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Demographic, reproductive, and dietary determinants of perfluorooctane sulfonic (PFOS) and perfluorooctanoic acid (PFOA) concentrations in human colostrum

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Abstract

To determine demographic, reproductive, and maternal dietary factors that predict perfluoroalkyl substance (PFAS) concentrations in breast milk, we measured perfluorooctane sulfonic (PFOS) and perfluorooctanoic acid (PFOA) concentrations, using liquid chromatography-mass spectrometry, in 184 colostrum samples collected from women participating in a cohort study in eastern Slovakia between 2002 and 2004. During their hospital delivery stay, mothers completed a food frequency questionnaire, and demographic and reproductive data were also collected. PFOS and PFOA predictors were identified by optimizing multiple linear regression models using Akaike's information criterion (AIC). The geometric mean concentration in colostrum was 35.3 pg/ml for PFOS and 32.8 pg/ml for PFOA. In multivariable models, parous women had 40%

lower PFOS (95% CI: –56 to –17%) and 40% lower PFOA (95% CI: –54 to –23%) concentrations compared with nulliparous women. Moreover, fresh/frozen fish consumption, longer birth intervals, and Slovak ethnicity were associated with higher PFOS and PFOA concentrations in colostrum. These results will help guide the design of future epidemiologic studies examining milk PFAS concentrations in relation to health endpoints in children.

Keywords

Perfluoroalkyl substances; PFAS; perfluoroalkyl acids; PFC; predictors; breastfeeding; polychlorinated biphenyl; lipid; correlation

Introduction

Perfluoroalkyl substances (PFASs) are a class of chemicals with unique hydrophobic and lipophobic properties that have numerous applications in a variety of consumer and industrial products. They are often used as surfactants to make nonstick coatings and repellents that are used in food packaging, textiles, and countless other consumer products.¹ As a result of their widespread use and their environmental persistence, PFASs have been detected in environmental samples from surface waters, air, sludge, soils, sediments, and ice caps around the globe.²

Human exposure to PFASs occurs through a variety of pathways, and exposure begins during gestation. For instance, the most prevalent PFAS homologues such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are readily detectable in the plasma of pregnant women,³ in cord blood,⁴ and in neonatal blood spots,⁵ and strong, positive correlations between maternal and cord blood PFAS concentrations have been documented.^{4, 6} Exposure also occurs during the postnatal period. A longer and more exclusive duration of breastfeeding during infancy increases exposure and resulting blood levels,^{7, 8} as do dietary sources in childhood and adulthood.^{9, 10} Once exposed, the estimated elimination half-life is approximately 5.4 years for PFOS, and 3.8 years for PFOA.^{5, 11} Although PFOS was voluntarily phased out by its major manufacturer in 2002, and PFOA was set to be phased out by six manufacturers in 2015,^{2, 12, 13} the stability of PFASs in the environment and their long elimination half-lives ensure that they will have a continued presence in biological media. Indeed, in surveys conducted in 2011–12 by the U.S. Centers for Disease Control and Prevention, almost a decade after PFOS was phased out in the United States, >99% of the U.S. population aged 12 years and older—including women of reproductive age—have detectable plasma PFOS levels.¹⁴

Some epidemiologic studies indicate that early life PFAS exposure is associated with altered health outcomes in infants and children, including immune, reproductive, motor, and neurobehavioral outcomes.^{15–20} When perinatal and early life PFAS concentrations are not directly measured (for instance, in maternal, cord, or children's blood samples), epidemiologic studies may rely on other measures of exposure, including concentrations in breast milk, to estimate early life PFAS exposure, since 1) milk concentrations are strongly and positively correlated with maternal and cord PFAS concentrations,^{21, 22} 2) sample collection is less invasive, and 3) the sufficient volumes needed for chemical analyses can be

easier to obtain. For these reasons, studies identifying the factors that are associated with PFAS concentrations in breast milk are needed. For example, identification of PFAS determinants will serve to guide the selection of variables that potentially confound PFAS—health outcome associations. Furthermore, studies of the determinants of PFAS in milk are needed to identify potential measures to reduce maternal and infant exposure to PFASs.

The determinants of early-life PFAS exposure have been investigated in other studies, however, this work has primarily focused on predictors of serum/plasma PFAS concentrations^{4, 23–29} as opposed to determinants of breast milk PFAS concentrations.^{30, 31} Furthermore, these studies have generally not included dietary factors. Given the paucity of data on determinants of PFAS concentrations in breast milk, and the need for this information to guide the design of epidemiologic studies of PFAS exposure and disease outcomes, the present study investigates demographic, reproductive, and dietary predictors of PFAS in colostrum among a cohort of mother-infant pairs in Slovakia.

Materials and Methods

Study population

Participants in the present PFAS study are a subset of mother-infant pairs recruited into a prospective birth cohort study in eastern Slovakia.³² Women were approached to participate in our study between 2002 and 2004 at the time they came to the local hospital in Michalovce, Slovakia to deliver their child. At that time, mothers gave written informed consent to participate. This cohort was originally assembled to examine the potential health effects of PCBs on child development, as Michalovce is an area with substantial PCB contamination,³³ which is in part due to a chemical manufacturing facility that produced PCBs from 1959 to 1984. Environmental PCB concentrations,³⁴ as well as concentrations in adults and children,³⁵ have been well-characterized in this population. Since the Michalovce district has only one hospital, the vast majority of women giving birth during 2002–2004 delivered at this hospital. We excluded 1) mothers with more than four previous births, 2) mothers less than 18 years of age, 3) mothers who had resided fewer than 5 years in Michalovce, 4) mothers with a major illness during pregnancy (including cancer, psychosis, toxoplasmosis, and rubella), and, after delivery, 5) mothers of infants who had severe birth defects.³² In total, 811 women were enrolled from the Michalovce district. From this cohort of 811, we randomly selected 184 families who had completed follow-up through 45 months of age. This was done to maximize the number of subsequent health endpoints (through 45 months) that could be examined in relation to colostrum PFAS concentrations. The original study protocol was approved by the Institutional Review Boards at the University of California, Davis and the Slovak Medical University.

Maternal colostrum collection

Colostrum samples were collected on day 4 or 5 postpartum before the mother's discharge from the maternity ward. Between 5 and 55 ml of colostrum was manually expressed by the mother into a 60 ml clear glass vial, immediately frozen at -20°C , and then later transported frozen in thermoboxes from eastern Slovakia to the Slovak Medical University in Bratislava where they were stored at -20°C .

