Maternal diet during pregnancy and micronuclei frequency in peripheral blood T lymphocytes in mothers and newborns (Rhea cohort, Crete)

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Abstract

Purpose: Assess if diet and adherence to cancer prevention guidelines during pregnancy were associated with micronucleus (MN) frequency in mothers and newborns. MN are biomarkers of early genetic effects that have been associated with cancer risk in adults.

Methods: 188 mothers and 200 newborns from the Rhea cohort (Greece) were included in the study. At early-mid pregnancy, we conducted personal interviews and a validated food frequency questionnaire was completed. With this information we constructed a score reflecting adherence to the World Cancer Research Fund/American Institute for Cancer Research cancer prevention guidelines on diet, physical activity and body fatness. At delivery, maternal and/or cord blood was collected to measure DNA and hemoglobin adducts of dietary origin and frequencies of MN in binucleated and mononucleated T lymphocytes (MNBN and MNMONO).

Results: In mothers, higher levels of red meat consumption were associated with increased MNBN frequency [2ndtertile IRR=1.34 (1.00, 1.80), 3rdtertile IRR=1.33 (0.96, 1.85)] and MNMONO frequency [2ndtertile IRR=1.53 (0.84, 2.77), 3rdtertile IRR=2.69 (1.44, 5.05)]. The opposite trend was observed for MNBN in newborns [2ndtertile IRR=0.64 (0.44, 0.94), 3rdtertile IRR=0.68 (0.46, 1.01)] and no association was observed with MNMONO. Increased MN frequency in pregnant women with high red meat consumption is consistent with previous knowledge.

Conclusions: Our results also suggest exposure to genotoxics during pregnancy might affect differently mothers and newborns. The predictive value of MN as biomarker for childhood cancer, rather than adulthood, remains unclear. With few exceptions, the association between maternal carcinogenic exposures during pregnancy and childhood cancer or early biologic effect biomarkers remains poorly understood.

Keywords

Maternal Nutrition; Cancer; Genotoxicity; Diet; Epidemiology

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Introduction

Cancer incidence among European children has been steadily increasing in the last decades [1] but the causes of this increase are not well identified. The NewGeneris project examined whether maternal exposure to carcinogens during pregnancy results in *in utero* exposure and early biological effects in the unborn child, possibly leading to increased risk of cancer in later childhood [2]. The NewGeneris project combined the use of biomarkers of exposure, such as DNA and hemoglobin (Hb) adducts in cord blood samples, and micronuclei (MN) as biomarkers of early genotoxic effects. MN frequency in peripheral blood lymphocytes reflects the effects of genotoxic exposures and of intrinsic genetic instability [3]. High MN frequency is positively associated with increased risk of cancer in adults [4]. Results from the NewGeneris analysis showed that newborns from the Rhea cohort (Crete, Greece) presented the highest mean frequencies of MN in cord blood compared to newborns from other European cohorts (i.e. Spain, England, Denmark and Norway) [2]. We have previously reported associations of ambient air pollution, water disinfection by-products and reproductive factors in relation to MN frequency in the Rhea cohort [5–7]. In the current analysis we focused on the associations between maternal dietary habits and MN frequency in maternal and cord blood.

Food, nutrition and physical activity are associated with approximately one third of all cancers occurring in high-income countries [8]. Certain foods can prevent or contribute to cancer risk. Processed meat and red meat have been recently classified by the International Agency for Research on Cancer (IARC) Monographs Program as carcinogenic to humans (Group 1) and as probably carcinogenic to humans (Group 2A), respectively [9]. Processed meat consumption has been positively associated with colorectal cancer and stomach cancer and red meat consumption has been positively associated with colorectal, pancreatic and prostate cancer [9]. On the contrary, low intake of fruits and vegetables are associated with increased risk of cancer from the respiratory and digestive tract in prospective studies [10].

Nitrosamines, polycyclic aromatic hydrocarbons (PAHs), oxidative fat metabolites and acrylamide are carcinogenic to humans [11–13]. Nitrosamines are group of chemicals with well characterized genotoxic and carcinogenic activity. The main dietary source of nitrosamines is processed meat [14–16]. PAHs can be absorbed from

contaminated environments (soils or atmosphere) and accumulated in raw foods, such as vegetables, fruits, grains, vegetable oils, dairy products, and seafood, or be formed during the cooking process (reviewed by *Diggs et al.*[17]). Acrylamide is formed when carbohydrate-rich foods are cooked at high temperatures [18]. Glycidamide is a metabolite of acrylamide and it is considered as the main cause of genotoxicity from acrylamide exposure [19].

