefore also applied to minimize the influence of outliers in associations of estimated dietary intakes with the dioxin-like activity and bulky DNA adduct levels (*results not shown*). The average MN frequencies were reduced to the nearest integer when used as dependent variable in negative binomial regression models for investigation of associations.

A priori potential confounding factors, i.e. maternal age, pre-pregnancy BMI, parity, previous breast feeding, gestational age, plasma lipid, maternal smoking habits, maternal exposure to environmental tobacco smoke, estimated residential traffic-density, level of education as well as characteristics identified in the univariate regression analyses to be associated with the biomarkers, i.e. *p*-values below 0.20, were included as covariates for the multiple regression analyses of associations between dietary predictors and the measured biomarkers (*not all results shown here*). Differences between groups were considered to be statistically significant if the *p*-value was below 0.05.

Main analyses were performed for the non-smoking study population ($n = 92$).

The statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Table 1
Maternal characteristics.[a]

Age at delivery (years), $n = 98$	33 (21–43)
Maternal ethnicity (Caucasian, other), $n = 97$	90/7
Pre-pregnancy BMI (kg/m^2), $n = 95$	22.2 (17.3–38.7)
General health (good or normal, poor), $n = 94$	92/2
Parity (0/1 +), $n = 92$	17/75
Previous breast feeding (months), $n = 78$	9 (0–48)
Marital status (married or cohabiting, other), $n = 92$	90/2
Maternal education (high, low)[b], $n = 95$	71/24
Occupation (employed, student, unemployed)[c], $n = 93$	72/18/8
Smoking status (never, ex, current), $n = 96$	41/49/6
Exposure to passive smoke (none, some)[d], $n = 95$	57/38
Residential traffic density (vehicles km/24 h)[e], $n = 86$	978 (48–17,710)
Season of birth (spring, summer, autumn, winter)[f], $n = 98$	26/32/16/24
Supplement intake (some, none)[g], $n = 94$	82/12
Fish oil consumption (none, some), $n = 78$	66/12
Blackness of meats (light or golden, dark)[h], $n = 90$	38/52
Alcohol consumption (none, some), $n = 93$	47/46
Coffee consumption (<a cup/>a cup per day), $n = 92$	54/38

[a] All characteristics were obtained using information from the end-pregnancy questionnaire,
[b] Education for 4 years above secondary school (~bachelor levels or more) was classified as high.
[c] Participants who were students were in some cases also in work.
[d] At home, at work or elsewhere.
[e] Estimated traffic density within 100 m radius from the home.
[f] Spring refers to March–May, summer refers to June–August, autumn refers to September–November and winter refers to December–February.
[g] Some includes any intake of regular multivitamins and minerals, pregnancy formulation, iron, folic acid, calcium, vitamin C, vitamin E, vitamin B6, selenium, zinc, antioxidants and others supplements, e.g. Kräuterblut.
[h] Preferred degree of surface blackness of steak, chicken and pork.

3. Results

3.1. Study population

Healthy, in the early-30 s, Caucasian women ($N = 98$) with singleton planned Cesarean section participated (Table 1). All, except six, women were non-smoking throughout the pregnancy. Women reporting ex-smoking ($n = 49$), quit their active smoking or party smoking in average 6.5 years prior to their pregnancy. Current active smokers ($n = 6$) smoked 20 (6.5–30) cigarettes per day and have been smoking for 23 (16–26) years while ex-smokers smoked 8 (0–30) cigarettes per day have been smoking cigarettes for ~10 (<1–26) years (*median* (*min–max*)). More than half of the women report no exposure to ETS ($n = 57$) and frequent or residential exposure to ETS was not common as only non-smoking women living in homes without smoking were included in the full study which included monitoring of the indoor air and dust in the homes five days prior to delivery [19]. The women had on average given birth to one previous child. Maternal median weight gain during pregnancy was 14 kg, ranging from 2 to 25 kg. Half of the women ($n = 51$) had a pre-pregnancy body-mass index (BMI) within 20–25, whereas 26 women had BMIs above 25 ($n = 26$) 18 women BMI below 20. Some women ($n = 33$) were frequently doing exercise while others ($n = 62$) performed no-exercise during pregnancy. Pregnancy-related changes ($n = 45$) in type and amount of exercise practiced during pregnancy was as common as no changes among the participating women ($n = 42$).

