Xiaofang Jia, Masahiro Tagawa, Hiroshi Yatsuya, Hisao Naito, Yumi Hayashi, Husna Yetti, Seiko Sasaki, Atsuko Araki, Chihiro Miyashita, Tamiko Ikeno, Reiko Kishi and Tamie Nakajima*

Association of maternal whole blood fatty acid status during the prenatal period with term birth dimensions: a cross-sectional study

Abstract

Objective: To investigate selected fatty acid (FA) profiles in maternal whole blood during normal pregnancy and to evaluate their associations with term birth dimensions.

Methods: We characterized nine major maternal blood FAs representing four FA families during the second and third trimester of pregnancy, and explored their associations with birth weight, length, and chest or head circumferences by multivariate regression models, using data from 318 mother-newborn pairs of the Hokkaido Study.

Results: The absolute and/or relative contents of maternal blood docosahexaenoic acid and arachidonic acid were lowest at 35–41 gestational weeks during pregnancy, as was the essential FA status index. Different from palmitic and stearic acids, palmitoleic and oleic acid contents were higher at 35–41 gestational weeks than those at 23–31 gestational weeks. Three FA components were identified through principal component analysis, and were used in association analysis. Component 3, which was positively and significantly loaded by eicosapentaenoic acid (EPA), was associated with chest circumference [β=0.281, 95% confidence interval (CI): 0.006, 0.556] at 35–41 gestational weeks (P=0.046). No significant associations were observed for Component 1 and 2 loaded by FAs except EPA.

Conclusion: Maternal blood EPA content may have an important influence on infant chest circumference.

Keywords: Association study; fatty acid status; pregnancy; term birth outcomes.

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***Corresponding author: Tamie Nakajima,** PhD, College of Life and Health Sciences, Chubu University, Kasugai 487–8501, Japan, Tel.: +81 568 51 9655, Fax: +81 568 51 5370, E-mail: tnasu23@med.nagoya-u.ac.jp; and Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan

Xiaofang Jia: Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan; and National Institute for Nutrition and Health (former National Institute for Nutrition and Food Safety), Chinese Center for Disease Control and Prevention, Beijing, China

Masahiro Tagawa, Hisao Naito and Husna Yetti: Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan

Hiroshi Yatsuya: Department of Public Health, Fujita Health University School of Medicine, Toyoake, Japan

Yumi Hayashi: Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan

Seiko Sasaki: Department of Public Health Sciences, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Atsuko Araki, Chihiro Miyashita, Tamiko Ikeno and Reiko Kishi: Center for Environmental Health and Sciences, Hokkaido University, Sapporo, Japan

Introduction

Fatty acids (FAs) are essential for life as major sources of energy and structural components of cell membranes [1]. In the human body, long chain saturated FAs (SFAs) and unsaturated FAs of the n-7 and n-9 series can be synthesized from palmitic acid (16:0) [1]. Essential FAs (EFAs) are vital for human health, but cannot be synthesized by humans. Therefore, they have to be consumed with food. There are two families (n-6 and n-3) of EFAs: the parent EFAs linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3), and their long chain polyunsaturated FA (LC-PUFA) derivatives, such as arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), respectively [1, 2].

The maternal FA status, especially of polyunsaturated FAs (PUFAs), during normal pregnancy is under active

investigation [3–5]. A longitudinal study using repeated blood samples of pregnant women from the tenth week of gestation until delivery indicated that the relative amount of LA in plasma phospholipids did not change during pregnancy, whereas that of AA decreased. The DHA steadily declined after a temporary increase until 18 weeks of gestation. The overall maternal EFA status also progressively decreased during pregnancy [3]. Several studies investigated the changes in total plasma FA profiles during pregnancy. There was a significant decrease in the proportion of n-3 PUFAs in plasma from the first to third trimester in a longitudinal study [6]. Several cross-sectional studies also reported changes in the composition of total plasmatic FAs during pregnancy: a significant increase in the proportion of palmitic acid and a significant decrease in AA occurred between the first and second trimesters, which were more marked between the second trimester and at delivery [7].

The human fetus is dependent on adequate placental transport of FAs from the maternal circulation, in addition to many other nutrients, for normal development and growth [2]. As birth dimensions have prognostic potential for later development and health [8], associations between neonatal birth dimensions at term birth and selected FA contents in phospholipids of maternal plasma throughout gestation have been increasingly investigated [9–11]. Lower concentrations of most n-3 PUFAs and higher concentrations of AA early in pregnancy were associated with lower birth weight, after adjustment for confounders [9]. Similarly, significant positive associations were observed between the proportion of DHA (especially early in pregnancy) and birth weight and head circumference [10], while the proportion of AA at late pregnancy and at delivery was negatively associated with birth weight and birth length [10]. These studies suggested that unlike AA, the proportion of DHA in maternal blood during early pregnancy may be positively associated with fetal growth. As FAs are metabolized in the body by the same enzymes, metabolic interactions are often observed [10]. Associations of birth outcomes with individual FA may be affected by metabolic interactions with other FAs. Taken together, to investigate the influence of maternal blood various FA statuses on infant birth outcomes, under the consideration of metabolic interaction of FAs, is required.

The present study aimed to investigate selected FA profiles in maternal whole blood during normal pregnancy and to evaluate their associations with term birth dimensions using a Japanese cohort of pregnant women.

Methods

General design of the study

This study was part of the "Hokkaido Study on Environment and Children's Health", a hospital-based prospective cohort study conducted by the Hokkaido University Graduate School of Medicine [12–14]. Briefly, this cohort study is based on pregnant women who delivered at the Sapporo Toho Hospital in Sapporo, Hokkaido, Japan. From July 2002 to October 2005, we approached pregnant women who were between 23 and 35 weeks of gestation, and had no serious illnesses and medical complications. All potential subjects were native Japanese living in Sapporo and the surrounding industrialized areas. The following were the exclusion criteria for study subjects: women with incomplete information regarding their partner, women who had decided to enroll in the Japanese cord blood bank, and women who had decided to deliver their baby at another hospital. Some of the women we approached did not express interest in our study, and some were unable or unwilling to participate in the study. Ultimately, 514 pregnant women were enrolled in the Sapporo cohort study by providing written informed consent. All the women took antenatal and perinatal examinations at the hospital. Medical records of the hospital were utilized to obtain maternal and infant medical information, including multiple births, infant gender, gestational age, birth weight, birth length, birth chest or head circumference, maternal age, maternal height, maternal weight before pregnancy, parity, and medical history during pregnancy. Participants also completed a self-administered questionnaire survey after the second trimester about potential confounders in relation to the past medical history of the mothers and their partners, demographic characteristics, health status during pregnancy, dietary intake during pregnancy, work history during pregnancy, smoking habits, alcohol intake, caffeine intake, household income, education level, and exposure to chemical compounds in their daily life, as described in detail elsewhere [12–14]. All this information was collected to form the Hokkaido study database.

The present study extracted relevant data of eligible mothers and their infants from the database. Measurements of selected FA contents in maternal whole blood were conducted for 493 maternal blood samples in Nagoya University. Approval for this study was obtained from the Institutional Ethical Board for Epidemiologic Studies of Hokkaido University Graduate School of Medicine and from the Ethics Review Committee of Nagoya University Graduate School of Medicine.

Blood sampling

A 40-mL blood sample was taken from the maternal peripheral vein during the antenatal hospital examinations following enrollment. If the blood could not be taken during pregnancy because of maternal anemia, it was obtained during a 1-week hospitalization after delivery. Blood was obtained once from each woman. All samples were stored at −80°C until analysis. Consistent with published reports of the "Hokkaido Study on Environment and Children's Health" [13], the blood sampling period was categorized into four groups: 23–31 weeks of gestation, 32–34 weeks of gestation, 35–41 weeks of gestation, and within a week after delivery.

FA profiles in maternal whole blood

FA levels in maternal blood were determined by GC-MS as described in detail in our earlier study [15] after extracting lipids according to the method of Folch et al. [16]. Nine FA species targeted for measurement included palmitic and stearic acids of SFAs, palmitoleic and oleic acids of monounsaturated FAs (MUFAs), LA and AA of n-6 PUFAs, and ALA, EPA and DHA of n-3 PUFAs. Under the experimental conditions, the detection limits were 2.4 μg/mL for palmitic acid, 1.3 μg/mL for stearic acid, 0.69 μg/mL for palmitoleic acid, 3.6 μg/mL for oleic acid, and 2.0 μg/mL for each of the others. The data of FAs were also included in the unpublished results of one of our previous studies.

Inclusion of participants

Ten women were excluded from the study because of miscarriage, stillbirth, moving away before delivery, or voluntary withdrawal from the study. Forty-three mother-infant pairs were excluded because: the mothers had developed pregnancy-induced hypertension (n=11) or gestational diabetes mellitus (n=1); the mothers had delivered multiple infants (n=7); infants had heart failure (n=1); or infants were born preterm (gestational age<37 weeks, n=23). One hundred and thirty-four mother-infant pairs were further excluded as maternal blood had been collected after delivery. Out of the remaining 327 mothers, we obtained whole blood FA levels from 318 subjects, as a result, leaving 318 pairs for the analysis.

Covariates

Maternal age, height, pre-pregnancy weight, parity, smoking habit and alcohol intake during pregnancy, annual household income as the socio-economic status, and the blood sampling period (gestational age at blood sampling) were maternal covariates [9, 10, 13, 14]. In addition, gestational age at birth, delivery type, and the sex of newborns were neonatal covariates [9, 10, 13, 14]. The blood sampling period was used to control variations in FA concentrations that normally occur during the course of pregnancy [3, 9]. Parity was classified into two groups: primiparous and multiparous. Both smoking and alcohol intake statuses during pregnancy were dichotomized (yes/no). Information on annual household income was obtained from a self-reported questionnaire. It was divided into four categories: ≤3 million yen, 3–5 million yen, 5–7 million yen, and ≥7 million yen. The delivery type was dichotomized: vaginal or cesarean section.