There are few studies on maternal diet during pregnancy and childhood cancer. Case-control studies suggest that diets rich in fruits and vegetables during pregnancy may reduce risk of brain tumour [20] and retinoblastoma [21, 22]. Previous studies have focussed on the association between diet and MN in adults, and have found positive and negative association between certain foods and nutrients and MN [23–26]. To our knowledge, only one small study focused on maternal diet during pregnancy and MN in 69 mothers and 54 newborns. Increased MN frequency was observed in newborns from mothers who consumed fish oil during pregnancy. However, the number of mothers who consumed fish oil (n=12) was too small as to make strong conclusions. No other association was found between any of the 18 assessed food groups and MN in mothers or newborns [27].

The aims of the present study were to assess if i) consumption of certain individual foods and dietary carcinogens during pregnancy and ii) adherence to the World Cancer Research Fund/American Institute for Cancer Research for Cancer (WCRF/AICR) prevention guidelines during pregnancy are associated with genotoxic effects in mothers and newborns from the Rhea cohort.

Participants

The present study included mothers and newborns from the Rhea cohort Heraklion, Crete, Greece, described elsewhere [28]. Briefly, women who became pregnant between February 2007 and February 2008 at the prefecture of Heraklion were asked to participate in the study at the time of the first ultrasound examination (10th–13th weeks of pregnancy). Women were invited to participate in the study if they were residents of the study area, older than 16 years of age, visiting a participating hospital or private clinic during the 10th–13th week of gestation, and did not have communication limitations. Participating women were contacted again during the 14th–18th and 28th–32nd weeks of pregnancy and at birth. During recruitment, 1,610 eligible women agreed to participate, and 1,459 (91%) were

followed through delivery. A random subset of 408 participants, among pregnancies with no complications such as preterm births, donated maternal and/or cord blood for biomarker measurements (MN, DNA adducts and Hb adducts amongst others) as part of the NewGeneris study [29]. Mothers and newborns with MN analysis in maternal and/or cord blood T lymphocytes from singleton pregnancies and with information on either frequency food questionnaires or exposure to dietary carcinogens (i.e. adducts analysis) were considered for this analysis. Overall, 188 mothers and 200 newborns were included. Of these, 145 were mother–child pairs.

The Ethics Committee of the University Hospital in Heraklion, Greece, approved the study and all participants provided written informed consent.

Methods

Exposures assessment

Individual foods. Information on maternal diet during pregnancy was collected between 14th- 18th weeks of pregnancy. We used a validated food frequency questionnaires (FFQ) which included 250 food items [28]. Briefly, for each food item, participants were asked about average portion size and frequency of consumption since the last menstrual period. The questionnaire included pictures of small, medium and large portion sizes for each food item and respondents chose the one that best captured their typical intake. To estimate the intake of each food item in grams, portion sizes were multiplied by daily frequencies of intake. For complex items (such as mixed dishes), we used standard recipes as described in the Composition Tables of foods and Greek dishes by Antonia Trichopoulou, 3rd edition. Individual portion sizes and recipes were used to calculate daily energy intake on the basis of the UK food tables (McCance & Widdowson's The Composition of Foods, 6th summary edition). For the analysis we generated four major groups and assessed their association with MN: fruits, vegetables, red meat and processed meat. Beef, pork, lamb, goat and veal meats were classified as red meat; sausages, bacon, salami and ham were classified as processed meat.