In average 3 daily meals plus morning, afternoon and evening snacks 5–6 times a week were consumed (Table 2). None of the participants in this study could be classified as vegetarians. Multivitamins, iron and folic acid were the dietary supplements most commonly consumed. Some women ($n = 24$) had frequent or exclusive intake of organic foods. There was a common preference for organic milk ($n = 72$) and eggs ($n = 65$).

Pan frying was a common method for preparation of meats ($n = 45$) and fish ($n = 34$) while roasting in oven or boiling was

Table 2
Estimated maternal dietary intakes.[a]

	Percentiles				
	5%	25%	50%	75%	95%
Fruits (g/day)[b]	104	257	379	588	1104
Vegetables (g/day)[c]	183	341	478	1139	2044
Potatoes (g/day)	52	197	243	342	384
Fish (g/day)[d]	1	16	32	51	103
Red meats (g/day)[e]	47	76	109	141	234
Poultry (g/day)[f]	8	24	40	68	138
Dairy products (g/day)[g]	112	291	555	784	1632
Eggs (g/day)	8	12	16	21	37
Breads and cereals (g/day)[h]	141	250	306	355	462
Fats (g/day)[i]	58	73	90	113	170
Proteins (g/day)[i]	80	101	121	148	185
Carbohydrates (g/day)[i]	284	377	429	542	717
Fibres (g/day)[i]	30	37	46	61	95
Energy (KJ/day)[i]	8604	10,876	12,644	14,843	20,017

[a] Estimated on the basis of information from the end-pregnancy food frequency questionnaire.
[b] Apples, pears, bananas, pineapples, berries, melons, kiwi, citrus, stone, tropical, nuts and dried fruits.
[c] Lettuces, cabbages, broccoli, carrot, root fruits, leak, onion, zucchini, beans, corn, peas, asparagus, celery, tomatoes, cucumber, bell pepper, avocado, egg plant, mushrooms, soy beans, garlic and olives.
[d] All fish and shellfish.
[e] Beef, veal, pork, lamb, liver paste and plucks.
[f] Chicken, turkey, duck and goose.
[g] Skimmed, semi-skimmed, whole milk products, buttermilk, cheeses, cream, yoghurt and ice creams.
[h] Breads, cereals, rice, pasta and bulgur.
[i] Estimated from the sum of all food and drink items.

as commonly or more frequently used for preparation of meats ($n = 49$) and fish ($n = 59$). Barbecuing of meats ($n = 69$), fish ($n = 84$), vegetables ($n = 81$) was very rarely used, monthly used ($n = 18$, $n = 8$, $n = 9$) or used each week ($n = 6$, $n = 0$, $n = 2$). Similar low frequencies of the use of deep frying and microwave ovens were reported. Maternal intakes of coffee, alcohol and to a smaller extent also raw fish, meats, tuna or salmon intakes were lower than before pregnancy. Increased intake of dairy products during pregnancy was common. A few participants reported higher intakes of candy, fruits, fats, cereals, meats and fish due to pregnancy. The majority reported, however, to have similar intakes as before pregnancy.

Preference of meats with dark surface did not significantly associate with any of the dietary variables investigated. Women with no supplement intake ate less fish than women with supplement intake (24 versus 48 g/day, $p = 0.04$). Women with fish oil intake had a higher intake of vegetables than those with no-fish oil intake (918 versus 711 g/day, $p = 0.04$). Higher intake of vegetable was associated with higher of red meat, poultry, fats, proteins, carbohydrates, fibres and energy intake. Higher intake of red meat associated with higher fat intake, proteins, carbohydrates, fibres and energy. Higher intake of poultry associated with higher intake of fats, proteins and energy. Higher intake of dairy products associated with higher protein and energy.