Statistical analysis

First, mean FA levels in maternal blood were calculated according to the blood sampling period in order to simulate maternal blood FA changes during pregnancy by one-way ANOVA, followed by Tukey's test for multiple comparisons. Next, the associations of maternal blood FA levels with neonatal birth dimensions such as birth weight, birth length, and chest or head circumferences were studied by linear regression analyses, including the aforementioned covariates as potential confounding factors.

We refer to the sum of the nine measured FAs as the total FA concentration. The relative proportion of a given FA to total FAs was calculated by dividing its concentration by the total concentration of FAs. The EFA status index was calculated, defined as the ratio of the sum of the n-3 and n-6 FAs to the sum of the n-7 and n-9 FAs [3]. Data of variables were presented as the mean±SEM. If the distribution of a variable was not normal, a logarithm or square root transformation was performed before analysis. Otherwise, a Kruskal-Wallis test was used if parametric test assumptions were not satisfied.

As the nine FAs were highly correlated with one another (Spearman correlation coefficient range: 0.112–0.919), principal component analysis (PCA) was performed on the correlation matrix of the nine measured FAs. Briefly, PCA is a data reduction technique that forms linear combinations of original variables into groups of correlated variables, each accounting for as much of the remaining variance of all of the FAs as possible, as reported elsewhere [17, 18]. Varimax rotations were used to obtain an orthogonal solution. The first three components were extracted from the present dataset depending on cumulatively explained total variance (>80%), whereas the rest accounted for a fraction of total variance and were excluded from the remaining analysis. The factor loadings of the nine FAs for the three components were outputted, representing the correlations of each component with its corresponding FA, and were used to calculate factor scores for each participant. Individuals with a higher score were indicated to have an FA pattern described by the component more commonly than those with a lower score. Factor scores for each component were treated as continuous variables with a nearly normal distribution. The associations of the three components and birth outcome measures were examined by simple linear regression analyses. Then, multivariate analyses were performed by consecutively including predefined sets of covariates. First, we included factor scores for other components and covariates for maternal physiological characteristics, and neonatal characteristics (Model 1). The delivery type was also included, but only for head circumference. Subsequently, we further added covariates for maternal lifestyle and socio-economic factors (Model 2). Linear regression analyses were also stratified by gestational age at blood sampling to examine the interaction with gestational age. No variables included in the multivariate models had a problem with multicollinearity according to a variance inflation factor >10. All statistical analyses were performed using SPSS 17.0 software (Chicago, IL, USA). Results were statistically significant if $P<0.05$.

Results

Characteristics of mothers and neonates

We included 318 mother-infant pairs in the study (Table 1). The differences in smoking status during pregnancy were significant among three blood sampling groups, and no differences were observed for other characteristics of mothers and neonates, which indicated that women with different blood sampling periods were almost homogenous.

Table 1 Characteristics of mothers and neonates by blood sampling period (n=318).

Characteristics	Blood sampling period (gestational weeks)			P-value
	23–31 (n=135)	32–34 (n=81)	35–41 (n=102)	
Maternal characteristics				
Age (years)[a]	30.3±0.4	29.7±0.5	29.4±0.5	0.219
Height (cm)[a]	159.0±0.4	157.9±0.6	158.2±0.5	0.242
Pre-pregnancy body weight (kg)[a,b]	53.7±0.7	53.2±0.9	51.7±0.7	0.259
Pre-pregnancy BMI (kg/m^2)[a,b]	21.2±0.3	21.3±0.3	20.7±0.3	0.245
Parity (times)[b]				
0	72 (53.3)	41 (50.6)	52 (51.0)	0.925
≥1	63 (46.7)	39 (48.1)	50 (49.0)	
Past conception (times)				
0	52 (38.5)	30 (37.0)	37 (36.3)	0.944
1	45 (33.3)	26 (32.1)	31 (30.4)	
≥2	38 (28.1)	25 (30.9)	34 (33.3)	
Education level (years)				
≤12	58 (43.0)	35 (43.2)	49 (48.0)	0.706
≥13	77 (57.0)	46 (56.8)	53 (52.0)	
Annual household income (million yen)[b]				
<3	26 (19.3)	20 (24.7)	22 (21.6)	0.880
3–5	69 (51.1)	37 (45.7)	47 (46.1)	
5–7	26 (19.3)	14 (17.3)	23 (22.5)	
≥7	13 (9.6)	10 (12.3)	9 (8.8)	
History of smoking				
Yes	79 (58.5)	49 (60.5)	57 (55.9)	0.816
No	56 (41.5)	32 (39.5)	45 (44.1)	
Smoking during pregnancy				
Yes	21 (15.6)	25 (30.9)	22 (21.6)	0.029[c]
No	114 (84.4)	56 (69.1)	80 (78.4)	
Alcohol intake during pregnancy				
Yes	49 (36.3)	26 (32.1)	31 (30.4)	0.611
No	86 (63.7)	55 (67.9)	71 (69.6)	
Alcohol intake among drinkers during pregnancy (g/day)[a]	3.7±0.7	8.0±5.8	7.2±3.4	0.737
Type of delivery				
Vaginal	113 (83.7)	67 (82.7)	93 (91.2)	0.170
Cesarean section	22 (16.3)	14 (17.3)	9 (8.8)	
Infant characteristics				
Sex				
Male	68 (50.4)	42 (51.9)	41 (40.2)	0.198
Female	67 (49.6)	39 (48.1)	61 (59.8)	
Gestational age (weeks)[a]	39.2±0.1	39.4±0.1	39.5±0.1	0.227
Birth weight (g)[a]	3097.0±31.0	3076.8±37.3	3156.8±33.2	0.277
Birth length (cm)[a]	48.2±0.2	48.3±0.2	48.4±0.2	0.771
Chest circumference (cm)[a]	31.6±0.1	31.5±0.1	31.9±0.1	0.098
Head circumference (cm)[a]	33.3±0.1	33.3±0.1	33.3±0.1	0.774

[a]Mean±SEM, otherwise n (%).

[b]The number of missing data: pre-pregnancy body weight (1 and 2 at 23–31 and 35–41 gestational weeks of blood sampling, respectively), pre-pregnancy BMI (1 and 2 at 23–31 and 35–41 gestational weeks of blood sampling, respectively), parity (1 at 32–34 gestational weeks of blood sampling), annual household income (1 at 23–31 and 35–41 gestational weeks of blood sampling, respectively).

[c]Indicates significant difference, P<0.05.

Maternal blood FA status according to different blood sampling periods

Pronounced differences in absolute FA concentrations in maternal blood were observed throughout pregnancy. Maternal blood stearic acid content at 35–41 weeks of gestation (532±18 μg/mL) was lower than that at 32–34 gestational weeks (629±24 μg/mL, P=0.011, Figure 1B). Both palmitoleic and oleic acid levels were significantly higher at 35–41 gestational weeks (143±9 μg/mL, P=0.007 and

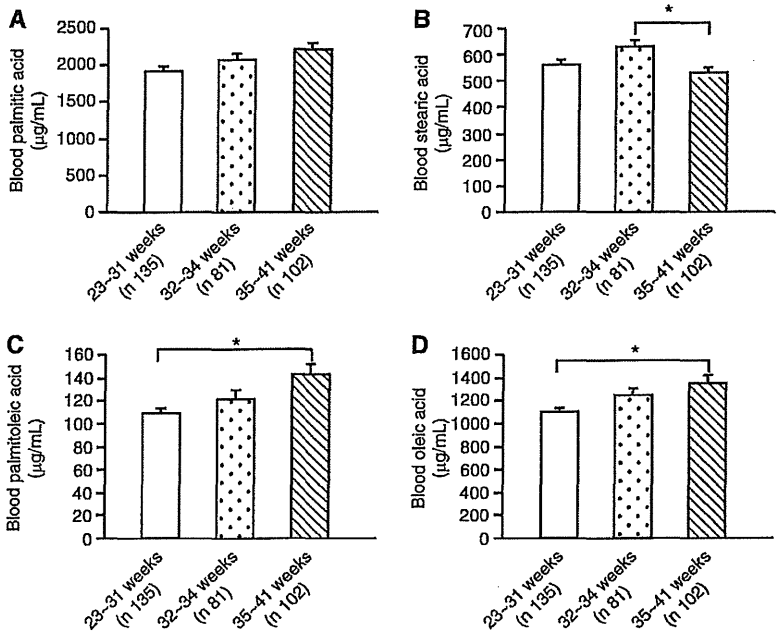

Figure 1 Concentrations of SFAs and MUFAs in maternal blood according to gestational age at blood sampling. Palmitic (A), stearic (B), palmitoleic (C) and oleic (D) acids were measured. *P<0.05. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids.

1350±70 μg/mL, P=0.010, respectively) compared to those at 23–31 weeks of gestation (109±5 μg/mL and 1095±39 μg/mL, respectively, Figure 1C and D). The DHA content at 35–41 weeks of pregnancy (25±2.0 μg/mL) was lower than that at 23–31 weeks of gestation (32±2.0 μg/mL, P=0.029, Figure 2E). Differences in the EFA status index of mothers were concordant with those observed for DHA content (Figure 2F). We also calculated FA families including SFAs, MUFAs, and n-6/n-3 PUFAs from individual FA (data not shown). The differences observed for total MUFA levels were comparable to those observed for palmitoleic and oleic acids. No significant differences were detected in the total levels of SFAs and of n-6 and n-3 PUFAs.