Dietary carcinogens. We used DNA and Hb adducts to assess exposure to genotoxic compounds of dietary origin. We used O6-methyldeoxyguanosine (O6-MedG) DNA adduct as a marker of exposure to nitrosamines; PAHs DNA adducts as a marker of exposure to PAHs; 3-(2'-deoxy-beta-D-erythro-pentofuranosyl)-pyrimido[1,2- α]purin-10(3H)-one (M1dG) DNA adduct as a marker of exposure to oxidative fat metabolites; and acrylamide (AA) and glycidamide (GA) Hb adducts as markers of exposure to acrylamide. Methods used to quantify DNA and Hb adducts are over-reviewed elsewhere [2]. Briefly, O6-MedG and PAHs DNA adducts were measured by ultrasensitive sandwich chemiluminescence immunoassays [30, 31]. The assay used for PAHs DNA adducts reflected exposure to benzo[a]pyrene and additional PAHs including chrysene, benzo[k]fluoranthene, dibenz[a,c]anthracene, and the bay-region and non-bay region benz[a]anthracenes [31, 32] M1dG was measured by immunoslot blot analysis [33]. AA and GA Hb adducts were determined by the adduct FIRE procedure using liquid chromatography tandem mass spectrometry [34]. All samples below the limits of detection (LOD) were replaced by 0.5xLOD. All above mentioned adducts were measured in cord blood. Given the significant correlation between the paired maternal and newborn DNA adduct levels reported in other studies [27, 35], we assumed that DNA/Hb adduct cord blood levels reflect transplacental exposure to genotoxic compounds present in maternal blood circulation and we used adduct levels in cord blood as proxy of both maternal and prenatal exposure.

World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) score. Maternal overall diet quality was evaluated with the WCRF/AICR score, that reflects adherence to the WCRF/AICR recommendations on diet, weight management and physical activity for cancer prevention as previously described [36]. Briefly, general recommendations from which we have related data available were evaluated and we assigned the score 1 when the recommendation was met, score 0.5 when the recommendation was partially met, and score 0 when the recommendation was not met at all (Online Resource 1). We grouped the WCRF/AICR scores in 3 categories: 0-3; >3 to <4; 4 to 7 (highest adherence).

Outcome assessment

We collected cord blood immediately after delivery and maternal peripheral blood within 24 hours after delivery. We carried out the cytokinesis-block micronuclei (CBMN) assay using whole blood according to the standardized

protocol for the semi-automated image analysis system [6, 37]. We recorded the total numbers of bi-nucleated T lymphocytes (i.e. cells that have divided once) with MN (MNBN) and the total number of bi-nucleated T lymphocytes scored for each participant, and we calculated MNBN frequencies per 1,000 bi-nucleated lymphocytes scored [37]. A minimum of 2500 bi-nucleated T lymphocytes were scored per person. Following the same procedure, we calculated the MN frequencies in mono-nucleated T lymphocytes (i.e. cells that did not divide; MNMONO). We calculated the cytokinesis-block proliferation index (CBPI) for each participant as follows: $CBPI = (\text{number of MONO} + 2 \times \text{number of BN} + 3 \times \text{number of polynucleated lymphocytes}) / \text{total number of lymphocytes counted}$ [37]. The design of the MN protocol aims at maximizing the number of binucleated lymphocytes, which results on average in a CBPI of 2. Lower CBPI frequencies reflect cell cycle delay due to toxicity or cell immaturity, and higher CBPI frequencies result from excessive mitogenic activation.

Potential confounders and effect modifiers

At study enrolment (10th–13th weeks of pregnancy), we used self-administered questionnaires to collect information on potential confounders such as maternal age, place of residence, maternal education, maternal origin and smoking habits. Daily energy intake (kcal/day) was derived from data collected in FFQ using food composition tables. Date of birth, sex and birth weight was obtained from medical records. We estimated gestational age as the interval between the last menstrual period and the date of delivery.

Additionally, for a sub-set of women with sufficient serum sample, we measured serum levels of iron and vitamin B12 (information available for 131 mothers and 133 newborns) and folate levels in red blood (information available for 39 mothers and 41 newborns) given their potential effect on MN formation.

Statistical analyses

We examined the distribution of study population characteristics, exposure and outcome and based on that, we categorized food groups and adducts variables in tertiles. We used negative binomial regression models to assess the association between exposures and MNBN frequency and MNMONO frequency. We used a natural log link, and the

model included the total number of cells scored as an offset. We estimated incidence rate ratios (IRR) and 95% confidence intervals [IRR (95%CI)] using robust standard errors.