3.2. Biomarkers

Dioxin-like activities above LOD were detected in plasma from 97% of the mothers and in 52% of the newborns, respectively (Table 3). Maternal and cord blood plasma dioxin-like activities were at similar levels (*median:* 37 versus 33 pg CALUX®-toxic equivalency quantity (TEQ)/g lipid, $p > 0.05$) (14). Bulky DNA adducts were detected in all samples, and the levels were similar in maternal DNA and cord blood DNA (*median:* 1.40 versus 1.37 n/10^8 nt; $p < 0.05$) (19). MN frequencies in the mothers ranged 2.50–16.50 MNBN‰, in percentage 47% of the cells was BN, and the cytokinesis-block proliferation index (CBPI) ranged 1.6–2.8 in the present study. MN frequencies in cord blood were lower than in their mothers (*median:* 3.22 versus 7.00 MNBN‰, $p < 0.001$). In newborns MN frequencies ranged 0–9.20 MNBN‰, 59% of the cells was BN, and cord blood CBPI ranged 1.8–2.6.

A significant positive correlation between maternal and cord blood bulky DNA adduct levels in WBCs (*Spearman Rank Correlations:* Rs = 0.9, $p < 0.01$; β(95% CI): 0.98 (0.96, 1.01) and a modest positive correlation between maternal and cord blood dioxin-like plasma activity levels were observed (*Spearman Rank Correlations:* Rs = 0.4, $p = 0.02$; β(95%CI); 0.16 (0.04, 0.27)), whereas no significant association was found between maternal and cord blood MN frequency in binucleated lymphocytes (*Spearman Rank Correlations:* Rs < 0.1, $p = 0.7$; β(95%CI); 0.01 (−0.04, 0.05)). A positive association between dioxin-like activity in plasma and MN in cord blood lymphocytes was found as previously reported [14], whereas no other significant associations between the bulky DNA adduct in WBCs and MN in maternal and umbilical blood lymphocytes were observed [19]. The associations between dioxin-like activity in plasma and bulky DNA adducts in WBCs were non-significant for the mothers (β (95%CI); 2.64 (−1.43, 6.71)) and their newborns (β (95%CI); 8.62 (−4.78, 22.02)).

3.3. Maternal diet and their associations with the biomarkers

Positive significant associations between maternal preference of darkly fried steak, chicken and pork and elevated bulky DNA adducts in both maternal and cord blood were found (Table 3). Hence, both maternal and fetal levels were significantly higher among consumers of darkly fried meat as compared with those who consumed lightly cooked meats (*median maternal and fetal levels;* 1.54 and 1.51 versus 1.27 and 1.16 n/10^8 nt, respectively). The differences were also statistically significant ($p = 0.041$ and $p = 0.042$) after excluding the smokers (*median maternal and fetal levels;* 1.52 and 1.51 versus 1.19 and 1.13 n/10^8 nt, respectively).

The majority of women took supplement before and/or during pregnancy (*some;* $n = 82$ versus *none;* $n = 12$). A significant association between intake of supplements and lower maternal levels of bulky DNA adducts was observed, while the association between maternal supplement intake and cord blood bulky DNA adducts was non-significant.

Table 3
Dioxin-like plasma activity, bulky DNA adducts and micronuclei levels in mother-newborns and their univariate associations with estimated maternal dietary habits from non-smokers ($n = 92$).