FA composition (% total FAs) in maternal blood during pregnancy is presented in Figures 3 and 4. The proportions of stearic acid in total FAs at 35–41 gestational weeks (11±0.3%) were lower than those at 23–31 (13±0.3%, P<0.001) and at 32–34 (13±0.4%, P<0.001) weeks of gestation (Figure 3B). Interestingly, differences in the proportion of palmitoleic acid were completely opposite to those of stearic acid concentrations in total FAs (Figure 3C). Similar to palmitoleic acid, the proportion of oleic acid was significantly higher at 35–41 weeks of gestation (26±0.3%) compared to the value at 23–31 weeks of gestation (24±0.3%, P<0.001, Figure 3D). The proportion of AA was lower at 35–41 weeks of gestation (1.2±0.1%) than at 23–31 (1.6±0.1%, P=0.006) and at 32–34 (1.6±0.1%, P=0.049) gestational weeks (Figure 4B). The proportion of DHA was lower at 35–41 weeks of gestation (0.49±0.03%) than at 23–31 (0.73±0.04%, P<0.001) or 32–34 (0.66±0.05%, P=0.021) gestational weeks (Figure 4E). We also studied changes in the proportions of FA families (data not shown). The proportion of total MUFAs sharply increased (28±0.3%, P=0.001) and was similar to the differences observed in oleic acid. The proportion of total n-3 PUFAs was lower at 35–41 weeks of gestation (0.91±0.04%) than at 23–31 gestational weeks (1.20±0.05%, P=0.002).

PCA-derived maternal blood FA components

Considering that total FA concentration was derived from the sum of all measured individual FA, rather than actually determined, the association study used absolute FA contents, not FA proportions, as independent variables. As there were strong correlations among the nine FA contents (Spearman correlation coefficient range: 0.112–0.919, data not shown), we performed PCA and identified three components which together represented 83.99% of the total variation in blood FA concentrations (Table 2). According to the factor loadings of the nine FAs for the three components, strong positive correlations (factor loadings≥approximately 0.70) were observed for Component 1 with palmitic, stearic, palmitoleic and oleic acids,

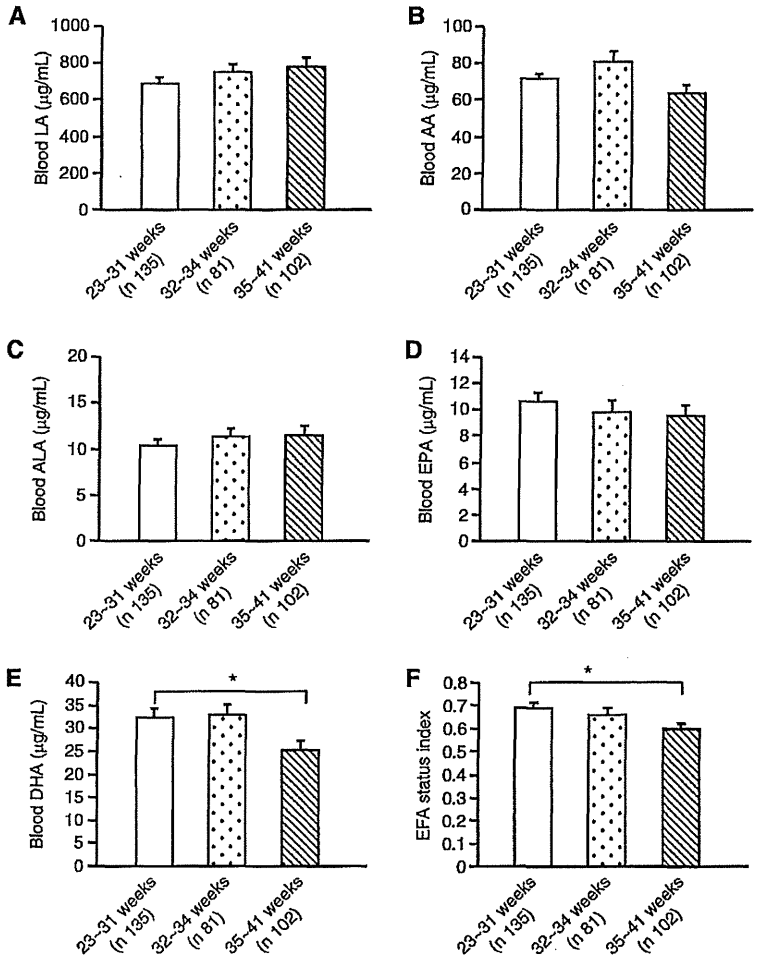

Figure 2 N-6 and n-3 PUFA concentrations in maternal blood according to gestational age at blood sampling. LA (A), AA (B), ALA (C), EPA (D) and DHA (E), and EFA status index (F) were included. *P<0.05. LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EFA, essential fatty acid.

for Component 2 with LA, AA, ALA and DHA, and for Component 3 with EPA.

Associations between term birth dimensions and PCA-derived maternal blood FA components

No significant associations were observed between any birth dimensions and FA components in maternal blood when considering mothers with various blood sampling periods as a whole (data not shown). After stratifying mothers by gestational age at blood sampling, significant associations were detected (Table 3). At 35–41 weeks of gestation, Component 3, which had strong positive correlation with EPA, was significantly and positively associated with chest circumference in the univariate model [β=0.285; 95% confidence interval (CI): 0.015, 0.554; P=0.039] and in multivariate Model 1 (β=0.281; 95% CI: 0.006, 0.556; P=0.046). The association was marginally significant in multivariate Model 2 (β=0.264; 95% CI: −0.006, 0.534; P=0.055). No significant associations were found between birth weight, birth length or head circumference and maternal blood FA components at each blood sampling period (all P>0.05, data not shown).

Discussion

Whole blood lipids are representative of the FA composition of all the circulating lipid classes, lipoproteins and

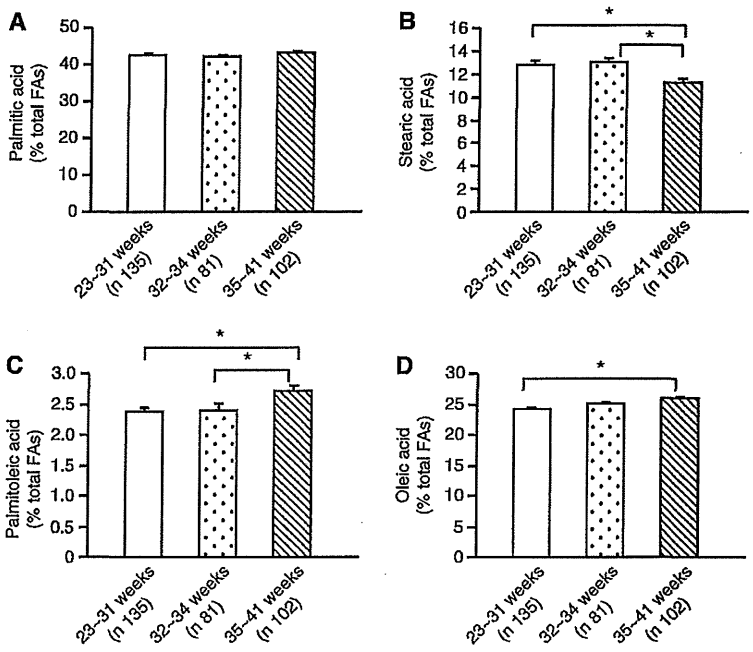

Figure 3 Compositions of SFAs and MUFAs in maternal blood according to gestational age at blood sampling. Palmitic (A), stearic (B), palmitoleic (C) and oleic (D) acids were included. *P<0.05. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids.

cells [19]. Unlike previous studies [3–5], the present study investigated the nine FAs in maternal whole blood at different gestational ages using blood samples from a Japanese pregnancy cohort. Several significant differences in absolute and relative contents of blood FAs were observed during the course of pregnancy. In contrast to stearic acid, levels of palmitoleic and oleic acids were considerably higher at 35–41 gestational weeks relative to 23–31 weeks of gestation. Interestingly, DHA content was lower at 35–41 gestational weeks than at 23–31 weeks of gestation, as was the EFA status index. Taken together, the maternal blood EFA status and DHA level seem to decrease as gestation progresses into the later period of pregnancy, which has also been demonstrated in an earlier longitudinal study [3]. We also explored potential associations between term birth dimensions, i.e., weight, length, and chest and head circumferences, and maternal blood FA status during pregnancy. At 35–41 gestational weeks, FA Component 3 was significantly and positively associated with chest circumference, suggesting a possible novel role of EPA in fetal growth.

Human brain structure, in large part, is composed of lipids (about 50–60% of dry matter) and includes high proportions of LC-PUFAs, especially DHA and AA [20]. During the last trimester of gestation, a brain growth spurt, accompanied by considerable lipid accretion, occurs in the human fetus [21]. Therefore, it is critical to preferentially transfer LC-PUFAs across the human placenta to support the rapid accretion of LC-PUFAs in nervous tissue during the period of brain growth spurt [4]. Similarly, the present study found that the absolute and relative contents of maternal blood DHA at 35–41 gestational weeks were lowest during pregnancy, as was the proportion of AA. EFA status index also indicated a lower EFA status in maternal blood in this period. Our findings were quite similar to the percentage differences found in total plasma DHA and AA during pregnancy in a cross-sectional study [7], as well as the patterns of maternal plasma phospholipid-associated DHA and AA in longitudinal studies of pregnant women [3, 4]. We interpreted that these changes in blood FA contents indicated their increased demands in later periods of pregnancy when the nervous system significantly develops.