To control for potential confounding effects, we included *a priori* covariates, selected using Directed Acyclic Diagrams, in the regression models. We adjusted all maternal models for age (age at birth (years) in maternal blood models and gestational age (weeks) in cord blood models), education (low: ≤ 9 years education; medium: 10-14 years education; high: University degree or higher), area of residence (rural, urban), season of delivery/birth (winter: December-February; spring: March-May; summer: June-August; autumn: September-November) and daily energy intake during pregnancy (kcal/day). We also explored the effects of other potential confounders (e.g. smoking during pregnancy (yes, no), maternal origin (Greek, others)) by entering covariates individually to the different models, if their inclusion changed the incidence rate ratio by more than 10% we kept them in the model.

All potential confounders except sex, gestational age and season of birth had missing values. The percentage of missing values ranged from 1 (birth weight) to 13 (area of residence). In order to increase efficiency and minimize selection bias, we applied multiple imputation methods as previously described [7]. We conducted all adjusted analyses using imputed covariates. Analyses using non-imputed data gave similar results (not shown).

We evaluated the association between iron, vitamin B12 and folate and MNBN frequency and MNMONO frequency. If we observed association between them, we explored the potential confounding effect of this microelements on the associations under study (i.e. individual foods, diet-related adducts, WCRF/AICR score and MN frequency).

Statistical analyses were done using Stata version 12.1 (StataCorp, College Station, TX, USA).

Results

Study Population Characteristics

Mothers included in this analysis were mainly of Greek origin (85%), had a mean (standard deviation ($\pm$ SD)) age of 29.5 $\pm$ 5.0 years, 28% reported active smoking during pregnancy, about half of them had medium educational level (51%) and lived in urban areas (64%). Half of newborns were boys (52%). Mean gestational age of newborns was 38.5 $\pm$ 1.3 weeks and their mean birth weight was 3.2 $\pm$ 4.2 kg. The prevalence of preterm birth was 6%.

MN in maternal and cord blood

The median MNBN frequency was 2.39‰ (interquartile range-IQR, 2.42) in the 188 maternal blood samples and 1.52‰ (1.65) in the 200 cord blood samples. There was no correlation between mother and newborn MNBN frequencies based on the 145 paired samples (Spearman correlation coefficient=0.13). The median MNMONO frequency was 0.42‰ (0.92) in the 118 maternal blood samples and 0.44 ‰ (0.97) in the 200 cord blood samples. Correlation was moderate between mother and newborn MNMONO frequencies based on the 145 paired samples (Spearman correlation coefficient=0.24). Correlation between MNBN and MNMONO was also moderate in mothers and newborns (Spearman correlation coefficient=0.40 and 0.3, respectively). The mean CBPI was 1.66 $\pm$ 0.22 (range, 1.11–2.16) for maternal cultures and 1.60 $\pm$ 0.18 (range, 1.11–1.98) for cord blood cultures (p-value<0.001). Similar results were obtained when we only considered the 145 paired samples: 1.66 $\pm$ 0.18 (range, 1.16–1.71) for maternal cultures and 1.60 $\pm$ 0.15 (range, 1.57–1.63) for cord blood cultures (p-value<0.001).

Median MNBN frequency was higher in older mothers than in younger ones, higher in more educated women than in less educated ones, and higher in women who delivered boys than in women who delivered girls. The highest median MNBN frequency was observed in summer and the lowest in autumn, in both mothers and newborns. Newborn girls had higher median MNBN than newborn boys (Table 1). Differences in MNMONO frequency were observed according to season in both mothers and newborns. Median MNMONO frequency was lower in newborns from underweight mothers (Table 1).

Consumption of individual foods and MN

Estimated median intake of red meat, processed meat, vegetables, and fruit was 42.8 (48.8), 5.8 (13.6), 200.7 (136.9) and 417.9 (353.9) g/day, respectively. Among those food items, only red meat consumption was associated with increased MNBN frequency in maternal blood after adjusting for confounders [2nd tertile: IRR=1.34 (1.00, 1.80); 3rd tertile: IRR=1.33 (0.96, 1.85)]. On the contrary, red meat consumption was associated with decreased MNBN frequency in cord blood in the crude and the adjusted analysis [2nd tertile: IRR=0.64 (0.44, 0.94); 3rd tertile IRR 0.68 (0.46, 1.01)]. Red meat consumption was also associated with increased MNMONO frequency in maternal blood after adjusting for confounders [2nd tertile: IRR=1.53 (0.84, 2.77); 3rd tertile IRR=2.69 (1.44, 5.03)]. Processed meat consumption was associated with increased MNMONO frequency in cord blood [2nd tertile: IRR=1.70 (1.12, 2.58); 3rd tertile IRR=1.95 (1.23, 3.10)]. Increased MNMONO frequency was observed in the 2nd tertile of vegetables consumption [2nd tertile: IRR=1.75 (1.09, 2.82); 3rd tertile IRR=1.28 (0.80, 2.05)] in maternal blood, whereas reduced MNMONO frequency was observed among newborns with higher consumption of vegetables [2nd tertile: IRR=0.56 (1.34, 0.91); 3rd tertile: IRR=0.63 (0.39, 1.00)]. No other associations were observed for any other food and MNBN frequency and MNMONO frequency in either maternal blood or cord blood in the crude (Online resource, table 1) or the adjusted analysis (Figure 1). Similar results were obtained when only paired samples were considered (Online resource, table 2).