	Dioxin-like plasma activity[a]		Bulky DNA adducts[b]		Micronuclei frequency[c]	
	Mothers ($n = 92$)	Newborns ($n = 67$)	Mothers ($n = 70$)	Newborns ($n = 65$)	Mothers ($n = 69$)	Newborns ($n = 54$)
Median (95%CI)	38 (19–78)	33 (17–107)	1.36 (0.42–3.09)	1.34 (0.43–3.17)	6.97 (2.89–13.73)	3.16 (0.00–7.12)
Regression estimates (β) and the 95% CI for categorical parameters and highest versus lowest quartile for continues parameters						
Blackness[d]	2.4 (−6.4 to 11.3)	12.0 (−4.2 to 28.1)	0.4 (0.1 to 0.8)*	0.4 (0.0 to 0.8)*	−0.2 (−0.4 to 0.0)	0.3 (−0.0 to 0.7)
Supplements[e]	−8.8 (−21.7 to 4.1)	18.7 (−5.0 to 42.4)	−0.4 (−1.0 to 0.3)	−0.4 (−1.1 to 0.3)	−0.1 (−0.4 to 0.3)	0.4 (0.3 to 1.0)
Fruits	−1.0 (−13.2 to 11.2)	13.4 (−9.3 to 36.2)	0.3 (−0.3 to 0.8)	0.3 (−0.3 to 0.9)	0.3 (0.0 to 0.6)*	0.1 (−0.4 to 0.6)
Vegetables	6.1 (−6.0 to 18.3)	−16.2 (−38.1 to 5.7)	0.8 (−0.8 to 2.4)	0.8 (−0.9 to 2.4)	0.1 (−0.2 to 0.4)	−0.3 (−0.8 to 0.3)
Fish	−1.6 (−13.7 to 10.6)	−4.3 (−26.0 to 17.5)	−0.5 (−1.0 to 0.0)	−0.5 (−1.6 to 0.1)	−0.1 (−0.4 to 0.2)	0.1 (−0.4 to 0.6)
Fish oil[f]	6.5 (−5.1 to 18.0)	−2.6 (−31.3 to 26.1)	−0.4 (−1.0 to 0.2)	−0.4 (−1.0 to 0.2)	0.1 (−0.2 to 0.4)	0.5 (0.0 to 0.9)*
Red meats	−4.6 (−16.8 to 7.6)	−11.3 (−33.9 to 11.2)	0.1 (−0.4 to 0.7)	0.2 (−0.4 to 0.7)	0.1 (−0.2 to 0.4)	−0.1 (−0.6 to 0.4)
Poultry	2.6 (−9.4 to 14.7)	−3.6 (−25.1 to 17.9)	0.1 (−0.5 to 0.7)	0.0 (−0.5 to 0.7)	0.1 (−0.2 to 0.4)	−0.1 (−0.5 to 0.3)
Dairy products	6.8 (−5.1 to 18.6)	12.4 (−9.3 to 34.2)	0.1 (−0.6 to 0.6)	0.0 (−0.6 to 0.6)	−0.1 (−0.4 to 0.2)	0.2 (−0.3 to 0.7)
Eggs	−8.3 (−20.4 to 3.7)	−2.0 (−25.6 to 21.6)	0.1 (−0.5 to 0.7)	0.1 (−0.5 to 0.8)	0.0 (−0.3 to 0.4)	−0.0 (−0.5 to 0.5)
Bread and cereals	−4.0 (−16.2 to 8.1)	8.5 (−13.9 to 30.9)	0.3 (−0.2 to 0.9)	0.4 (−0.1 to 1.0)	−0.1 (−0.3 to 0.2)	0.2 (−0.3 to 0.6)
Alcohol[g]	2.7 (−5.9 to 11.3)	7.0 (−8.9 to 22.8)	0.3 (−0.1 to 0.6)	0.2 (−0.2 to 0.6)	0.0 (−0.2 to 0.2)	−0.1 (−0.4 to 0.3)
Coffee[h]	−9.1 (−17.7 to −0.4)*	6.8 (−9.0 to 22.6)	0.2 (−0.2 to 0.6)	0.2 (−0.3 to 0.6)	0.2 (−0.0 to 0.4)	0.3 (−0.1 to 0.6)
Fats	−7.9 (−20.0 to 4.2)	11.5 (−9.3 to 32.3)	0.3 (−0.2 to 0.8)	0.4 (−0.1 to 0.9)	−0.1 (−0.4 to 0.2)	0. 0 (−0.5 to 0.5)
Proteins	1.8 (−10.3 to 13.9)	−9.1 (−14.6 to 32.7)	0.0 (−0.5 to 0.6)	0.1 (−0.5 to 0.7)	0.1 (−0.1 to 0.4)	0.1 (−0.4 to 0.5)
Carbohydrates	6.6 (−5.6 to 18.7)	12.1 (−11.0 to 35.2)	0.6 (0.7 to 1.2)*	0.6 (0.7 to 1.2)*	−0.1 (−0.2,0.1)	0.1 (−0.3 to 0.6)
Fibres	11.9 (−0.1 to 23.8)	−2.9 (−25.5 to 19.8)	0.1 (−0.6 to 0.9)	0.3 (−0.6 to 0.9)	−0.0 (−0.4 to 0.2)	0.1 (−0.4 to 0.7)
Energy	3.5 (−8.6 to 15.6)	16.5 (−6.1 to 39.0)	0.3 (−0.3 to 0.9)	0.4 (−0.2 to 1.0)	0.2 (−0.1 to 0.5)	0.1 (−0.4 to 0.5)