The differences in relative SFA and MUFA compositions in the course of normal pregnancy have been examined in total plasma, or plasma phospholipids and cholesteryl esters. Relative contents of palmitic acid in maternal total plasma or plasma phospholipids were higher, while those of stearic acid were lower at delivery compared to early and middle pregnancy [4, 7, 22]. Maternal blood stearic acid content and its proportion in our study also were significantly lowered at 35–41 gestational weeks during the course

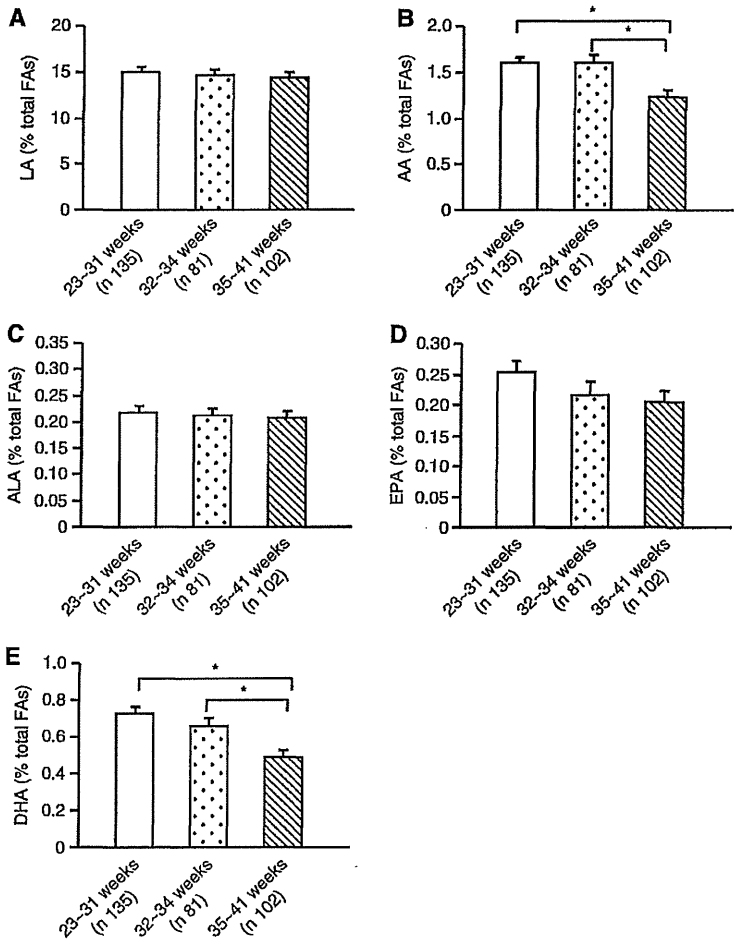

Figure 4 N-6 and n-3 PUFA Compositions in maternal blood according to gestational age at blood sampling. LA (A), AA (B), ALA (C), EPA (D) and DHA (E), were included. *P<0.05. LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FAs, fatty acids.

Table 2 Factor loadings of the nine fatty acids (FAs) for the three principal components of FA combinations identified.

	Component 1	Component 2	Component 3
Variance explained (%)	57.15	18.35	8.49
Eigenvalue	5.14	1.65	0.76
Fatty acids		Factor loadings[a]	
Palmitic acid	0.95	0.25	0.02
Stearic acid	0.70	0.27	0.22
Palmitoleic acid	0.88	0.10	0.03
Oleic acid	0.91	0.26	−0.02
Linoleic acid	0.57	0.72	−0.05
Arachidonic acid	0.21	0.89	0.20
α-linolenic acid	0.52	0.68	−0.04
EPA	0.06	0.21	0.94
DHA	0.07	0.85	0.42

[a]Factor loading denotes coefficient of the corresponding fatty acid (FA) in the linear combinations of nine FAs for the principal component, and represents the correlation of the component with the corresponding FA.

EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid.

Table 3 Associations between fatty acid principal components and chest circumference.

Gestational age at blood sampling	Independent variable	Univariate model[a]	Multivariate model[a]	
			Model 1[b]	Model 2[c]
23–31 weeks	Component 1	0.057 (−0.270, 0.383)	0.060 (−0.240, 0.360)	0.068 (−0.238, 0.373)
	Component 2	−0.057 (−0.344, 0.229)	−0.048 (−0.315, 0.218)	−0.089 (−0.365, 0.187)
	Component 3	0.002 (−0.279, 0.284)	−0.142 (−0.407, 0.122)	−0.149 (−0.419, 0.121)
32–34 weeks	Component 1	−0.077 (−0.381, 0.227)	−0.068 (−0.380, 0.245)	−0.076 (−0.376, 0.224)
	Component 2	0.118 (−0.128, 0.365)	0.106 (−0.150, 0.362)	0.156 (−0.092, 0.405)
	Component 3	−0.216 (−0.477, 0.045)	−0.185 (−0.475, 0.105)	−0.201 (−0.481, 0.079)
35–41 weeks	Component 1	−0.133 (−0.359, 0.092)	−0.128 (−0.347, 0.092)	−0.096 (−0.320, 0.127)
	Component 2	−0.212 (−0.499, 0.075)	−0.226 (−0.530, 0.079)	−0.174 (−0.475, 0.126)
	Component 3	0.285 (0.015, 0.554)[d]	0.281 (0.006, 0.556)[d]	0.264 (−0.006, 0.534)

[a]Linear regression analysis with chest circumference as dependent variable and principal component as independent variable. All values are partial regression coefficients β (95% CI), representing the expected change in chest circumference as a result of a unit change in component score.

[b]Adjusted for maternal age, height, weight before pregnancy, parity, gestational age at birth, gestational age at blood sampling, and infant gender.

[c]As in Model 1 with additional adjustment for smoking and alcohol intake during pregnancy, and annual household income.

[d]Indicates significant associations, P<0.05.

of pregnancy. Unlike aforementioned studies [4, 7, 22], no significant increment was observed in absolute and relative contents of palmitic acid during pregnancy. Additionally, the proportions of plasma cholesteryl ester-associated and total plasma oleic acid increased as gestation progressed [4, 7]. In the present study, absolute and relative amounts of blood oleic and palmitoleic acids were higher at 35–41 gestational weeks compared to 23–31 and/or 32–34 weeks of gestation. Thus, a loss of LC-PUFAs such as DHA and AA in maternal blood at 35–41 weeks of gestation might have been replaced by MUFAs rather than SFAs [4, 22].

The observed differences in maternal blood FA status during pregnancy could be related to changes in dietary intake of FAs. However, this would be unlikely as others reported that dietary habits remained unaltered during pregnancy. Neither the amount and type of fat nor the FA composition of the maternal diet changed during pregnancy until 1 month postpartum [23, 24]. Other previous studies indicated changes in estrogen levels and intrahepatic cholestasis during normal pregnancy possibly explained the altered patterns of palmitic, stearic, and oleic acids, LA and AA in maternal plasma phospholipids during pregnancy [4, 5]. This speculation remains to be clarified as we did not measure estrogen levels or markers for cholestasis.

The associations of maternal plasma EFAs and their LC-PUFA derivatives in the course of pregnancy with anthropometric parameters of newborns have been extensively investigated, especially for DHA and AA in terms of birth weight, birth length and head circumference [3, 9–11, 25]. It would be worth emphasizing that our study examined nine FAs representative for SFAs, MUFAs and PUFAs simultaneously in relation to the anthropometric parameters. Namely, in an attempt to control potential confounding of metabolic interactions among these nine FAs, multivariate association analysis using principal components derived from the nine FAs was performed. One novel finding that a unit increase in the Component 3 at 35–41 gestational weeks was associated with an increase in chest circumference might indicate potential influence of falling EPA in maternal blood as gestation processes on fetal chest circumference. The finding may be consistent with an earlier studies that low maternal plasma phospholipid-associated EPA during early pregnancy was associated with lower birth weight [9, 25]. However, caveat is needed to interpret the present finding as there could be a possibility of chance finding as a result of multiple tests done in the present study, although the comparisons had been determined before conducting the study. Also, we did not solely rely on the statistical significance of the findings, but on biological plausibility and consistency.

Meanwhile, it is not clear why only associations with chest circumference, without other birth dimensions, were observed in the present study. One speculation may be our use of PCA-derived components in the multivariate analysis instead of absolute FA levels. Another reason may be related to the fact that the present study was carried out in Japanese whose fish consumption is generally much higher than other ethnicities. Indeed, a previous study indicated that blood n-3 PUFA level was much higher in Korean people – who eat a lot of marine products, as do Japanese people – than in Americans [26]. There may be a threshold in the association between n-3

PUFA and anthropometric parameters. Finally, statistical power might have been insufficient to detect small effect so larger studies would be needed in future.

There are some limitations in this study. First, although this is a part of the established Hokkaido Cohort Study, the analyses of maternal blood FA status in the course of gestation are cross-sectional in nature. Second, this study has a small sample size that may limit its statistical efficiency. Third, selection bias may have occurred because this cohort was based in a single hospital that treated pregnant women in Sapporo and the surrounding areas. Fourth, this study lacks dietary assessment of pregnant women.

Conclusions

In conclusion, several significant differences in blood FA status were observed during normal pregnancy. In particular, the absolute and/or relative contents of DHA and AA in maternal blood were lower at 35–41 weeks of gestation, while those of MUFAs were higher. Moreover, the blood FA Component 3 representing a strong positive correlation with EPA was significantly and positively associated with chest circumference at 35–41 gestational weeks after adjustment for confounders. These results may well suggest that PUFA intake during pregnancy should be increased to meet the fetal requirement for growth and development, and that maternal blood EPA content may be involved in fetal growth.

Authors' contributions: XJ carried out the analysis, interpreted the results, and drafted the manuscript. HY carried out the analysis. MT, HN, YH, and HY participated in the measurement of maternal blood fatty acid levels. SS, AA, CM, TI, and RK participated in the design of the Hokkaido Cohort Study and carried it out. TN conceived the study, interpreted the results, and contributed to drafting the manuscript. All authors read and approved the final manuscript.