Diet-related adducts and MN

Cord blood median levels of M1dG, O⁶-MedG and PAHs were 18.8 (62.0), 0.2 (0.4), 1.0 (2.0) DNA adducts per 10⁸ nucleotides, respectively. Median levels of AA and GA Hb adducts were 14.1 (9.6) and 10.8 (7.1) pmol/g Hb, respectively. 18% (24/131), 41% (58/143) and 25% (31/124) of samples analyzed for M1dG, O⁶-MedG and PAHs, respectively, had levels below the LOD. The lowest tertile of M1dG, O⁶-MedG and PAHs included samples with adduct levels below the LOD.

No statistically significant association was detected between any adduct and MNBN frequency and MNMONO frequency in either maternal blood or cord blood (crude analysis: Online resource, table 3, adjusted analysis: Figure 2). A positive association was suggested between levels of O⁶-MedG-DNA adduct and MNBN frequency and

MNMONO frequency in maternal blood (p-value_{linear trend adjusted}=0.183 and 0.06, respectively). Similar results were obtained when only paired samples were considered (Online resource, table 4).

Adherence to WCRF/AICR score and MN

There was no association between adherence to the WCRF/AICR guidelines and MNBN frequency in maternal blood [adjusted IRR for moderate adherence =1.06 (0.76, 1.47), adjusted IRR for high adherence=1.21 (0.87, 1.67)] or in cord blood [adjusted IRR for moderate adherence 1.00 (0.73, 1.38), adjusted IRR for high adherence=1.10 (0.80, 1.53)]. Similarly, there was no association between adherence to the WCRF/AICR guidelines and MNMONO frequency in maternal blood [adjusted IRR for moderate adherence=0.56 (0.29, 1.07), adjusted IRR for high adherence=0.59 (0.34, 1.04)] or in cord blood [adjusted IRR for moderate adherence=0.75 (0.47, 1.21), adjusted IRR for high adherence=0.62 (0.37, 1.06)]. Similar results were obtained for MNBN and MNMONO when we also adjusted for active smoking (not shown).

Iron, vitamin B12, folate and MN

Median serum levels of iron and vitamin B12 were 65 (57.5) µg/dl and 157.3 (136.2) µg/dl, respectively. Median level of red blood cell folate was 804.5 (455.5) ng/ml. No association was detected between levels of iron, vitamin B12 and folate and MNBN frequency or MNMONO frequency in mothers and newborns (Online resource, table 5).

Discussion

The present study assessed diet during pregnancy in association with MNBN and MNMONO frequency in both maternal blood and cord blood. This is the largest single country cohort study to date, in which these associations have been studied. Red meat consumption seemed to be positively associated with increased MMBN frequencies and MNMONO frequencies in mothers. The opposite trend was observed for MMBN frequencies in newborns. Processed meat consumption was associated with increased MMBN frequencies in newborns. We observed increased levels of MNMONO frequency in mothers with higher vegetables intake, whereas we observed decreased levels in newborns with higher vegetable intake.