Questionnaires and medical record abstraction

During the hospital delivery stay, mothers completed a questionnaire administered by trained staff that elicited socio-demographic information, questions related to maternal health, family living environment, past pregnancies, and tobacco use. Women were considered smokers if they reported smoking during pregnancy or stated that they were a current smoker at the delivery interview. Romani ethnicity was assigned if the ethnic origin of either of the mother's parents was Romani, the Romani language was spoken at home, or the mother was planning to raise her child with the Romani language. Otherwise, ethnicity was assigned as Slovak/other European. From the infant's medical record, study staff abstracted child's birth weight and gestational age. Gestation length was based on the date of the woman's last menstrual period and the judgment of her physician. For parous women, inter-pregnancy interval was calculated as the duration (in months) between birth of a previous child and the beginning of the target pregnancy. From questionnaires and medical records, we identified the following determinants of interest: maternal BMI, marital status, education, parity, total previous nursing (months), inter-pregnancy interval, age at child's birth, and smoking during pregnancy; as well as child ethnicity (Roman vs. Slovak/other European), gestation length, birthweight, and infant sex.

Maternal dietary data collection

During the mother's hospital delivery stay, a food frequency questionnaire was administered to mothers by trained nurses to obtain detailed information on dietary habits and sources of various foods. The questionnaire comprised 96 items, and was developed to reflect the national dietary habits in Slovakia. The questionnaire has been used to assess dietary habits in previous studies in Slovakia,^{36–38} including those of women in this cohort.³⁷ Questionnaire items were designed to ascertain information about the dietary habits of the women over the past 12 months, including the grams consumed per day, per week, or per month, whichever was appropriate (see Supporting Information). With respect to PFAS exposure, thirteen specific food types [fish (fresh or frozen), canned fish, beef, pork, chicken, liver, eggs, milk, yogurt, cheese, butter/margarine, fresh fruit/vegetables, and bread] were selected for our analysis based on previous literature examining dietary predictors of PFAS.^{24–27, 31} The selected foods also represent common items in the typical diet of this area. Data processing included conversion into individual daily consumption units by an experienced dietitian (KB). Subsequently, the daily intake of individual foods (g/day) was calculated using software specifically designed for the study.

Measurement of PFOS and PFOA in colostrum

The analytic procedure involved solid phase extraction in combination with liquid chromatography-mass spectrometry (LC-MS) on a triple quadrupole mass spectrometer using a previously-reported method.^{31, 39, 40} Specifically, after thawing, the colostrum samples were homogenized after stabilizing them at a temperature of 38°C. For the analyses, a 0.5 ml sample was used to which labeled internal standards (13C4-PFOA and 13C4-PFOS, Wellington Laboratories, Guelph, Canada) were added. Protein denaturation was performed by the addition of 0.5 ml 1 M formic acid and sonication for 30 minutes, followed by solid phase extraction using 1 ml, 30 mg Oasis WAX cartridges. After loading the sample

mixture, washing of the cartridges was done with ammonium acetate at pH 4 (25 mM, 1 ml) and tetrahydrofuran in methanol (25% v/v, 0.5 ml). Elution of PFOS and PFOA was achieved with 0.4 ml of a 1% ammonium hydroxide solution in methanol. To this eluate, 0.4 ml 0.1M formic acid was added before injection of the whole extract onto a C8 (Xterra MS C8, 10 mm × 4.6 mm, particle size 5 µm) trapping column in a column switching LC-MS/MS system. For the analytical separation, a Betasil C8 column was used (50 mm × 2.1 mm, particle size 3 µm). Separation was obtained by gradient elution at a flow rate of 0.3 ml/min using 20 mM NH₄AC pH4 and acetonitril. The LC system was an Agilent 1200 Series (Palo Alto, CA, USA) coupled with an Agilent 6410 electro spray interface (ESI) operated in the negative ion mode prior to triple-quadrupole mass spectrometric detection. The ion transitions used for quantification were m/z 413 – m/z 369 (PFOA) and m/z 499 – m/z 80 (PFOS).

All colostrum specimens were analyzed at the Institute for Environmental Studies at VU University in the Netherlands, under accreditation of the Dutch accreditation council (ISO17025). This laboratory also participates in inter-laboratory proficiency testing. To assess quality control/assurance, in every series of samples (n<16), a procedure blank, an enriched sample (similar/same matrix, within 20%), and a sample from a previous series were included (z-values <|2|). If the procedure blank was greater than the limit of detection (LOD), then the series was repeated. The coefficient of variation was 9% for PFOS and 8% for PFOA concentrations. The LOD for PFOS was 1.4 pg/ml and the LOD for PFOA was 0.4 pg/ml: all samples were above the LOD for PFOS and PFOA.

PCB and lipid measurement

To examine the correlations between serum PCB and colostrum PFAS concentrations, maternal and 6-month infant serum PCB concentrations were determined at the Department of Toxic Organic Pollutants at the Slovak Medical University in Bratislava using high-resolution gas chromatography with electron capture detection or high-resolution mass spectrometry, the specifics of which have been documented previously.^{41, 42} For the present analysis, we focused on serum concentrations of PCB-153 because serum PCB-153 concentrations are strongly correlated with total (sum) PCB concentrations in this cohort ($\rho > 0.99$ for maternal and 6-month serum concentrations).⁴³ Lipid concentrations were estimated by the enzymatic summation method.⁴⁴ Total serum cholesterol (TC) and triglyceride (TG) concentrations were determined at the Department of Clinical Biochemistry of TOP-MED General Hospital Bratislava using a DuPont Automatic Clinical Analyzer III (DuPont, Jonesboro, AR, USA), and cholesterol oxidase without cholesterol esterase was used to detect free cholesterol (FC). The method by Takayama and colleagues was used to determine serum choline-containing phospholipids (PL).⁴⁵ Total serum lipids were calculated using the formula: $TL = 1.677 \times (TC - FC) + FC + TG + PL$.