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REGULAR ARTICLE

Prenatal maternal blood triglyceride and fatty acid levels in relation to exposure to di(2-ethylhexyl)phthalate: a cross-sectional study

Xiaofang Jia · Yukiko Harada · Masahiro Tagawa · Hisao Naito · Yumi Hayashi · Husna Yetti · Masashi Kato · Seiko Sasaki · Atsuko Araki · Chihiro Miyashita · Tamiko Ikeno · Reiko Kishi · Tamie Nakajima

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Abstract
Objectives The hypolipidemic effects of di(2-ethylhexyl)phthalate (DEHP) exposure in humans have not been investigated. And the influences of maternal prenatal DEHP exposure on birth outcomes are not well-known. We aimed to estimate prenatal DEHP exposure in maternal blood, and evaluate its relationships to maternal blood triglyceride (TG) and fatty acid (FA) levels and to birth outcomes.
Methods We studied 318 mother–newborn pairs residing in Sapporo, Japan. Blood was taken one time during pregnancy for each mother. Maternal and infant characteristics were obtained from medical records and questionnaire survey. We measured DEHP metabolite, mono(2-ethylhexyl) phthalate (MEHP), along with TG and 9 FAs using maternal blood, and analyzed associations of MEHP level with maternal blood TG/FA levels and infant birth dimensions.
Results Maternal blood TG and palmitoleic/oleic acid levels were higher, but stearic/docosahexaenoic acids and MEHP were lower during late pregnancy. Maternal blood MEHP levels inversely correlated with TG and palmitic/palmitoleic/oleic/linoleic/α-linolenic acids. After adjustment for confounders, we found that a tenfold increase in blood MEHP levels correlated with a decrease in TG of 25.1 mg/dl [95 % confidence interval (CI) 4.8–45.3 mg/dl], and similar relations in palmitic ($\beta = -581.8$; 95 % CI −906.5, −257.0), oleic ($\beta = -304.2$; 95 % CI −518.0, −90.5), linoleic ($\beta = -348.6$; 95 % CI −510.6, −186.6), and α-linolenic ($\beta = -6.3$; 95 % CI −9.5, −3.0) acids. However, we observed no correlations between maternal blood MEHP levels and infant birth weight, length, chest circumference, or head circumference.
Conclusions Ambient DEHP exposure during pregnancy inversely correlated with maternal blood TG and 4 FA levels, but not birth outcomes.

X. Jia and Y. Harada have contributed equally to this work.

X. Jia · M. Tagawa · H. Naito · H. Yetti · M. Kato · T. Nakajima
Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

X. Jia
National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention, 29 Nanwei Rd., Xuanwu District, Beijing 100050, China

Y. Harada
Department of Pediatrics, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan

Y. Hayashi
Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, 1-1-20 Daiko-Minami, Higashi-ku, Nagoya 461-8673, Japan

S. Sasaki
Department of Public Health Sciences, Hokkaido University Graduate School of Medicine, Kita 15, Nish 7, Kita-ku, Sapporo 060-8638, Japan

A. Araki · C. Miyashita · T. Ikeno · R. Kishi
Center for Environmental Health and Sciences, Hokkaido University, Kita 12, Nish 7, Kita-ku, Sapporo 060-0812, Japan

T. Nakajima (✉)
College of Life and Health Sciences, Chubu University, 1200 Matsumoto-cho, Kasugai 487-8501, Japan
e-mail: tnasu23@med.nagoya-u.ac.jp

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Introduction

Di(2-ethylhexyl)phthalate (DEHP) is the most widely used plasticizer of polyvinylchloride in the manufacture of a wide variety of consumer goods, such as food packaging, building products, clothing, car products, medical devices and children's products (but not in toys intended for mouthing) [1, 2], although the use of DEHP in the latter two goods has been decreasing recently due to several government restrictions [3, 4]. DEHP is not chemically bound to polyvinylchloride and leaks from polyvinylchloride items with time and use. Consequently, it is a ubiquitous environmental contaminant [1, 2]. Ambient exposure to DEHP in the general adult population may be in the range of 3–30 μg/kg body weight/day [5], while that in Japanese pregnant women has been estimated to be 3.45–41.6 μg/kg/day [6]: no difference in the exposure levels between the former and the latter. Fujimaki et al. [6] identified that the maximum estimated intake level per body weight reached the old Tolerable Daily Intake level of 40–140 μg/kg/day set by Japanese Ministry of Health, Labour and Welfare. Importantly, the intake level was more than the latest Tolerable Daily Intake level of 30 μg/kg/day set by Food Safety Commission of Japan [7]. Therefore, ambient DEHP exposure and potential adverse effects in Japanese warrants close concerns. After entering the human body, DEHP is first metabolized to the monoester, mono(2-ethylhexyl) phthalate (MEHP), which can be oxidized further to oxidative metabolites [8].

DEHP is a known reproductive and developmental toxicant in animals [1, 5, 8]. The growing relevant toxicity reports from experimental animals, together with widespread human exposure, raise serious concerns over the potential risks from human exposure to DEHP. There have been emerging studies conducted of human health outcomes in relation to prenatal DEHP exposure in recent years. Prenatal DEHP exposure has been associated with shorter gestational age at birth [9, 10], lower mental and psychomotor development indices [11], and lower birth weight [12]. Moreover, prenatal DEHP exposure was reported to correlate with shorter anogenital distance, reduced penile size and incomplete testicular descent [13, 14], more non-optimal reflexes [15] and reduced masculine play behavior [16] in male infants, suggesting that possible sex difference exists in DEHP toxicity. Additionally, our group recently reported that maternal exposure to DEHP decreased plasma levels of triglycerides (TG) [1] and four fatty acid (FA) components [17] in prepartum mice, including palmitic acid, oleic acid, linoleic acid (LA), and α-linolenic acid (ALA), which was suspected to correlate with adverse effects of DEHP. However, no information regarding humans is available.

To date, the potential health hazards from exposure to DEHP and/or its main metabolite MEHP in humans at risk, such as pregnant women and infants, have not been well-documented and warrant extensive investigation. Thus, this study aimed to estimate MEHP levels in the blood of pregnant women as a biomarker of ambient DEHP exposure, and to evaluate potential associations with blood levels of TG and FAs in pregnant women and term birth outcomes in infants.

Materials and methods

Study population

This study was part of the "Hokkaido Study on Environment and Children's Health" [18–20]. Briefly, from July 2002 to October 2005, we approached pregnant women who were at 23–35 weeks of gestation and had no serious illnesses and medical complications to register with a hospital-based prospective cohort study at the Sapporo Toho Hospital in Sapporo, Hokkaido, Japan. The following were the exclusion criteria for study subjects: women with incomplete partner's information, women who had decided to enroll in the Japanese cord blood bank, or women who had decided to deliver the baby at another hospital. Some of the women we approached did not express interest in our study, and some were unable or unwilling to participate in the study. Ultimately, 514 pregnant women (30 % of those approached) were enrolled in this study by providing written informed consent. Maternal and infant medical information were obtained from medical records of antenatal and perinatal examinations at the hospital. A self-administered questionnaire survey was completed after the second trimester to collect potential confounders, as described in detail elsewhere [18–20]. This study was approved by the Institutional Ethical Board for Epidemiologic studies of Hokkaido University Graduate School of Medicine, and Ethics Review Committee of Nagoya University Graduate School of Medicine.

Blood sampling

A blood sample of approximately 40 ml was taken from the maternal peripheral vein at the time of the next prenatal hospital examination after recruitment. If the blood could not be taken during pregnancy due to maternal anemia, it was obtained during hospitalization within a week after delivery. As blood was obtained one time for each woman, the analyses using blood samples were cross-sectional in

nature. Consistent with published reports of "Hokkaido Study on Environment and Children's Health" [19], the blood sampling period was categorized into four groups: 23–31 weeks of gestation, 32–34 weeks of gestation, 35–41 weeks of gestation, within a week after delivery. All samples were stored at −80 °C until analysis.

MEHP level in maternal blood

Blood samples (30 μl) were mixed with 120 μl 1 N HCl, 350 μl saturated saline solution and 50 μl of 10 μM MEHP-d as an internal standard. MEHP was then extracted twice with 500 μl ethyl acetate after shaking for 15 min. The ethyl acetate layer was evaporated, and the sediments were dissolved into 40 μl ethyl acetate. After adding 20 μl *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide, the tube was left at room temperature for 60 min, and the MEHP tert-butyldimethylsilyl derivative concentration formed was measured by gas chromatography–mass spectrometry (GC/MS) (6890N, 5973N; Agilent Technologies, CA, USA) under the analytical conditions mentioned previously by Ito et al. [21]. For each sample, duplicate analysis was performed. Ultimately, MEHP levels were available from 493 maternal blood samples. The detection limit of MEHP was 1 pmol/ml. Coefficient of variation (CV) of MEHP measurements within a day was 2.0–7.8 % for 6 days, and CV of day to day for 6 days was 6.2 % at 5 pmol/ml of concentration.

TG concentration in maternal blood

The TG level in blood was measured using TG-IE kits (Wako, Osaka, Japan) after extracting lipids as described by Folch et al. [22].

FA profiles in maternal blood

FA levels in maternal blood were determined duplicately by GC–MS as described in detail in our earlier study [17] after extracting lipids according to the method of Folch et al. [22]. Nine FA species targeted for measurement included palmitic and stearic acids of saturated FAs, palmitoleic and oleic acids of monounsaturated FAs, LA and arachidonic acid (AA) of the n-6 family, and ALA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of the n-3 family. Under the experimental conditions, the detection limits were 2.4 μg/ml for palmitic acid, 1.3 μg/ml for stearic acid, 0.69 μg/ml for palmitoleic acid, 3.6 μg/ml for oleic acid, and 2.0 μg/ml for the others.