* $p < 0.05$.
[a] Linear regression model with dioxin-like plasma activity per gram lipid (pg CALUX®-TEQ/g lipid) as dependent variable.
[b] Linear regression model with bulky DNA adducts per 10^8 nucleotides ($n/10^8$ nt) as dependent variable.
[c] Negative binomial regression model with rounded MN frequencies per 1000 binucleated cells (MNBN ‰) as dependent variable.
[d] Degree of preferred blackness (light or golden, dark) of steak, chicken and pork.
[e] Intake of supplements (none, some) during pregnancy.
[f] Fish oil intake during pregnancy (none, some).
[g] Alcohol consumption during last month of pregnancy (none, some).
[h] Coffee consumption during last month of pregnancy (<a cup, ≥a cup or more per day).

To avoid potential effects related to maternal active smoking multiple regression analysis restricted to non-smokers were performed (Table 4). Surface darkness of meat, maternal exposure to ETS, estimated residential traffic density and maternal education levels associated with both maternal and cord blood bulky DNA adduct levels ($p < 0.20$) in the multivariate models, while the protective effect estimates of supplements intake on the adduct levels were less significant ($p > 0.20$) in the further adjusted models.

The effect estimates of maternal preference of meats with dark surface associated with higher bulky DNA adduct levels remained significant after additional adjustment for maternal pre-pregnancy BMI, maternal age and alcohol intake and birth weight for the cord blood model (*results not shown*). Additional adjustment of for example fruits and vegetable intake as proxy of dietary antioxidants did not modify the associations between preferred meats with dark surface and bulky DNA adduct levels (*Coef. (95%CIs) for maternal and cord blood bulky DNA adduct levels:* 0.46 (0.07–0.85) and 0.46 (0.05–0.88)).

One MN more per 1000 BN was observed in newborns born by mothers who preferred darkly fried meat surfaces (*medians:* 4 versus 3 MNBN‰; $p = 0.7$), whereas the maternal median MN frequency was similar in the dichotomized groups.

In the present study evaluation of the potential effects of fish oil is limited by the small number of participants with reported fish oil intake (*some;* $n = 12$ versus *none;* $n = 66$), however, an increased MN frequency was observed in newborns born by women who reported some intake of fish oil (*medians*; 4 versus 2.5 MNBN‰; $p = 0.05$). The

Table 4
Adjusted associations between maternal intake of cooked meat and the bulky DNA adduct levels ($n/10^8$ nt) in maternal and cord blood collected from non-smokers.