We found some evidence that red meat consumption increased MN frequency (both MNBN and MNMONO) in pregnant women. Several studies have suggested an association between red meat consumption and increased colorectal cancer [38, 39], but few studies have focused on the association between food groups and MN frequency. In concordance with our results, a recent study recently reported lower MNBN in blood reticulocytes among vegetarians compared to non-vegetarians [40]. A mother-child study from Denmark (n<100), found no significant associations between maternal red meat intake during the last month of pregnancy and MNBN frequency in maternal or cord blood [27]. There is evidence that consumption of red meat results in increased endogenous formation of N-nitroso compounds (NOC), and that NOC can be also found in foods containing nitrates and nitrites such as red meat [14, 15, 39]. High levels of nitrates and nitrites can be also found vegetables. This could explain the association observed between higher intake of vegetables and MNMONO in mothers. However, the association between endogenous formation of NOC is less marked with nitrate intake from vegetable origin [41] and vegetables also contain high amounts of vitamin C and specific polyphenols that directly inhibit endogenous nitrosation reactions [14]. Interestingly, our results suggested a positive association between cord blood O⁶-MedG-DNA adduct and MNBN frequency and MNMONO frequency in maternal blood, although results were not statistically significant. These findings support the hypothesis that endogenous NOC may contribute to the genotoxic burden. Contrary as seen in mothers, red meat consumption during pregnancy was associated with decreased MNBN frequency in newborns and no association was observed with MNMONO frequency. Similarly, higher intake of vegetables was associated with reduced MNMONO frequency in newborns, opposite as observed in mothers. Other studies on maternal exposures during pregnancy and MN frequencies in mothers and newborns have detected association in mothers but not in newborns or opposite trends between mothers and newborns [5, 7, 35]. This observation suggests that exposure to genotoxics during pregnancy might affect differently MN induction in maternal blood and in cord blood. Consequently, levels genotoxic exposures identified in mothers, or adults in general, might not be necessarily genotoxic for newborns and vice-versa. First, MN in maternal blood circulating T lymphocytes reflect accumulated exposures during several years, not only during pregnancy. Second, genome stability during foetal development might be different than later in life, therefore the biological significance of MN in newborns might be different than in adults [42]. However, differences observed between mothers and newborns, could be also attributed to differences

in T lymphocyte subtypes (CD4+ cells versus CD8+ cells) or maturation stages of same T lymphocyte subtypes in maternal and cord blood, which are reflected in the CBPI [42].

We did not observe an association between adherence to WCRF/AIRC guidelines and MNBN or MNMONO in mothers or cord blood either. Concordance to the WCRF/AIRC score has been associated to reduced risk of cancer in adults [36] but no previous studies have assessed the potential association with biomarkers of genotoxicity.

The strengths of this analysis are the prospective design, the temporal proximity between exposure and effect, and the use of a valid cancer biomarker in adults. MNBN and MNMONO frequencies were available from a relatively large and well characterized sample of maternal blood and cord blood. We processed samples using a pre-defined protocol [43] and we examined fixed cells using a semi-automated image base scoring system that reduced potential variation and subjectivity of manual scoring [37]. We adjusted our models for several *a priori* and possible confounders although we cannot completely rule out the effect of residual confounding of other unmeasured factors. This study also has limitations. First, the evaluation of several maternal dietary exposures was done at the first or second trimesters of pregnancy. This period corresponds to the establishment of maternal-placental-foetal circulation that occurs around the 10th week of gestation, but does not fully correspond with the time of generation of cord blood lymphocytes that occurs mostly at the third trimester [44]. Second, we did not have levels of DNA/Hb adducts from maternal blood and we used cord blood levels as a proxy measure. However, we do not think this might have affected much our results as good correlation between paired maternal and newborn samples has been previously reported [27, 35]. In conclusion, red meat consumption during pregnancy was associated with increased MNBN and MNMONO frequencies in pregnant women; the opposite trend was observed for MNBN in newborns. Our results suggest exposure to genotoxics during pregnancy might affect differently mothers and newborns. Early genetic changes are only one of the possible mechanisms of cancer induction and the predictive value of MN (both MNBN and MNMONO) as biomarker for childhood cancer, rather than adulthood, remains unclear. With few exceptions, the association between maternal carcinogenic exposures during pregnancy and childhood cancer or early biologic effect biomarkers remains poorly understood.

317 **Conflict of interest statement**

318 On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figure Captions

Fig 1 Association between individual food groups and MNBN frequency and MNMONO frequency in maternal blood and cord blood samples. Models for maternal blood were adjusted for age at birth, education, residence, season of delivery and daily energy intake during pregnancy. Models for cord blood were adjusted for gestational age, maternal education, residence, season of birth and maternal daily energy intake during pregnancy. T1: lower tertile, T2: middle tertile, T3: upper tertile.