Data analysis

Ten registered women were excluded due to miscarriage, stillbirth, relocation, or voluntary withdrawal from the study before follow-up. The following subjects were excluded from analysis: those with maternal pregnancy-induced hypertension ($n = 11$), diabetes mellitus ($n = 1$), fetal heart failure ($n = 1$), or multiple births ($n = 7$). We also excluded premature births ($n = 23$)—defined as birth at less than 37 weeks of gestation—from the data analysis to keep the focus on fetal growth [19, 23], and excluded postnatal blood samplings ($n = 134$) from the analysis to focus on prenatal subjects, resulting in a sample size of 327. Ultimately, the sample size available for analysis from the 327 was 318 subjects who completed the measurements of MEHP, TG and FAs.

Correlations between MEHP concentration or TG and FA levels in maternal blood and characteristics of subjects were analyzed by Spearman's rank correlation test, Mann–Whitney *U* test and Kruskal–Wallis test. Finally, unadjusted and multivariable-adjusted linear regression analyses were performed to evaluate the association between MEHP levels and concentrations of TG and FAs in maternal blood. TG and FA components were dependent variables; MEHP level was independent variable. In the multivariable-adjusted models, maternal age [24], smoking and alcohol intake during pregnancy [25], inshore fish and deep-sea fish intake during pregnancy [24], and the blood sampling period (based on the correlation analyses) were included as potential confounders. We also performed unadjusted and multivariable-adjusted linear regression analyses to evaluate the association between maternal blood MEHP levels and birth outcome measures. Birth weight, birth length, chest circumference or head circumference were dependent variables; maternal blood MEHP was independent variable. In the multivariable-adjusted models, maternal age, height and pre-pregnancy weight, parity, smoking and alcohol intake during pregnancy, socioeconomic status (annual household income), gestational age, infant sex [19, 20, 23], and the blood sampling period were included as potential confounders. For head circumference, the adjusted model also included delivery type [19]. The linear regression analyses were also stratified by infant gender to clarify the interaction with infant gender. Because of the skewed distribution, we treated maternal blood MEHP level as a continuous variable on a $\log_{10}$ scale. All statistical analyses were performed using SPSS software. Results were statistically significant if $p < 0.05$.

Results

Maternal and infant characteristics and their association with maternal blood MEHP level

We included 318 mother-infant pairs in the study (Table 1). The women aged less than 30 years accounted for 48.1 %. Approximately, 51.9 % of the women were primiparous, and 37.4 % of them did not conceive before.

Table 1 Maternal and infant characteristics, and their association with maternal blood MEHP level ($n = 318$)

Characteristics	n (%)	MEHP (nmol/ml)[a]	p value
Maternal characteristics			
Age (years)			
<30	153 (48.1)	0.040	0.959
≥30	165 (51.9)	0.038	
Height (cm)			
<158.0	138 (43.4)	0.037	0.164
≥158.0	180 (56.6)	0.041	
Pre-pregnancy body weight (kg)[b]			
<52	152 (47.8)	0.041	0.726
≥52	163 (51.3)	0.037	
Pre-pregnancy BMI (kg/m^2)[b]			
<20.6	163 (51.3)	0.039	0.990
≥20.6	152 (47.8)	0.037	
Parity (times)[b]			
0	165 (51.9)	0.038	0.479
≥1	152 (47.8)	0.040	
Past conception (times)			
0	119 (37.4)	0.038	0.710
1	102 (32.1)	0.042	
≥2	97 (30.5)	0.038	
Educational level (years)			
≤12	142 (44.7)	0.038	0.590
≥13	176 (55.3)	0.040	
Annual household income (million yen)[b]			
<3	68 (21.4)	0.040	0.332
3–5	153 (48.1)	0.033	
5–7	63 (19.8)	0.042	
≥7	32 (10.1)	0.045	
History of smoking			
Yes	185 (58.2)	0.038	0.657
No	133 (41.8)	0.041	
Smoking during pregnancy			
Yes	68 (21.4)	0.035	0.438
No	250 (78.6)	0.039	
Alcohol intake during pregnancy			
Yes	106 (33.3)	0.039	0.797
No	212 (66.7)	0.038	
Alcohol intake among drinkers during pregnancy (g/day)			
<1.5	53 (50.0)	0.038	0.284
≥1.5	53 (50.0)	0.042	
Caffeine intake during pregnancy (mg/day)			
<116.5	159 (50.0)	0.036	0.062
≥116.5	159 (50.0)	0.041	
Fish intake during pregnancy			
Inshore fish			
≤1–2 times/month	174 (54.7)	0.037	0.513
≥1–2 times/week	144 (45.3)	0.042	
Deep-sea fish			
≤1–2 times/month	155 (48.7)	0.037	0.355
≥1–2 times/week	163 (51.3)	0.041	
Blood sampling period (gestational weeks)			
23–31	135 (42.5)	0.043	0.002[c]
32–34	81 (25.5)	0.046	
35–41	102 (32.1)	0.028	
Type of delivery			
Vaginal	273 (85.8)	0.039	0.916
Cesarean section	45 (14.2)	0.045	
Infant characteristics			
Sex			
Male	151 (47.5)	0.038	0.374
Female	167 (52.5)	0.041	
Gestational age (weeks)			
≤39	171 (53.8)	0.040	0.990
≥40	147 (46.2)	0.038	
Birth weight (g)			
<3098.0	157 (49.4)	0.041	0.588
≥3098.0	161 (50.6)	0.037	
Birth length (cm)			
<48.3	156 (49.1)	0.038	0.458
≥48.3	162 (50.9)	0.039	
Chest circumference (cm)			
<31.5	121 (38.1)	0.040	0.534
≥31.5	197 (61.9)	0.038	

Table 1 continued

Characteristics	*n* (%)	MEHP (nmol/ml)[a]	*p* value
Head circumference (cm)			
<33.2	157 (49.4)	0.039	0.934
≥33.2	161 (50.6)	0.039	

BMI body mass index, *MEHP* mono(2-ethylhexyl) phthalate, *SD* standard deviation

[a] Median

[b] Missing data: pre-pregnancy body weight (3), pre-pregnancy BMI (3), parity (1), annual household income (2)

[c] p value <0.05, indicating statistically significant correlation

Mothers had more than 13 years of education (55.3 %), and 3–5 million yen of annual household income (48.1 %). Mothers who smoked during pregnancy were 21.4, and 33.3 % had alcohol intake during pregnancy. The number of women who ate inshore fish and deep-sea fish at least 1–2 times/week was 144 (45.3 %) and 163 (51.3 %), respectively. Blood sampling was conducted during 23–31 gestational weeks for 42.5 % of women. Two-hundred seventy-three women had vaginal births (85.8 %). One hundred and fifty-one infants (47.5 %) were male, and the gestational age of 53.8 % pregnancy was less than 40 weeks. The number of infants with birth weight, birth length, chest circumference and head circumference less than 3098.0 g, 48.3, 31.5 and 33.2 cm, respectively, was 157 (49.4 %), 156 (49.1 %), 121 (38.1 %), and 157 (49.4 %), respectively. Additionally, we observed statistically significant differences in medians of MEHP levels by blood sampling period ($p = 0.002$), but did not find significant correlations with other characteristics. Thus, we adjusted the blood sampling period in the multivariate regression models.

MEHP level in blood of pregnant women

The mean (± SD) MEHP level was 0.049 ± 0.040 nmol/ml, ranging from 0.007 to 0.316 nmol/ml with a median of 0.039 nmol/ml.

TG and FA levels in maternal blood

Mean (± SD) TG was 94.1 ± 53.7 mg/dl (Table 2). Mean palmitic and stearic acids were 2052.4 (±853.4) and 569.1 (±206.3) μg/ml, respectively. Mean palmitoleic and oleic acids were 122.9 (±75.8) and 1215.5 (±562.7) μg/ml, respectively. Mean LA and AA were 735.6 (±426.6) and 71.4 (±42.6) μg/ml, respectively. Mean ALA, EPA, and DHA were 11.0 (±8.5), 10.0 (±8.4), and 30.2 (±21.8) μg/ml, respectively. Altogether, the mean (± SD) total FAs was 4818.2 ± 1982.0 μg/ml. Highly abundant and common FAs from the total FA pool were palmitic acid (42.8 %), oleic acid (25.0 %), LA (14.8 %), and stearic acid (12.4 %).

Table 2 Concentrations of TG and FA components in maternal blood ($n = 318$)

Parameters	Mean ± SD
TG (mg/dl)	94.1 ± 53.7
FA components (μg/ml)	
Palmitic acid	2052.4 ± 853.4 (42.8)[b]
Stearic acid	569.1 ± 206.3 (12.4)[b]
Palmitoleic acid	122.9 ± 75.8 (2.5)[b]
Oleic acid	1215.5 ± 562.7 (25.0)[b]
LA	735.6 ± 426.6 (14.8)[b]
AA	71.4 ± 42.6 (1.5)[b]
ALA	11.0 ± 8.5 (0.2)[b]
EPA	10.0 ± 8.4 (0.2)[b]
DHA	30.2 ± 21.8 (0.6)[b]
Total FAs (μg/ml)[a]	4818.2 ± 1982.0

AA arachidonic acid, *ALA* α-linolenic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *FA* fatty acid, *LA* linoleic acid, *SD* standard deviation, *TG* triglycerides

[a] Total FAs correspond to the sum of all FA measured

[b] Values in parentheses denote the percentage of each FA in total FAs

TG and FA relationships to subject characteristics

The relationships between TG and FA levels and subject characteristics, which were potential confounders of TG and FAs, were checked (data not shown). We found significant differences in the ALA level by maternal age ($p = 0.044$), in the EPA level by inshore fish intake ($p = 0.001$), and in TG ($p < 0.001$), stearic acid ($p = 0.002$), palmitoleic acid ($p = 0.012$), oleic acid ($p = 0.003$), and DHA ($p = 0.020$) levels by the blood sampling period. Specially, except for stearic acid and DHA, the levels of TG, palmitoleic and oleic acids increased in the late gestational age when blood sampling.