	β^*	(95% CIs)	p-value
Model for maternal levels			
Degree of preferred blackness of meats[a]	0.46	(0.08 to 0.83)	0.02
Supplements[b]	−0.25	(−0.84 to 0.34)	0.40
Environmental tobacco smoke exposure[c]	0.22	(−0.17 to 0.61)	0.26
Residential traffic density exposure[d]	0.39	(−0.01 to 0.79)	0.05
Maternal education level[e]	0.43	(−0.07 to 0.93)	0.09
Model for cord blood levels			
Degree of preferred blackness of meats	0.46	(0.05 to 0.86)	0.03
Supplements	−0.28	(−0.92 to 0.36)	0.38
Environmental tobacco smoke exposure	0.18	(−0.22 to 0.59)	0.38
Residential traffic density exposure	0.37	(−0.05 to 0.78)	0.08
Maternal education level	0.50	(−0.07 to 1.07)	0.08

* Effect estimates from linear regression models for each of the personal characteristics included the multivariable models.
[a] Degree of preferred blackness (light or golden, dark) of steak, chicken and pork.
[b] Intake of supplements (none, some) during pregnancy.
[c] At home, at work or elsewhere.
[d] Estimated traffic density within 100 m radius from the home (<400 vehicle km/24 h, >400 vehicle km/24 h).
[e] Education (>4 years above secondary school, <4 years above secondary school).

results were similar after exclusion of smokers (*medians*; 3.9 versus 2.5 MNBN‰; $p = 0.039$).

Higher dioxin-like plasma activities were observed in mother-newborn samples from those who preferred well-done meat as compared with the other group, but not at significant levels (*medians:* 37 and 51 versus 33 and 25 pg CALUX®-TEQ/g; $p = 0.3$ and $p = 0.4$, respectively).

Dioxin-like activities were similar in plasma from the subset of 12 mother–newborns who reported some fish oil intake as compared to those who did not report fish oil intake (*medians*: 42 and 48 versus 35 and 31 pg CALUX®-TEQ/g; $p = 0.4$ and $p = 0.5$). No significant associations between fish or fatty fish, i.e. salmon, herring, mackerel, eel, trout, and Greenland halibut, and dioxin-like plasma activity were observed (*results not shown*). A significant inverse association between frequent coffee intake and maternal dioxin-like activity was observed.

No other significant associations between maternal dietary habits and the biomarkers were found.

4. Discussion

Our study indicates that maternal intake of fried meats with dark surface contributes to the bulky DNA adduct levels in maternal and umbilical cord blood. The association was significant in univariate and multiple regression analyses. Intake of supplements was associated with lower maternal bulky DNA adducts levels. No other significant associations between the dietary variables included in the multiple linear regression models and the biomarkers measured in maternal and cord blood samples were identified. The significant associations between frequent consumption of coffee and lower maternal dioxin-like plasma activity; intake of supplements and lower maternal bulky DNA adducts as well as between intake of fish oil and higher cord blood MN frequency could be potential chance findings as no obvious biological mechanisms as far as we are aware can explain the associations.

The strengths of this study include the measurements of dioxin-like activity, bulky DNA adducts and micronuclei frequencies, i.e. two well-established cancer-related biomarkers, in both cord blood and maternal blood, the ability to estimate maternal dietary intake on the basis of a validated FFQ and to control for potential confounding by maternal education and effects related to the traffic density within 100 m radii of the participants' homes. The limitations of the present study were related to the risk of chance findings as multiple testing were conducted, potential misclassification of the maternal diet and the relative small size of the study population.