Vegetables (g/day): T1 ≥ 13 - ≤ 15 ; T2 >152 - ≤ 251 ; T3 >251 - ≤ 1039 . Fruit (g/day): T1 ≥ 0 - ≤ 325 ; T2 >325 - ≤ 552 ; T3 >552 - ≤ 1665 . Red meat (g/day): T1 ≥ 0 - ≤ 19 ; T2 >19 - ≤ 51 ; T3 >51 - ≤ 221 . Processed meat (g/day): T1 ≥ 0 - ≤ 2 ; T2 >2 - ≤ 11 ; T3 >11 - ≤ 80

Fig 2 Association between DNA and haemoglobin adducts in cord blood and MNBN frequency and MNMONO frequency in maternal blood and cord blood samples. Models for maternal blood were adjusted for age at birth, education, residence, season of delivery and daily energy intake during pregnancy. Models for cord blood were adjusted for gestational age, maternal education, residence, season of birth and maternal daily energy intake during pregnancy. T1: lower tertile, T2: middle tertile, T3: upper tertile. M1dG ($n/10^8$ nt): T1 ≥ 2.5 - ≤ 12.8 ; T2 >12.8 - ≤ 39.1 ; T3 >39.1 - ≤ 276.5 . O⁶-MedG ($n/10^8$ nt): T1 ≥ 0.08 - ≤ 0.15 ; T2 >0.15 - ≤ 0.40 ; T3 >0.40 - ≤ 2.40 . PAHs ($n/10^8$ nt): T1 ≥ 0.2 - ≤ 0.5 ; T2 >0.5 - ≤ 1.5 ; T3 >1.5 - ≤ 12.7 . AA (pmol/g Hb): T1 ≥ 5.2 - ≤ 11.6 ; T2 >11.6 - ≤ 18.9 ; T3 >18.9 - ≤ 145.0 . GA (pmol/g Hb): T1 ≥ 4.2 - ≤ 9.4 ; T2 >9.4 - ≤ 13.9 ; T3 >13.9 - ≤ 59.9

MNBN in maternal blood

MNMONO in maternal blood

MNBN in cord blood

MNMONO in cord blood

Table 1. Median (IQR) MNBN frequency and MNMONO frequency in maternal blood and cord blood samples according to characteristics of study participants ^a

Characteristics of mothers and newborns	Maternal blood (n=188)			Cord blood (n=200)		
	MNBN		MNMONO	MNBN		MNMONO
	n (%)	Median (IQR)	Median (IQR)	n (%)	Median (IQR)	Median (IQR)
Maternal age (years)						
<35	156 (83)	2.2* (2.5)	0.4 (0.9)	169 (84)	1.5 (1.7)	0.5 (1.0)
≥ 35	29 (15)	3.6 (3.3)	0.60(1.2)	28 (14)	1.7 (1.7)	0.2 (1.0)
Missing	3 (2)	2.6 (2.5)	0 (0.35)	3 (1)	1.0 (2.9)	0.1 (0.9)
Residence ^b						
Urban	124 (66)	2.9 (2.5)	0.5 (1.0)	129 (64)	1.5 (1.6)	0.5 (0.9)
Rural	43 (23)	1.9 (2.5)	0.3 (0.9)	47 (23)	1.5 (1.6)	0.3 (0.85)
Missing	21 (11)	2.0 (1.6)	0.4 (0.9)	24 (12)	1.5 (1.9)	0.5 (1.1)
Maternal education ^b						
Low (≤9 years)	40 (21)	1.8 (2.5)*	0.2 (1.1)	45 (22)	1.7 (1.5)	0.5 (1.1)
Medium (10-14 years)	100 (53)	2.3 (2.7)	0.4 (0.8)	101 (50)	1.5 (1.9)	0.4 (1.0)
High (University degree or higher)	42 (22)	3.4 (2.5)	0.6 (1.1)	44 (22)	1.6 (1.0)	0.5 (0.9)
Missing	6 (3)	1.9 (1.1)	0.3 (0.9)	10 (5)	1.6 (2.0)	0.1 (0.6)
Ethnic origin ^b						
Greek	158 (84)	2.6 (2.5)	0.45 (1.0)	172 (86)	1.6 (1.7)	0.4 (1.0)
Others	26 (14)	1.9 (2.4)	0.3 (0.7)	25 (12)	1.6 (1.1)	0.6 (1.0)
Missing	4 (2)	1.5 (3.6)	0 (0.4)	3 (1)	0.7 (2.6)	0 (0.4)
Maternal Active Smoking ^b						
No	115 (61)	2.4 (2.4)	0.4 (0.8)	13 (6)	1.6 (1.6)	0.4 (0.9)
Yes	57 (30)	2.3 (2.9)	0.4 (1.1)	172 (86)	1.4 (1.9)	0.45 (1.1)
Missing	16 (9)	2.3 (1.7)	0.45 (1.1)	15 (4)	1.5 (1.4)	0.5 (0.8)
Pre-pregnancy BMI (kg/m ²)						