Relationships between MEHP levels and TG/FA concentrations in maternal blood

Table 3 showed univariate and multivariate regression model results for maternal blood TG and FAs on $\log_{10}$-transformed MEHP concentration. Multivariate models of TG and FAs were adjusted for confounders correlated with TG and FAs at p values <0.05, factors known to be related to TG and FAs from previous reports [24, 25] and the blood sampling period. In the crude model, we found significant negative correlations of MEHP exposure with TG, palmitic

Table 3 Regression coefficients (95 % confidence interval, CI) between $\log_{10}$-transformed MEHP level (nmol/ml) and TG/FA in maternal blood

Dependent variable	Crude model β^c (95 % CI)	Adjusted model β^c (95 % CI)
TG (mg/dl)[a]	−30.4 (−51.0, −9.8)	−25.1 (−45.3, −4.8)
Palmitic acid (μg/ml)[a]	−635.7 (−959.6, −311.7)	−581.8 (−906.5, −257.0)
Stearic acid (μg/ml)[a]	−33.3 (−113.3, 46.8)	−38.8 (−119.9, 42.3)
Palmitoleic acid (μg/ml)[a]	−34.1 (−63.3, −5.0)	−27.5 (−56.6, 1.6)
Oleic acid (μg/ml)[a]	−355.8 (−570.7, −140.8)	−304.2 (−518.0, −90.5)
LA (μg/ml)[a]	−363.0 (−523.8, −202.2)	−348.6 (−510.6, −186.6)
AA (μg/ml)[a]	−8.3 (−24.8, 8.2)	−9.8 (−26.5, 6.9)
ALA (μg/ml)[a]	−6.4 (−9.7, −3.2)	−6.3 (−9.5, −3.0)
EPA (μg/ml)[b]	0.8 (−2.5, 4.1)	0.4 (−2.9, 3.6)
DHA (μg/ml)[b]	4.9 (−3.6, 13.4)	3.3 (−5.2, 11.8)

AA arachidonic acid, *ALA* α-linolenic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *FA* fatty acid, *LA* linoleic acid, *TG* triglycerides

[a] Adjusted for blood sampling period, maternal age, smoking and alcohol intake during pregnancy

[b] Adjusted for blood sampling period, maternal age, smoking and alcohol intake during pregnancy, inshore fish and deep-sea fish intake during pregnancy

[c] Partial regression coefficient represents the expected change in dependent variables as a result of a tenfold change in MEHP level, because MEHP level was $\log_{10}$-transformed

acid, palmitoleic acid, oleic acid, LA and ALA in maternal blood. After full adjustment for potential confounders, we found that a tenfold increase in MEHP levels correlated with a decrease in TG of 25.1 mg/dl [95 % confidence interval (CI), 4.8–45.3 mg/dl], and similar relations in palmitic acid ($\beta = -581.8$; 95 % CI −906.5, −257.0), oleic acid ($\beta = -304.2$; 95 % CI −518.0, −90.5), LA ($\beta = -348.6$; 95 % CI −510.6, −186.6), and ALA ($\beta = -6.3$; 95 % CI −9.5, −3.0). We also evaluated the relationships of MEHP with TG and FA levels in maternal blood by blood sampling periods (Table 4). Maternal blood MEHP level significantly and negatively associated with palmitic acid, oleic acid, LA and ALA levels at 23–31 weeks of gestation, and TG, palmitic acid, palmitoleic acid, oleic acid, LA and ALA levels at 32–34 weeks in univariate and multivariate regression models. MEHP level in maternal blood negatively associated with LA in univariate regression model at 35–41 weeks, which was insignificant after adjusting for confounders.

Relationships between maternal blood MEHP levels and infant birth outcomes

Table 5 showed the results of univariate and multivariate regression analyses for the association between maternal blood MEHP levels and birth weight or birth size of infants. Multivariate models were adjusted for known risk factors correlated with birth outcomes from previous reports [19, 20, 23] and the blood sampling period. No significant associations with birth weight, birth length, chest circumference, or head circumference were found for prenatal MEHP level in maternal blood in all infants, males or females.

Discussion

To our knowledge, this study was the first to focus on the potential effects of DEHP exposure on blood levels of TG and FA components in pregnant women, and to find significant inverse associations between DEHP principal metabolite MEHP levels, and TG, palmitic acid, oleic acid, LA and ALA levels in maternal blood after adjustment for confounders, using a Japanese pregnancy cohort. TG is an ester derived from glycerol and three FAs. Since palmitic acid, oleic acid and LA were predominant FA components, accounting for 82.6 % of the total FA pool, it was conceivable that these three FAs also similarly declined upon DEHP exposure, as TG decreased. These findings were quite similar to the effects of DEHP exposure on TG and FA levels in the plasma of prepartum mice in our earlier study [1, 17]. We also observed significant differences in maternal blood MEHP, TG, stearic/palmitoleic/oleic acid and DHA levels by blood sampling period (gestational weeks): MEHP, stearic acid and DHA were lower, whereas TG and palmitoleic/oleic acids were higher, in late gestation (35–41 weeks). Additionally, maternal plasma free FAs are an important source of essential FAs to the developing fetus [26]. Although MEHP exposure negatively correlated with TG and several FAs in maternal

Table 4 Regression coefficients (95 % confidence interval, CI) between $\log_{10}$-transformed MEHP level (nmol/ml) and TG/FA in maternal blood by blood sampling periods

Dependent variable	23–31 weeks (n = 135)		32–34 weeks (n = 81)		35–41 weeks (n = 102)	
	Crude model β^c (95 % CI)	Adjusted model β^c (95 % CI)	Crude model β^c (95 % CI)	Adjusted model β^c (95 % CI)	Crude model β^c (95 % CI)	Adjusted model β^c (95 % CI)
TG (mg/dl)[a]	−17.8 (−44.1, 8.5)	−19.9 (−46.3, 6.6)	−72.1 (−116.1, −28.0)	−71.3 (−115.0, −27.6)	0.7 (−38.7, 40.2)	5.0 (−34.9, 44.9)
Palmitic acid (μg/ml)[a]	−553.3 (−1033.4, −73.2)	−601.1 (−1080.5, −121.6)	−932.9 (−1482.1, −383.6)	−923.5 (−1488.1, −358.8)	−391.8 (−1062.3, 278.7)	−263.2 (−928.7, 402.3)
Stearic acid (μg/ml)[a]	−68.5 (−208.1, 71.0)	−75.7 (−216.69, 65.2)	−27.9 (−196.1, 140.2)	−26.0 (−199.0, 146.9)	−59.8 (−177.8, 58.2)	−44.2 (−163.0, 74.7)
Palmitoleic acid (μg/ml)[a]	−29.9 (−67.9, 8.1)	−32.2 (−70.6, 6.2)	−59.4 (−113.3, −5.4)	−58.9 (−114.2, −3.5)	−0.8 (−62.6, 61.0)	13.0 (−47.8, 73.8)
Oleic acid (μg/ml)[a]	−357.7 (−647.4, −67.9)	−385.1 (−675.3, −94.9)	−445.2 (−813.4, −77.1)	−450.0 (−827.2, −72.2)	−178.4 (−640.9, 284.1)	−101.1 (−564.1, 361.9)
LA (μg/ml)[a]	−385.2 (−630.9, −139.5)	−396.9 (−642.5, −151.4)	−370.5 (−661.7, −79.2)	−394.3 (−686.8, −101.8)	−323.8 (−645.8, −1.8)	−280.9 (−603.3, 41.6)
AA (μg/ml)[a]	−19.7 (−45.9, 6.4)	−19.5 (−45.3, 6.3)	−26.0 (−63.7, 11.6)	−26.7 (−65.3, 12.0)	4.4 (−21.5, 30.4)	6.6 (−19.6, 32.7)
ALA (μg/ml)[a]	−5.9 (−11.1, −0.7)	−6.1 (−11.3, −0.8)	−7.6 (−13.4, −1.9)	−7.8 (−13.6, −2.1)	−6.2 (−12.4, 0.0)	−4.8 (−10.8, 1.2)
EPA (μg/ml)[b]	−0.5 (−6.3, 5.2)	−1.0 (−6.7, 4.7)	−1.1 (−7.8, 5.7)	−1.1 (−7.8, 5.6)	2.9 (−2.2, 8.0)	3.0 (−2.3, 8.2)
DHA (μg/ml)[b]	1.0 (−14.1, 16.2)	1.0 (−14.0, 15.9)	−5.8 (−23.0, 11.4)	−6.7 (−24.8, 11.3)	10.8 (−1.7, 23.2)	11.9 (−0.9, 24.7)

AA arachidonic acid, *ALA* α-linolenic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *FA* fatty acid, *LA* linoleic acid, *TG* triglycerides

[a] Adjusted for maternal age, smoking and alcohol intake during pregnancy

[b] Adjusted for maternal age, smoking and alcohol intake during pregnancy, inshore fish and deep-sea fish intake during pregnancy

[c] Partial regression coefficient represents the expected change in dependent variables as a result of a tenfold change in MEHP level, because MEHP level was $\log_{10}$-transformed