To our best knowledge no previous studies have investigated possible associations between maternal diet estimated on the basis of FFQs and bulky DNA adducts and MN detected in cord blood. FFQs which can relatively easily be used in population studies to provide comprehensive information on diet are considered to be among the best methods available for assessment of dietary habits. However, estimation of dietary habits on the basis of FFQs suffers from uncertainty due to e.g. recall bias, assumptions made on food composition, recipies, portion sizes, and toxicokinetics which may result in misclassification. Additionally, the range of food items and the period covered in FFQs can be a limiting factor for evaluation of associations with effects induced by accumulated exposures during time periods preceding the period covered by the FFQs.

Information on active and passive tobacco smoke exposure were obtained from medical records, questionnaire and time-activity diaries. The validity and accuracy of such self-reported tobacco exposure, especially exposure to ETS, has been questioned because of a common belief that smoking pregnant women would underestimate the amount smoked or deny smoking at all the and the limitations of questionnaire related to e.g. the amount of ETS exposure and recall bias. In a Swedish study found that validity of self-reported active smoking during pregnancy was high according to comparisons with cotinine measurements [37] and in terms of exposure to ETS by the end the pregnancy 8% was misclassified as non-exposed instead of exposed.

In the present study effects of smoking can not be evaluated due to the small numbers of smokers participating. It was, however, observed that the six mothers who smoked during pregnancy and at time of blood collection differed in terms of being four years older as compared with the median of the study population ($p = 0.01$) and by having a significantly lower estimated intake of fruits and vegetables than the non-smoking mothers ($p = 0.03$). These variations and potential not observed variations may, in addition to their habit of smoking, have contributed to the levels of bulky DNA adducts and MN frequencies observed in these six mother–newborn pairs [19], and main statistical analyses were performed with and without the smokers. Due to the short half-life of cotinine in plasma it would had been difficult to interpret levels at the time of birth (with almost certain no-exposure for the preceding days).

The significant associations between maternal intake of fried meats with a high degree of surface darkness and bulky DNA adduct in both maternal and fetal WBCs found in the present study could be attributable to genotoxic compounds, e.g. HCAs and PAHs, formed and adhered to the meat during cooking at high temperatures, such as pan-frying, and fat dripping on hot surfaces in higher degrees to the meats with high degree of surface darkness compared to meats with lighter surfaces [4]. In consistency with our findings, intake of darkly fried meats and fish have been associated with high-bulky DNA adduct WBC levels, at non-significant levels, in a Danish middle aged population ($N = 375$) in which the adducts were detected by the same laboratory as in the present study [38]. Significantly higher bulky DNA adduct levels in female breast tissue ($N = 44$) have also been positively correlated with higher intake of fried meat [39]. A positive significant correlation between the total estimated HCA intakes and bulky DNA adducts was also found in that study of German women, although the authors concluded that because the adducts were not specific only for HCAs other genotoxic exposures contribute to the detected adduct levels.

The ^{32}P-postlabelling technique used in the present study detects a broad range of adducts, whilst other studies have used more PAH specific assays. Analogously to the present study, positive significant associations between high frequency of charbroiled food intake and high PAH-DNA adducts and anti-B[a]PDE-adducts, respectively, in WBCs have been described in Californian firefighters ($N = 47$) and an Italian adult population ($N = 291$) [40,41].

Protective effects of supplement intake in terms of maternal bulky DNA adducts levels were indicated. The adducts, measured by the postlabelling technique, represent both adducts formed directly between a chemical carcinogen but also indirectly by oxidative processes. A recent study shows that nanoparticles induce adducts and the formation could be inhibited by antioxidants [42].

Although it is biologically plausible that intake of multivitamins and minerals is associated with lower adduct levels, the present finding could be due to chance or confounded, particularly due to the asymmetry in group size. In newborns ($N = 235$) from Krakow, Poland, protective effects of high cord blood plasma levels of antioxidants, i.e. α-tocopherol, carotenoids and retinol, on the effect of the association between maternal exposure to carcinogenic PAHs measured in the air during 48 h in second trimester and cord blood anti-B[a]PDE-adducts WBC levels have been reported in adjusted multiple linear regression models [43]. Similarly, using FFQ responses to estimate individual intakes of antioxidants, high intakes of beta-carotene, vitamin C and vitamin E, have been associated with low bulky DNA adducts in WBCs from an adult Italian population ($N = 309$) [44]. In contrast to this, no significant

associations between estimated vitamins A, C, and E from FFQ responses on diet and supplements and bulky DNA adduct WBC levels were found in a Danish adult population ($N = 375$) [38]. Is it possible that an association can be observed for use of supplement only as many supplements have quite high levels of antioxidants compared to what can be achieved through food intake alone.