Statistical methods

We first compared selected maternal and infant characteristics among the 184 mother-infant pairs in the present PFAS study to the 811 mother-infant pairs enrolled in the Michalovce cohort at birth. Subsequently, descriptive statistics and Spearman correlation coefficients comparing PFASs, PCB-153, and lipid concentrations were estimated. To examine

demographic, reproductive, and dietary determinants of colostrum PFAS concentrations, we first estimated the geometric mean (and the 95% confidence interval of the geometric mean) of PFOS and PFOA within strata of each demographic, reproductive, and dietary factor. Then, to directly compare concentrations across each factor's strata, we estimated the ratio of PFAS concentration for a given stratum relative to a reference group (e.g., PFOS concentration among Slovak/other European women versus Romani women). Tests of trend in bivariate models were based on the quantitative form of the predictor where appropriate, despite categorization for the purposes of ratio estimation, and PFOS and PFOA concentrations were transformed by the natural logarithm in all regression models. Finally, to identify the most meaningful predictors of PFAS concentrations, we fit two multivariable models (one for PFOS and one for PFOA) that included the 12 demographic and reproductive predictors and the 13 dietary predictors examined in bivariate models. We then applied stepwise variable selection using Akaike's information criterion (AIC) for optimal model fit, as was done in a previous study of PFAS determinants.²⁵ All continuous variables were treated as such in the multivariable models. Finally, as a sensitivity analysis, we fit an additional model that included total energy intake (kJ/day), in addition to the variables identified through the selection procedure, to determine whether total energy intake altered the association between dietary predictors and colostrum PFAS concentrations.⁴⁶

Results

Study population

Mother-infant pairs in whom colostrum PFAS concentrations were measured were similar to the overall Michalovce cohort with regard to most demographic, maternal, and child characteristics (Table 1). Infants in the PFAS study were more likely to be female sex and have their family identify as Slovak/other European, and women in the PFAS study were also slightly more likely to be multiparous.

Colostrum PFAS concentrations and association with serum PCB and lipid concentrations

Of the 184 mothers with at least one measured PFAS, 176 had a measured PFOS, 166 had a measured PFOA, and 158 mothers had measured concentrations of both PFASs. The geometric mean concentration was 35.3 pg/ml for PFOS and 32.8 pg/ml for PFOA (Table 2). PFOS concentrations ranged from 9.4 to 650.0 pg/ml; the range was narrower for PFOA (8.0 to 160.0 pg/ml). PFOS and PFOA concentrations in colostrum were strongly and positively correlated ($\rho=0.66$; $p<0.001$) (Table 3). PFAS concentrations were not correlated with either maternal or 6-month infant PCB-153 serum concentrations (either on a wet-weight or lipid basis) in this sample ($\rho<|0.08|$). There was some suggestion of a weak positive correlation between colostrum PFAS concentrations and maternal serum lipid concentrations (0.11 ρ 0.14).

Predictors of colostrum PFAS concentrations—Bivariate analyses

Demographic determinants—Slovak/other European women had approximately 55% (95% CI: 17 to 107%) greater concentrations of PFOS and 94% (95% CI: 50 to 151%) greater PFOA concentrations compared with Romani women (Table 4). Further, greater educational attainment was associated with higher PFOS (p for trend=0.02) and PFOA (p for

trend=0.001) concentrations in colostrum. Specifically, compared with women with only basic schooling, women with a college or university education had approximately 2-fold higher PFAS concentrations (Table 4). Marital status and maternal body mass index (BMI) were not meaningful predictors of colostrum PFAS concentrations.

Reproductive determinants—Compared with nulliparous women, parous women had lower concentrations of both PFASs in their colostrum. Specifically, women with one previous birth had a 23% lower PFOS concentration in colostrum compared with nulliparous women (95% CI: −39 to −3%), while women with two or more previous births had a 27% lower PFOS concentration compared with nulliparous women (95% CI: −44 to −4%). A similar pattern of results was observed for PFOA (Table 4).

Longer previous nursing was associated with lower PFOS and PFOA concentrations (Table 4). On average, women who previously breastfed for more than 12 months had a 27% (95% CI: −46 to −1%) lower PFOS (p for trend=0.15) and a 24% (95% CI: −41 to −1%) lower PFOA (p for trend=0.36) concentration compared with women who reported no previous breastfeeding.

Inter-pregnancy interval, maternal age, infant gestation length and birthweight did not show noteworthy associations in bivariate models with either PFAS homologue, but variability, particularly in gestation length, was limited in this study. In addition, there was some suggestion that maternal smoking during pregnancy was associated with slightly lower PFAS concentrations (approximately 11–17% lower PFAS concentrations among smokers compared with non-smokers) (Table 4).

Dietary determinants—The most noteworthy associations were observed for fish consumption. Greater fresh/frozen fish intake was associated with higher PFOS (p for trend=0.13) and PFOA concentrations (p for trend=0.01) (Table 5). Those consuming >10 grams of fresh or frozen fish per day had approximately 20–30% higher PFAS concentrations. The direction of association was similar for canned fish consumption, but the magnitude of the association was not as strong. Butter and margarine consumption was inversely associated with both PFOS and PFOA concentrations (p for trend 0.19 and 0.04, respectively), but estimates were relatively imprecise.

Predictors of colostrum PFAS concentrations—Multivariable analyses

For both PFOS and PFOA, parity was associated with lower PFAS concentrations in colostrum, and Slovak ethnicity, longer birth intervals, and maternal fish consumption were associated with higher PFAS concentrations (Table 6). Specifically, compared with nulliparous mothers, parous mothers had 40% lower PFOS concentrations (95% CI: −56 to −17%) and 40% lower PFOA concentrations (95% CI: −54 to −23%). Slovak women had approximately 28% and 57% higher concentrations of PFOS and PFOA, respectively, compared with Romani women. An inter-pregnancy interval of 18 months was associated with approximately 40% higher PFOS and PFOA concentrations, relative to an interval of <18 months. Each 50 g/day increase in fresh or frozen fish consumption was associated with a 101% increase in PFOS (95% CI: 33 to 203%), and a 55% increase in PFOA (95% CI: 11 to 116%); canned fish consumption was not retained in the stepwise model.

For PFOS, the stepwise procedure also identified birthweight and fresh fruit/vegetable consumption as predictors of lower PFOS levels, though the estimated association was relatively small (e.g., 2% lower PFOS concentrations per 100 g of birthweight, and 5% lower PFOS concentrations per 100 g/day of fresh fruit/vegetable consumption). For PFOA, greater butter/margarine consumption was associated with lower concentrations, though this association was relatively small (5% lower PFOA per 10 g/day). Additionally, each 1000g of milk consumed per day was associated with a 22% higher PFOA concentration. Finally, in our sensitivity analysis, adjustment for total energy intake did not materially change the estimated associations (e.g., the contribution of fish consumption was changed by <2%).