Normal (≥ 18.5 - ≤ 25)	117 (62)	2.4 (2.4)	0.4 (1.0)	111 (55)	1.5 (1.4)	0.4 (1.0)*
Underweight (< 18.5)	12 (6)	3.3 (3.2)	0.2 (0.7)	13 (6)	1.5 (2.0)	0.7 (0.5)
Overweight (> 25 - ≤ 30)	36 (19)	2.4 (2.4)	0.5 (1.1)	36 (18)	1.6 (1.6)	0.4 (0.7)
Obese (> 30)	17 (9)	2.4 (2.3)	0.4 (0.7)	30 (15)	1.6 (1.7)	0.7 (1.0)
Missing	6 (3)	1.9 (1.1)	0.3 (0.9)	10 (5)	1.6 (2.0)	0.1 (0.6)
Physical activity before pregnancy						
No	146 (78)	2.4 (2.8)	0.4 (0.95)	146 (73)	1.5 (1.8)	0.5 (1.0)
Yes	37 (20)	2.4 (1.9)	0.35 (0.7)	44 (22)	1.6 (1.4)	0.3 (0.8)
Missing	5 (3)	2.6 (1.5)	0 (0.9)	10 (5)	1.6 (2.4)	0.6 (0.9)
Sex						
Male	96 (51)	2.9 (2.8) *	0.4 (1.0)	103 (51)	1.2 (1.4) *	0.4 (0.9)
Female	92 (49)	2.1 (2.4)	0.4 (0.9)	97 (48)	1.7 (1.7)	0.5 (1.1)
Gestational age (weeks)						
< 37	12 (6)	1.9 (2.1)	0.7 (0.9)	11 (5)	1.0 (1.0)	0.5 (0.7)
≥ 37	176 (94)	2.4 (2.4)	0.4 (0.9)	189 (94)	1.5 (1.7)	0.4 (1.0)
Birth weight (kg)						
Normal (> 2.5 - < 4)	172 (91)	2.6 (2.4)	0.4 (1.0)	184 (92)	1.5 (1.6)	0.5 (1.0)
Low (≤ 2.5)	9 (5)	2.1 (1.1)	0.3 (0.7)	7 (3)	1.5 (1.6)	0.3 (0.7)
High (≥ 4)	7 (4)	1.9 (2.5)	0 (0.3)	6 (3)	2.1 (1.2)	0.4 (0.9)
Missing				3 (1)	1.0 (3.9)	0.1 (0.9)
Season of delivery/birth (months)						
Winter (Dec-Feb)	73 (39)	2.6 (2.3)*	0.3 (0.7)	70 (35)	1.5 (1.4)*	0.5 (0.8)*
Spring (Mar-May)	58 (31)	2.7 (2.5)	0.7 (1.3)	78 (39)	1.5 (1.5)	0.5 (1.3)
Summer (Jun-Aug)	21 (11)	3.9 (2.6)	0.8 (0.4)	26 (13)	3.4 (2.1)	0.4 (0.6)
Autumn (Sep-Nov)	36 (19)	1.6 (1.7)	0 (0.3)	26 (13)	0.8 (1.3)	0 (0.3)

^a Comparison between groups were performed using Krustal-Wallis test. ^b Self reported data. * p-values based on Krustal-Wallis test < 0.05

MNBN in maternal blood

MNMONO in maternal blood

MNBN in cord blood

MNMONO in cord blood