In a pooled study with European adults ($N = 1086$), a significant association between high dietary fiber intakes and low bulky DNA adducts WBC levels was found in adjusted multiple linear regression models [45]. Likewise an inverse correlations between high fruits and vegetable and low bulky DNA adduct levels were found in the Italian population [44]. High fruits and vegetable intakes were, however, not associated with lower DNA adduct levels in the pooled European population [45] or in the Danish population [38]. Increased alcohol intake has been correlated lower DNA levels [45] and other studies found no significant associations [44].

The associations between preferred meats with dark surface and bulky DNA adduct levels observed in our study was robust to additional adjustment of fruits and vegetable intake and other personal characteristics potentially influencing the formation and repair of bulky DNA adducts.

In the present study no significant dietary predictors of dioxin-like plasma activity and MN levels appeared. A positive correlation between estimated intake of animal fat and increased dioxin-like activity have been found in cord blood from Belgian newborns ($N = 871$) [13]. Similarly, in nulliparous pregnant women ($N = 100$) with differential fish intake, estimated total dietary fat was significantly associated with increased dioxin-like plasma activity [46], while, as in the present study, high estimated intake of food groups in which the dioxin-like compounds are most commonly detected, i.e. fish, meat, dairy products, and eggs [5], were not correlated with dioxin-like activity [13,46]. Significant positive associations between estimated high fatty fish intake and high levels of PCBs, including PCB 118, have, however, been found in plasma and adipose tissue [47] from nulliparous pregnant ($n = 100$) and middle-aged women ($N = 402$), respectively, living in Denmark.

Higher MN frequency in fathers ($N = 129$) with high blood mercury levels as compared with those with lower levels has been found in a Spanish population [22]. In mothers ($N = 129$) and their newborns ($N = 102$) the differences were non-significant. The authors proposed that intake of contaminated fish could have been the source of mercury. Estimated high intake of fish fat and high MN frequency in adults ($N = 773$) living in Belgium has been found to be positively associated [48]. Higher MN frequencies was observed in newborns ($N = 12$) born by mothers with reported fish oil in the present study population. The present finding could be due to change finding or confounded. If observed in other studies it could also indicate that consumption of fish oil result in exposures associated with MN induction such as contamination with POPs.

In the Belgian adults ($N = 773$) a positive correlation between high vegetable intakes and high MN frequency was found [48]. Possibly, this indicates that consumption of locally grown vegetables lead to consumption of polluted vegetables. In the present study population consumption of home grown vegetables was low. For future studies in pooled analyses with other study population resulting in larger statistical power it could be of relevance to investigate estimated intake of micronutrients from food and supplements in populations like the present. High intakes of vitamin E, vitamin A, folate, preformed nicotinic acid and calcium estimated from FFQ intake have e.g. been significantly associated with low MN frequency in Australian adults ($N = 190$), whereas high intakes of riboflavin, patothenic acid and biotin were associated with high MN frequency [49].

In conclusion, the results of the present study suggest that maternal intake of darkly fried meats contributes to the bulky DNA adduct levels in maternal and umbilical cord WBCs. FFQs were for assessment of meat surface in our study useful whereas the limited study size or methodological complexities may have hindered the assessment of more comprehensive dietary information of relevance for the biomarkers measured in mother-newborn pairs.

Conflict of interest statement

The authors declare that there are no conflicts of interest

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