Discussion

PFAS homologues, specifically PFOS and PFOA, were detectable in the colostrum of women in eastern Slovakia in 2002–2004. In multivariable models, we observed that Slovak ethnicity, nulliparity, longer birth intervals, and greater maternal fish consumption were predictors of higher PFOS and PFOA concentrations in colostrum. These results are consistent with at least one previous study investigating reproductive and dietary determinants of PFAS concentrations in maternal plasma.²⁵

The median concentration of colostrum PFOS in our study was 33.5 pg/ml. This concentration was lower than median concentrations reported in geographically similar areas around the same time period. For instance, concentrations determined in samples collected in 2006 in Munich (113 pg/ml) and Leipzig (123 pg/ml), Germany were considerably greater, as were concentrations among women living in Győr, Hungary (330 pg/ml) in 1996–1997.⁴⁷ Like PFOS, the median concentration of PFOA in our study was 33.5 pg/ml. This median concentration was similar to one study in Massachusetts, which reported median concentrations of 36.1 pg/ml among milk samples collected in 2004.³¹ It should be noted however that the reported concentrations in these populations were measured in mature breast milk. While the comparability between concentrations measured in colostrum and mature milk have been demonstrated for some lipophilic persistent organic pollutants^{48, 49} (though not all⁵⁰), we were unable to identify studies comparing PFAS concentrations in colostrum to those in mature milk, and thus the comparability between PFAS concentrations in colostrum versus mature milk is unknown. Similarly, few studies have reported on the comparability between paired maternal serum and breast milk PFAS concentrations. A study in Sweden observed that breast milk PFOS concentrations were approximately 1% of the PFOS concentrations measured in maternal serum, and milk PFOA concentration was approximately 2% of the serum PFOA concentration, though only 1 sample of the 12 had a PFOA concentration above the limit of detection.⁵¹ These numbers are consistent with a study of 20 matched maternal serum and milk samples from Seoul.⁵² In that study, PFOS concentrations in breast milk were approximately 1% of those in maternal serum, and for PFOA, 2%. These findings suggest that in the Slovak cohort, estimated median maternal serum PFOS concentrations would be approximately 3.5 ng/ml, and PFOA concentrations 6.6 ng/ml. While the estimated median PFOA concentration is similar to those reported in Norway²⁵ and in the United States²⁹ around the same time period, the estimated median PFOS concentration appears to be 3 to 7-fold lower in this Slovak population.

We did not observe any evidence of an association between PFAS concentrations and PCB-153 concentrations. Specifically, colostrum PFOS and PFOA concentrations were not correlated with maternal PCB-153 or 6-month infant serum PCB-153 concentrations, either on a wet-weight or lipid basis ($\rho < |0.08|$). This suggests that further investigation into health outcomes associated with milk PFAS concentrations, in this cohort, should not be confounded by PCB-153 concentrations.

We also examined correlations between PFASs and serum lipid concentrations to determine whether PFAS concentrations were associated with lipid profiles based on several lipid parameters (total cholesterol, HDL cholesterol and LDL cholesterol). In the present study we observed small, but positive correlations between maternal serum lipid concentrations and PFOS ($\rho = 0.11$) and PFOA ($\rho = 0.14$). One study of pregnant women reported associations between increased PFAS levels and increased cholesterol concentrations, where PFOS and PFOA were associated with total cholesterol, HDL cholesterol, and LDL cholesterol.⁵³ Similar associations have also been observed in other cross-sectional analyses in different populations. For instance, among a Danish cohort of middle-aged persons, PFOS and PFOA were positively associated with total cholesterol levels,⁵⁴ and in a U.S. study of children and adolescents, PFOS and PFOA concentrations were positively associated with total cholesterol levels.⁵⁵ The mechanism by which this occurs in humans (if causal) is not well understood. While the authors of the study in pregnant women⁵³ acknowledged that PFAS may activate the peroxisome proliferator-activated receptor alpha (PPAR α), a receptor implicated in the regulation of lipid and glucose metabolism, PPAR α -activating drugs typically lower serum lipids in humans.⁵⁶ This association is intriguing, and warrants further investigation, especially once the relationships among PFAS, serum lipids, and PPAR α are clarified for humans.

In our multivariable model, ethnicity was associated with PFAS concentrations, where Slovak women had 28% and 57% higher PFOS and PFOA concentrations, respectively. Compared with Roma women, Slovak/other European women generally have greater economic resources to purchase consumer products that may contain PFASs.⁵⁷ This interpretation is consistent with results from other studies that have observed positive associations between income and maternal PFAS concentrations.^{25, 29}

Consistent with findings from previous studies examining reproductive determinants of maternal PFAS concentrations,^{4, 23–25, 29, 30} we found strong associations with parity and inter-pregnancy interval in our multivariable analyses. With each pregnancy and cycle of breastfeeding, more PFAS leaves the mother and is transferred to the fetus or infant.^{7, 8, 58–60} This occurs during gestation as PFASs readily pass across the placenta to the fetus. This also occurs during infancy when the mother nurses the child and PFASs are transferred from mother to infant via breast milk. Thus with each subsequent pregnancy, maternal PFAS concentrations are reduced. Subsequently, between pregnancies (i.e., the inter-pregnancy interval), ongoing maternal PFAS exposures are expected to replenish or re-equilibrate serum PFAS levels.⁵⁸ Such mechanisms explain the inverse association between parity and colostrum PFAS, and the positive association between longer birth interval and PFASs. Additionally, we observed a weak inverse association between birthweight and PFOS (approximately 2% lower PFOS levels with each 100g increase in birthweight). While this

may be a causal association (as reported by some^{18, 19, 61–64}), others have posited that these observed associations may be the result of confounding, attributable to glomerular filtration rate (GFR).⁶⁵ Whether this association is causal or spurious remains to be determined.

Finally, in multivariable analyses we observed associations between greater fresh/frozen fish consumption and higher colostrum PFAS concentrations. Similarly, the MoBa study observed increased median concentrations of plasma PFAS concentrations with greater fish consumption.²⁵ Seafood consumption was also found to be positively associated with serum PFOS concentrations in the Northern Norway Mother-and-Child Contaminant Cohort study (MISA) (n=174).²⁴ Although we also had information on canned fish consumption, this predictor was not retained in the multivariable model, suggesting that it did not contribute meaningfully to the prediction of colostrum PFAS concentrations beyond existing parameters. Too, we were unable to more finely model fish/seafood consumption. At the time of milk collection in this cohort, fish consumption was generally low in this population, and the choices of fish available in food markets was extremely limited, thus additional fish/seafood determinants could not be derived from the dietary questionnaire.