Table 5 Regression coefficients (95 % confidence interval, CI) between maternal blood $\log_{10}$-transformed MEHP concentration (nmol/ml) and infant birth outcomes

Dependent variable	Overall[a] (n = 318)		Male[b] (n = 151)		Female[b] (n = 167)	
	Crude model β^c (95 % CI)	Adjusted model β^c (95 % CI)	Crude model β^c (95 % CI)	Adjusted model β^c (95 % CI)	Crude model β^c (95 % CI)	Adjusted model β^c (95 % CI)
Birth weight (g)	−77.7 (−212.3, 56.8)	−62.6 (−189.6, 64.4)	−28.1 (−249.1, 192.9)	−27.5 (−249.4, 194.4)	−101.2 (−272.5, 70.0)	−74.6 (−232.6, 83.5)
Birth length (cm)	−0.098 (−0.801, 0.605)	0.081 (−0.593, 0.755)	−0.022 (−1.311, 1.268)	−0.014 (−1.283, 1.256)	−0.046 (−0.815, 0.723)	0.072 (−0.673, 0.816)
Chest circumference (cm)	−0.181 (−0.746, 0.384)	−0.149 (−0.691, 0.393)	0.010 (−0.830, 0.850)	−0.162 (−1.004, 0.681)	−0.271 (−1.048, 0.506)	−0.075 (−0.819, 0.669)
Head circumference (cm)	0.079 (−0.392, 0.550)	0.117 (−0.343, 0.578)	0.402 (−0.368, 1.172)	0.244 (−0.558, 1.046)	−0.013 (−0.582, 0.557)	0.037 (−0.551, 0.626)

[a] Adjusted for maternal age, height, weight before pregnancy, parity, smoking and alcohol intake status during pregnancy, annual household income, gestational age, infant gender and blood sampling period in a multiple linear regression model. For head circumference, adjusted model also included delivery type

[b] Adjusted for maternal age, height, weight before pregnancy, parity, smoking and alcohol intake status during pregnancy, annual household income, gestational age and blood sampling period in a multiple linear regression model. For head circumference, adjusted model also included delivery type

[c] Partial regression coefficient represents the expected change in dependent variables as a result of a tenfold change in MEHP level, because MEHP level was $\log_{10}$-transformed

blood, no significant relationships of MEHP with birth weight and birth size of newborns were detected in the present study.

Maternal hypertriglyceridemia appears late in normal pregnancy [27]. DHA status steadily declines after a temporary increase until 18 weeks of gestation in maternal blood throughout normal pregnancy [28]. Here, maternal blood TG and palmitoleic/oleic acid levels were indeed significantly higher, whereas DHA was lower in women during late gestation (35–41 weeks) as the blood sampling period proceeded. Maternal physiological changes that occur normally in early pregnancy but are most pronounced in the third trimester have the potential to alter xenobiotic distribution and elimination [29]. The lower maternal blood MEHP level in late gestation most likely corresponded with increased renal blood flow and glomerular filtration rate, and unchanged renal tubular resorption, which enhanced the clearance of MEHP through the kidney. Additionally, the activity of hepatic DEHP metabolizing enzymes may have changed during pregnancy, which warrants further study. Taking the various alterations in maternal blood TG/FA concentrations during pregnancy into consideration, we did not consider that great influences by increased plasma volume and total body water would be likely.

The hypolipidemic effects of DEHP were first described in rats and mice by Reddy et al. [30]. Other studies confirmed the hypotriglyceridemic effects of DEHP in rats [31–33] and pregnant mice [1]. Most importantly, and in keeping with our finding of hypotriglyceridemic effects after DEHP exposure in pregnant mice [1], we further found that dietary DEHP exposure significantly reduced plasma levels of palmitic acid, oleic acid, LA and ALA in these pregnant mice with the same experimental protocol [17]. In humans, the maternal FA/lipid homeostasis environment may have dramatic changes upon exposure to DEHP during pregnancy, e.g. the composition and distribution of maternal FAs in the blood. However, no information is available, even though exposure to DEHP is common in the general population. In the present study, it was noteworthy that after adjustment for confounders including the blood sampling period, MEHP levels inversely correlated with TG, palmitic acid, oleic acid, LA and ALA levels in maternal blood. Moreover, maternal blood MEHP level inversely associated with palmitic acid, oleic acid, LA and ALA at 23–31 weeks of gestation, as well as TG, palmitic acid, palmitoleic acid, oleic acid, LA and ALA at 32–34 weeks in multivariate models. Short chain FAs with less than 20 carbon atoms may be susceptible to DEHP exposure in pregnant women. Among them, ALA may be more sensitive to DEHP, even though it accounted for only 0.2 % of total FAs. These findings were quite similar to those from pregnant mice [1, 17]. On the other

hand, at 35–41 weeks of gestation, no such relationships between maternal blood MEHP and TG/some FAs were observed. This may be due to significant low concentrations of MEHP in blood compared to those of 23–34 weeks of gestation, which mitigates the effects of DEHP on TG or FAs. Of course, the exact reason must be warranted. Therefore, we could not conclude whether effects of DEHP exposure on maternal blood TG/some FA levels are related to the physiological status of pregnancy or not. To answer this question, similar epidemiological study using non-pregnant women is also required.

The effects of DEHP on TG and FA levels were observed at lower exposure level than that of birth defects: DEHP exposure decreased ALA concentration in blood of wild-type mice by 50 % at a dose of 0.01 % [17], however, birth defects were observed at 0.05 % DEHP [1]. Plasma MEHP concentrations of mice at 0.01 % DEHP exposure were 0.98 ± 0.36 nmol/ml (unpublished data). The concentrations of MEHP in blood of pregnant women were 0.049 ± 0.040 nmol/ml (0.039 nmol/ml of median, ranging from 0.007 to 0.316 nmol/ml) in the present study. Lipase activity for DEHP was fivefold higher in mice than that of non-pregnant women [34]. Body burden in the pregnant women with highest MEHP level may be roughly estimated to be similar to that of mice exposed to 0.01 % DEHP. Therefore, it may be plausible that DEHP at the exposure levels influenced some FA levels in blood of pregnant women of current study. Of course, further study is warranted to clarify the exact exposure level of pregnant women. Ait Bamai et al. [35] compared DEHP level in floor dust among several countries and reported that the levels in Sapporo, Japan, where the current study was conducted, were higher than those in USA, Germany and Denmark. Although it is questionable that whether DEHP levels in the floor dust reflects the exact body burden in humans, the subjects in the current study may be exposed to relatively high level of DEHP. The present study not only for the first time observed the hypolipidemic effects of DEHP exposure in pregnant women, but got such findings at a mean level of MEHP exposure (0.049 ± 0.040 nmol/ml, that is 0.014 ± 0.011 μg/ml), lower than a mean MEHP concentration of 0.68 ± 0.85 μg/ml reported in the human maternal plasma at term in healthy subjects [36].

We further considered the possible mechanisms by which maternal MEHP level negatively correlated with blood levels of TG and four individual FAs, although the present study did not conduct mechanism study. It is well-understood that administration of DEHP to rodents in vivo and in vitro produces pleiotropic response in the liver, which is responsible for hypolipidemic effects [1, 37, 38], especially, a hallmark response of peroxisome proliferation to DEHP in the liver [30], the activation of peroxisome proliferator-activated receptor α (PPARα)-dependent hepatic FA catabolism by DEHP exposure [39], and a decrease in microsomal triglyceride transfer protein-mediated TG transport from liver to the blood after DEHP exposure [1]. Conversely, limited DEHP-specific human data are available. Previous study further found that distinct from wild-type mice expressing PPARα in several organs including liver, DEHP exposure did not influence the plasma levels of palmitic acid, oleic acid, LA and ALA in pregnant humanized PPARα and PPARα knockout mice [17]. Humanized PPARα mice over-expressed human PPARα only in liver, suggesting that PPARα in other organs but not in liver or species difference of PPARα function may be involved in the influence of DEHP on blood TG and FAs in the pregnant women. It may be difficult to infer the mechanism of DEHP influences on TG or FAs in pregnant women from our previous animal studies [1, 17]. Thus, studies aimed at elucidating the mechanism by which DEHP exposure inversely correlated to TG and FA levels in blood of pregnant women are required.

The growth and development of the fetus and its organs depend on a sufficient supply of nutrients including FAs and lipids crossing the placenta, and fetal growth determines the birth outcomes of newborns [40]. Given the significant negative correlations of DEHP exposure with maternal blood TG and four FA levels in this Japanese pregnancy cohort, we further evaluated the effects of maternal DEHP exposure on term birth outcomes of newborns. However, we did not find any significant relationships to birth weight, birth length, chest circumference, or head circumference in univariate and multivariate linear regression analyses. No abnormal birth outcomes were noted in newborns whose mothers had a relatively greater exposure to MEHP (mean ± SD, 0.68 ± 0.85 μg/ml in maternal plasma) during the prenatal period in Italy [36]. However, under a higher maternal MEHP exposure (e.g. median, 2.9 mg/L in maternal blood with low body weight infants), the cord blood MEHP level was associated with low birth weight in a nested case–control study of Chinese newborns [12], where a higher median MEHP (2.5 mg/l) level was found in cord blood with low birth weight infants. These varied findings do not necessarily support the consideration of race difference. The insignificant association in the present study was primarily related to lower MEHP exposure level, which further lowered during late pregnancy. MEHP effects on fetal growth and infant birth outcomes may occur at a much higher exposure level. On the other hand, maternal plasma levels of AA, EPA and DHA during pregnancy were reported to be associated with birth weight, birth length or head circumference [23, 41], while maternal blood MEHP level had no influences on maternal blood levels of these long-chain polyunsaturated FAs in the present study, as a result, MEHP did not influence birth outcomes.