Only two other studies simultaneously adjusted for reproductive and demographic factors (both in maternal plasma),^{25, 29} and of these, only one²⁵ also considered dietary factors. Multivariable results from that investigation indicated that parous women had 63% lower PFOS concentrations and 121% lower PFOA concentrations compared with nulliparous women,²⁵ and total estimated dietary PFOS and PFOA intake was positively associated with maternal plasma concentrations. The present study is therefore the first investigation of colostrum/breast milk PFAS determinants to examine for demographic, reproductive, and dietary factors simultaneously.

Our study was limited in its sample size, and as a result, some of our estimates of association are imprecise. In addition, since we did utilize a biomarker of maternal smoking (e.g., cotinine), misclassification may have impacted our analyses with regard to self-reported smoking. In addition, retrospective recall of dietary habits likely introduced misclassification. Since women were unaware of their PFAS concentrations at the time of the dietary survey, this bias is likely non-differential with respect to dietary—PFAS associations.

Most epidemiologic studies have focused on the determinants of PFASs in maternal or cord blood samples, and fewer studies have investigated the determinants of PFASs in breast milk. Understanding the determinants of PFAS concentrations in breast milk is important for future research. Studies of human health effects often rely on breast milk concentrations as a proxy of perinatal exposure, thus understanding the determinants of PFASs in breast milk will permit studies to accurately account for potential confounding factors and ultimately estimate less biased associations. In addition, a better understanding of the determinants of PFAS concentrations will help identify factors that may mitigate maternal and infant exposure. Finally, to our knowledge, this is the first study to analyze PFAS concentrations in the Slovak Republic. Thus, these data will provide important baseline concentration information for PFAS biomonitoring in Slovakia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of infants and mothers in the PFAS substudy and those enrolled in the Michalovce cohort at delivery.

Characteristic	PFAS study (n=184)	Michalovce cohort (n=811)
	n (%) ¹	n (%)
Ethnicity of child		
Romani	27(15)	174(21)
Slovak/other European	157(85)	637(79)
Maternal BMI (kg/m ²)		
<18.5 (underweight)	23(13)	112(15)
18.5–24.9 (normal weight)	117(67)	503(67)
≥25 (overweight/obese)	34(20)	138(18)
Missing ²	10	58
Marital Status		
Married	171(94)	718(92)
Never married	11(6)	57(7)
Divorced/separated	0	7(1)
Missing	2	29
Maternal education ³		
Basic Schooling (primary education)	33(18)	214(27)
High School Without Degree	42(23)	176(22)
High School With Degree	101(55)	340(43)
College/University	6(3)	59(7)
Missing	2	22
Parity		
0	67(36)	330(41)
1	75(41)	286(35)
2	33(18)	132(16)
3	9(5)	61(8)
4	0	2(0)
Total previous nursing (months)		
0	79(43)	419(53)
>0–3	33(18)	121(15)
>3–12	43(23)	146(18)
>12	28(15)	109(14)
Missing	1	16
Inter-pregnancy interval (months)		
<18	96(53)	444(57)
18+	84(47)	332(43)
Missing	4	35
Maternal age at child's birth (years)		
18–<20	12(7)	68(8)

	PFAS study (n=184)	Michalovce cohort (n=811)
Characteristic	n (%)¹	n (%)
20–30	140(76)	613(76)
31	32(17)	130(16)
Gestation length (weeks)		
<37	4(2)	18(2)
37–41	174(95)	761(95)
42	5(3)	23(3)
Missing	1	9
Birthweight		
<2500	7(4)	39(5)
2500–3499	105(57)	459(57)
3500	71(39)	310(38)
Missing	1	3
Maternal smoking during pregnancy		
Yes	51(29)	194(28)
No	125(71)	491(72)
Missing	8	126
Infant sex		
Female	106(58)	389(48)
Male	78(42)	422(52)

¹ Percentages may not sum to 100 because of rounding.

² Missing values not included in percentages.

³ Basic schooling includes 8–9 years of schooling (primary schooling)

Table 2

Colostrum PFAS and serum PCB descriptive statistics (n=184).

	n	n (%)>LOD ¹	GM ²	Min	P10	P25	P50	P75	P90	Max
Colostrum PFOS (pg/ml)	176	176 (100)	35.3	9.4	15.0	24.0	33.5	48.0	84.0	650.0
Colostrum PFOA (pg/ml)	166	166 (100)	32.8	8.0	16.0	23.0	33.5	48.0	67.0	160.0
Maternal serum PCB-153 (ng/g lipid)	172	172 (100)	203.1	50.6	92.0	132.3	198.7	292.8	472.9	1161.9
Infant 6-month serum PCB-153 (ng/g lipid)	169	169 (100)	130.7	1.7	23.2	58.5	151.7	297.3	512.9	2640.8

¹ Number and percent of participants above the assay's limit of detection

² Geometric mean

Table 3

Spearman correlation coefficients between colostrum PFAS, serum PCB, and serum lipid concentrations (n=184).

Variables	Spearman Correlations (*p<0.10; **p<0.001)							
	1	2	3	4	5	6	7	8
1. Colostrum PFOS (pg/ml)	1.00							
2. Colostrum PFOA (pg/ml)	0.66**	1.00						
3. Maternal Serum Lipid ^l	0.11	0.14*	1.00					
4. 6-Month Infant Serum Lipid ^l	-0.02	-0.03	0.11	1.00				
5. Maternal Serum PCB-153 (ng/ml)	0.05	0.05	0.25**	-0.05	1.00			
6. Maternal Serum PCB-153 (ng/g lipid)	0.03	0.02	-0.01	-0.11	0.95**	1.00		
7. Infant 6-Month Serum PCB-153 (ng/ml)	-0.08	-0.04	0.04	0.12	0.43**	0.42**	1.00	
8. Infant 6-Month Serum PCB-153 (ng/g lipid)	-0.08	-0.05	0.03	-0.07	0.45**	0.44**	0.97**	1.00

^lTotal serum lipid calculated using the formula by Takayama et al., 1977

Table 4

Bivariate associations between demographic and reproductive predictors and maternal colostrum PFAS concentrations (n=184).

Characteristic	Colostrum PFOS (n=176)			Colostrum PFOA (n=166)		
	n (%) ¹	GM (95% CI) ²	Ratio (95% CI) ³	n (%)	GM (95% CI)	Ratio (95% CI)
Ethnicity of child						
Romani	25 (14)	24.2 (18.6, 31.4)	Reference	18 (11)	18.2 (13.6, 24.2)	Reference
Slovak/other European	151 (86)	37.6 (33.7, 41.9)	1.55 (1.17, 2.07)	148 (89)	35.2 (32.4, 38.3)	1.94 (1.50, 2.51)
Maternal BMI (kg/m ²)						
<18.5 (underweight)	22 (13)	40.5 (30.7, 53.4)	1.16 (0.85, 1.58)	22 (14)	32.4 (25.4, 41.3)	0.97 (0.75, 1.25)
18.5–24.9 (normal weight)	112 (68)	34.9 (31.0, 39.2)	Reference	107 (66)	33.4 (30.1, 37.0)	Reference
≥25 (overweight/obese)	32 (19)	37.9 (28.0, 51.3)	1.09 (0.83, 1.42)	33 (20)	31.2 (25.3, 38.6)	0.94 (0.75, 1.16)
Marital Status						
Married	163 (94)	34.8 (31.4, 38.6)	Reference	155 (95)	32.7 (29.9, 35.7)	Reference
Never married	11 (6)	33.5 (19.3, 58.0)	0.96 (0.63, 1.46)	9 (5)	32.6 (19.1, 55.7)	1.00 (0.68, 1.46)
Maternal education						
Basic schooling	31 (18)	26.4 (20.3, 34.5)	Reference	22 (13)	21.9 (16.5, 28.9)	Reference
High school without degree	40 (23)	38.7 (29.8, 50.2)	1.46 (1.06, 2.02)	40 (24)	32.6 (27.3, 39.1)	1.49 (1.13, 1.98)
High school with degree	98 (56)	36.3 (32.2, 41.0)	1.37 (1.04, 1.81)	96 (59)	35.6 (32.3, 39.2)	1.63 (1.27, 2.09)
College/university	5 (3)	55.8 (21.8, 142.5)	2.11 (1.10, 4.04)	6 (4)	44.1 (18.3, 106.0)	2.02 (1.24, 3.28)
			p for trend=0.02 ⁴			p for trend=0.001
Parity						
0	64 (36)	42.2 (36.1, 49.2)	Reference	62 (37)	38.0 (33.3, 43.4)	Reference
1	73 (42)	32.5 (27.6, 38.2)	0.77 (0.61, 0.97)	66 (40)	29.7 (26.0, 33.8)	0.78 (0.64, 0.94)
2+	39 (22)	30.9 (24.3, 39.2)	0.73 (0.56, 0.96)	38 (23)	30.6 (25.0, 37.5)	0.80 (0.64, 1.01)
			p for trend=0.02			p for trend=0.06
Total previous nursing (months) ⁵						
0	76 (43)	41.5 (35.8, 48.1)	Reference	73 (44)	37.2 (33.0, 42.0)	Reference
>0–3	32 (18)	33.8 (26.0, 44.0)	0.82 (0.61, 1.08)	32 (19)	28.2 (23.4, 33.9)	0.76 (0.60, 0.95)
>3–12	40 (23)	29.7 (24.5, 35.9)	0.71 (0.55, 0.93)	37 (22)	31.8 (26.6, 38.1)	0.86 (0.69, 1.07)
>12	27 (15)	30.3 (21.9, 41.8)	0.73 (0.54, 0.99)	23 (14)	28.4 (21.0, 38.4)	0.76 (0.59, 0.99)
			p for trend=0.15			p for trend=0.36

Characteristic	Colostrum PFOS (n=176)			Colostrum PFOA (n=166)		
	n (%) ¹	GM (95% CI) ²	Ratio (95% CI) ³	n (%)	GM (95% CI)	Ratio (95% CI)
Inter-pregnancy interval (months) ⁶						
<18	92 (53)	35.1 (30.6, 40.1)	Reference	83 (51)	32.6 (28.7, 37.2)	Reference
18+	80 (47)	34.4 (29.3, 40.3)	0.98 (0.80, 1.20)	79 (49)	33.2 (29.5, 37.2)	1.02 (0.85, 1.21)
Maternal age at child's birth (years)						
18–25	84 (48)	33.4 (28.8, 38.8)	Reference	74 (45)	31.4 (27.6, 35.8)	Reference
26–30	61 (35)	38.9 (32.0, 47.5)	1.16 (0.93, 1.46)	61 (37)	33.1 (28.5, 38.4)	1.05 (0.87, 1.28)
31+	31 (18)	33.7 (28.0, 40.5)	1.01 (0.76, 1.34)	31 (19)	35.7 (29.8, 42.8)	1.14 (0.90, 1.44)
			p for trend=0.46			p for trend=0.15
Gestation length (weeks)						
<37	4 (2)	40.9 (9.1, 185.1)	1.16 (0.58, 2.33)	4 (2)	28.1 (7.4, 106.8)	0.85 (0.49, 1.49)
37–41	166 (95)	35.5 (31.9, 39.4)	Reference	156 (95)	33.0 (30.2, 36.1)	Reference
42	4 (2)	34.6 (9.8, 122.9)	0.98 (0.49, 1.97)	5 (3)	29.5 (20.6, 42.0)	0.89 (0.54, 1.48)
Birthweight						
<3000	48 (27)	37.5 (29.5, 47.7)	Reference	40 (24)	32.3 (26.4, 39.7)	Reference
3001–3499	63 (36)	33.7 (28.8, 39.4)	0.90 (0.69, 1.17)	62 (38)	31.7 (27.8, 36.2)	0.98 (0.78, 1.23)
3500	64 (37)	35.4 (30.1, 41.7)	0.94 (0.73, 1.23)	63 (38)	34.2 (29.8, 39.2)	1.06 (0.84, 1.32)
			p for trend=0.27			p for trend=0.81
Maternal smoking during pregnancy						
No	121 (72)	36.0 (31.8, 40.7)	Reference	117 (74)	34.1 (30.9, 37.6)	Reference
Yes	48 (28)	32.0 (26.1, 39.2)	0.89 (0.71, 1.12)	41 (26)	28.2 (23.4, 33.9)	0.83 (0.68, 1.01)
Infant sex						
Female	104 (59)	34.4 (30.0, 39.6)	Reference	91 (55)	33.5 (29.9, 37.6)	Reference
Male	72 (41)	36.6 (31.4, 42.7)	1.06 (0.90, 1.31)	75 (45)	31.9 (28.0, 36.4)	0.95 (0.80, 1.13)

¹ Percents may not sum to 100 because of rounding

² Geometric mean and 95% confidence interval of the geometric mean

³ Ratios are the exponentiated beta coefficients from a linear model with ln(PFOS) or ln(PFOA) as the outcome. Ratios represent the multiplicative difference in PFAS concentrations from the reference category.

⁴ p for trend represents the linear trend from a regression model where ln(PFAS) is a function of the quantitative (continuous) covariate.

⁵ Total previous nursing estimated for all women, where duration=0 for nulliparous women.

Inter-pregnancy interval only calculated for parous women.

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Table 5
Bivariate associations between dietary predictors and maternal colostrum PFAS concentrations.

Food group (grams/day)	Colostrum PFOS (n=172)			Colostrum PFOA (n=163)		
	n (%) ¹	GM (95% CI) ²	Ratio (95% CI) ³	n (%)	GM (95% CI)	Ratio (95% CI)
Beef						
0	86 (50)	34.5 (29.4, 40.5)	Reference	83 (50.9)	31.4 (27.6, 35.8)	Reference
>0–10	51 (30)	33.8 (28.5, 40.1)	0.98 (0.77, 1.24)	46 (28.2)	36.9 (32.0, 42.6)	1.18 (0.96, 1.44)
>10	35 (20)	37.9 (30.9, 46.5)	1.10 (0.84, 1.44)	34 (20.9)	30.5 (25.3, 36.9)	0.97 (0.78, 1.22)
			p for trend=0.56 ⁴			p for trend=0.36
Chicken						
0–<18	83 (48)	32.4 (28.2, 37.2)	Reference	78 (47.9)	31.1 (27.6, 35.0)	Reference
18–<40	59 (34)	40.3 (33.6, 48.3)	1.24 (0.99, 1.56)	58 (35.6)	37.0 (31.7, 43.1)	1.19 (0.98, 1.44)
40	30 (17)	32.6 (24.9, 42.6)	1.01 (0.76, 1.33)	27 (16.6)	29.0 (23.2, 36.2)	0.93 (0.73, 1.19)
			p for trend=0.78			p for trend=0.36
Pork						
0–<8	41 (24)	30.9 (25.9, 36.9)	Reference	38 (23.3)	30.2 (24.6, 37.1)	Reference
8–<15	43 (25)	33.5 (27.1, 41.4)	1.08 (0.81, 1.45)	41 (25.2)	32.4 (27.3, 38.4)	1.07 (0.84, 1.38)
15–<28	12 (7)	38.9 (23.5, 64.4)	1.26 (0.81, 1.95)	12 (7.4)	30.4 (20.7, 44.7)	1.01 (0.70, 1.45)
28	76 (44)	37.6 (32.0, 44.2)	1.22 (0.94, 1.58)	72 (44.2)	34.7 (30.6, 39.3)	1.15 (0.92, 1.44)
			p for trend=0.46			p for trend=0.61
Liver						
0	103 (60)	33.1 (29.2, 37.5)	Reference	99 (60.7)	30.1 (27.0, 33.5)	Reference
>0–5	41 (24)	38.5 (29.8, 49.6)	1.16 (0.91, 1.49)	37 (22.7)	38.6 (31.9, 46.7)	1.28 (1.04, 1.58)
>5	28 (16)	37.2 (29.6, 46.8)	1.13 (0.85, 1.50)	27 (16.6)	35.2 (28.1, 44.2)	1.17 (0.92, 1.48)
			p for trend=0.47			p for trend=0.27
Fresh/frozen Fish						
0	57 (33)	32.2 (27.6, 37.6)	Reference	53 (32.5)	31.5 (27.1, 36.6)	Reference
>0–10	69 (40)	33.3 (28.6, 38.8)	1.03 (0.81, 1.31)	68 (41.7)	30.2 (26.5, 34.5)	0.96 (0.79, 1.17)
>10	46 (27)	41.5 (32.6, 52.8)	1.29 (0.99, 1.67)	42 (25.8)	38.9 (32.4, 46.6)	1.23 (0.98, 1.55)
			p for trend=0.13			p for trend=0.01
Canned Fish						

Food group (grams/day)	Colostrum PFOS (n=172)				Colostrum PFOA (n=163)			
	n (%) ^f	GM (95% CI) ²	Ratio (95% CI) ³		n (%)	GM (95% CI)	Ratio (95% CI)	
0	78 (45)	34.2 (29.1, 40.1)	Reference		73 (44.8)	30.2 (26.5, 34.4)	Reference	
>0–10	58 (34)	33.4 (28.9, 38.5)	0.98 (0.77, 1.23)		56 (34.4)	34.7 (30.0, 40.3)	1.15 (0.95, 1.40)	
>10	36 (21)	39.5 (30.3, 51.4)	1.15 (0.88, 1.51)	p for trend=0.14	34 (20.9)	35.1 (28.8, 42.9)	1.16 (0.93, 1.46)	p for trend=0.17
Eggs								
0–<7	46 (27)	33.6 (28.0, 40.4)	Reference		41 (25.2)	31.3 (26.3, 37.2)	Reference	
7–<15	67 (39)	33.9 (28.2, 40.7)	1.01 (0.78, 1.30)		64 (39.3)	33.0 (28.4, 38.4)	1.06 (0.85, 1.32)	
15–<30	29 (17)	40.8 (31.4, 53.1)	1.21 (0.88, 1.67)		29 (17.8)	36.3 (30.7, 43.0)	1.16 (0.89, 1.52)	
30	30 (17)	34.1 (27.7, 42.1)	1.02 (0.74, 1.39)	p for trend=0.65	29 (17.8)	30.7 (24.7, 38.3)	0.98 (0.75, 1.29)	p for trend=0.95
Milk ⁵								
0–<65	41 (24)	40.7 (30.3, 54.5)	Reference		38 (23.3)	32.3 (26.2, 39.7)	Reference	
65–<161	36 (21)	28.4 (23.7, 34.0)	0.70 (0.52, 0.94)		31 (19)	30.1 (24.8, 36.5)	0.93 (0.72, 1.21)	
161–<300	45 (26)	29.6 (25.2, 34.8)	0.73 (0.55, 0.96)		45 (27.6)	28.1 (24.1, 32.8)	0.87 (0.69, 1.11)	
300	50 (29)	41.6 (35.4, 48.9)	1.02 (0.78, 1.35)	p for trend=0.11	49 (30.1)	40.0 (34.5, 46.3)	1.24 (0.98, 1.56)	p for trend=0.01
Yogurt								
0–<30	35 (20)	32.9 (26.1, 41.4)	Reference		35 (21.5)	26.2 (21.7, 31.7)	Reference	
30–<60	35 (20)	31.6 (24.8, 40.2)	0.96 (0.70, 1.32)		34 (20.9)	33.0 (27.0, 40.4)	1.26 (0.97, 1.63)	
60–<100	39 (23)	39.3 (33.0, 46.7)	1.19 (0.87, 1.63)		35 (21.5)	40.1 (34.4, 46.6)	1.53 (1.18, 1.98)	
100	63 (37)	35.6 (29.5, 42.9)	1.08 (0.82, 1.43)	p for trend=0.91	59 (36.2)	32.9 (28.2, 38.2)	1.25 (1.00, 1.58)	p for trend=0.84
Cheese								
0–<4	42 (24)	31.0 (25.4, 37.8)	Reference		38 (23.3)	29.3 (24.0, 35.7)	Reference	
4–<12	40 (23)	33.5 (27.1, 41.5)	1.08 (0.81, 1.45)		39 (23.9)	32.6 (28.0, 38.0)	1.11 (0.87, 1.43)	
12–<23	47 (27)	40.2 (32.1, 50.3)	1.30 (0.98, 1.72)		45 (27.6)	36.8 (30.8, 44.0)	1.26 (0.99, 1.60)	
23	43 (25)	35.1 (29.1, 42.3)	1.13 (0.85, 1.51)	p for trend=0.39	41 (25.2)	31.9 (26.8, 37.9)	1.09 (0.85, 1.39)	p for trend=0.91
Butter/Margarine								
>0–<14	43 (25)	36.5 (29.4, 45.4)	Reference		37 (22.7)	37.9 (31.6, 45.5)	Reference	

Food group (grams/day)	Colostrum PFOS (n=172)			Colostrum PFOA (n=163)		
	n (%) ¹	GM (95% CI) ²	Ratio (95% CI) ³	n (%)	GM (95% CI)	Ratio (95% CI)
14–<23	44 (26)	36.7 (29.8, 45.1)	1.00 (0.75, 1.34)	44 (27)	35.2 (29.6, 41.8)	0.93 (0.73, 1.18)
23–<31	45 (26)	36.4 (28.8, 46.0)	1.00 (0.75, 1.33)	43 (26.4)	30.6 (25.0, 37.5)	0.81 (0.63, 1.03)
31	40 (23)	30.2 (25.8, 35.4)	0.83 (0.62, 1.11)	39 (23.9)	28.1 (24.7, 31.9)	0.74 (0.58, 0.95)
Fresh Fruit/Vegetables			p for trend=0.19			p for trend=0.04
110–<289	42 (24)	37.4 (28.2, 49.6)	Reference	39 (23.9)	33.9 (27.3, 42.2)	Reference
289–<428	47 (27)	35.8 (30.0, 42.8)	0.96 (0.72, 1.27)	41 (25.2)	34.4 (29.1, 40.6)	1.01 (0.79, 1.30)
428–<610	41 (24)	34.6 (28.0, 42.7)	0.92 (0.69, 1.24)	41 (25.2)	29.3 (24.6, 35.0)	0.86 (0.67, 1.11)
610	42 (24)	32.0 (27.5, 37.3)	0.86 (0.64, 1.15)	42 (25.8)	33.5 (28.9, 38.8)	0.99 (0.77, 1.26)
Bread			p for trend=0.33			p for trend=0.83
0–80	98 (57)	35.5 (30.8, 41.0)	Reference	90 (55.2)	34.4 (30.4, 38.8)	Reference
>80–120	44 (26)	32.2 (26.7, 38.7)	0.91 (0.71, 1.15)	43 (26.4)	29.4 (25.1, 34.5)	0.86 (0.70, 1.05)
>120	30 (17)	37.4 (29.5, 47.4)	1.05 (0.80, 1.39)	30 (18.4)	32.8 (26.7, 40.2)	0.95 (0.76, 1.20)
			p for trend=0.34			p for trend=0.91

¹ Percents may not sum to 100 because of rounding

² Geometric mean and 95% confidence interval of the geometric mean

³ Ratios are the exponentiated beta coefficients from a linear model with ln(PFOS) or ln(PFOA) as the outcome. Ratios represent the multiplicative difference in PFAS concentrations from the reference category.

⁴ p for trend represents the linear trend from a regression model where ln(PFAS) is a function of the quantitative (continuous) covariate.

⁵ Liquid milk consumption; does not include milk consumption from yogurt or cheese intake.

Table 6

Multivariable determinants of colostrum PFOS and PFOA concentrations.

Determinant	% change (pg/ml) in PFOS (95% CI)	p	% change (pg/ml) in PFOA (95% CI)	p
Ethnicity				
Slovak/Other European	27.5 (−11.1, 83.0)	0.18	57.1 (18.1, 109.0)	0.002
Romani	Reference		Reference	
Parity				
Parous	−39.8 (−56.4, −16.9)	0.002	−40.4 (−53.9, −22.8)	0.006
Nulliparous	Reference		Reference	
Birth interval				
18 months	39.4 (1.4, 91.7)	0.04	41.6 (9.9, 82.5)	0.008
<18	--			
Fresh/frozen fish consumption (per 50g/day)	100.6 (−32.9, 202.9)	0.001	54.9 (−11.0, 116.1)	0.01
Birthweight (per 100g)	−2.3 (−4.2, −0.4)	0.02	--	
Fresh fruit/vegetables (100g/day)	−4.7 (−8.4, −0.7)	0.02	--	
Butter/margarine consumption (per 10g/day)	--		−5.0 (−10.2, 0.6)	0.08
Milk consumption (per liter/day)	--		22.1 (−3.1, 54.0)	0.09