analysis, decision to publish, or preparation of the manuscript.

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Introduction

The ratio of the 2nd finger to 4th finger lengths (2D/4D) in humans has been reported to be smaller in males than in females [1]. This sexual difference has been attributed to the prenatal hormonal environment, such as exposure to higher levels of androgens and some other gonad-specific hormones [2] through androgen receptors, which are located in fetal cartilaginous tissue [3]. This hypothesis for the underlying mechanism for this difference is supported by the following findings; lower 2D/4D in girls with congenital adrenal hyperplasia [4], higher 2D/4D in individuals with complete androgen insensitivity syndrome [5], and the existence of a relationship between 2D/4D and polymorphisms in androgen receptors [6].

Prenatal exposure to sex hormones is known to affect human development, including that of the fetal digits, and one of the most important periods for the fetus is from the first to second trimester of pregnancy. Although most organ systems are developing during this period, the endocrine control systems have already been formed. The sexual difference in 2D/4D has already been established during early prenatal development under the influence of sex hormones [7, 8], and 2D/4D is considered to be stable after the early prenatal stages. Therefore, 2D/4D has been used as an easily measurable and stable anthropometric index of prenatal androgen exposure. However, the mechanism responsible for the sexual difference in 2D/4D has not yet been elucidated in detail.

There is currently no established approach for measuring prenatal hormone exposure when investigating the relationship between 2D/4D and the hormonal environment earlier in pregnancy in order to elucidate the mechanism underlying the sexual difference in 2D/4D; measuring prenatal hormone levels is difficult and not feasible for ethical reasons during a normal pregnancy. On the other hand, umbilical cord blood is obtained immediately after delivery, and its hormone levels are broadly considered to reflect the hormonal environment of the fetus at late gestation [9, 10]. Previous studies have been performed using cord blood to investigate the relationship between fetal hormonal exposure and human development [11–13].

In the present study, as a part of the Sapporo Cohort, Hokkaido Study on Environment and Child Health [14, 15], we investigated whether sex hormone levels in cord blood influenced 2D/4D in school-aged children.

Participants and Methods

Participants

This prospective birth cohort study was based on the Sapporo Cohort, Hokkaido Study on Environment and Child Health [14, 15]. Study details regarding the population, data collection, sampling of biological specimens, and contents of the questionnaire have been described previously [14, 15]. Briefly, native Japanese women living in Sapporo City or its surrounding areas were enrolled into the study at 23–35 weeks of gestation at Sapporo Toho Hospital between July 2002 and October 2005. Of the 1796 women approached, 25% were excluded as they decided to enroll in the Japanese cord blood bank or deliver the baby at another hospital; therefore, 514 pregnant women were enrolled in this cohort study (participation rate of 28.6%).

This study was approved by the Institutional Ethical Board for Epidemiological Studies at Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environmental and Health Sciences. All participants provided written informed consent. Informed consent on behalf of the children enrolled was provided by their parents.

Measurement of 2D/4D

Ten out of 514 participants were excluded from the study due to miscarriage, stillbirth, relocation, or voluntary withdrawal from the study before delivery. As 7 sets of twins were born, a total of 511 children (246 males and 265 females) were finally included in the Sapporo Cohort study. Of these, 350 children (68.1%), who are currently school-aged and could be contacted for this survey, were requested via a mail to send black-and-white photocopies of the palms of both the left and right hands. Measurements of digits were made from photocopies of the ventral surface of the right and left hands. The participants were instructed to straighten their fingers and lightly place their hands palm down on the photocopy machine. Measurements were made to the nearest 0.5 mm from the mid-point of the finger crease proximal to the palm to the tip of the finger using steel Vernier calipers. The ratio was calculated by dividing the length of the second digit by that of the fourth digit[1]. All measurements were taken twice by two observers blinded to participants' information in order to confirm the measurements obtained.

Sex hormone measurements in cord blood samples

At the time of delivery, a blood sample of 10–30mL was collected from the umbilical cord and stored at -80°C for later analysis.

The following hormone levels in 294 stored cord blood samples (135 boys and 159 girls) were measured. Testosterone (T), estradiol (E), and progesterone (P) levels were measured using LC-MS/MS [16, 17]. An immunoradiometric assay was used to measure luteinizing hormone (LH) (Spac-S LH Kit, TFB, Inc., Tokyo Japan) and follicle-stimulating hormone (FSH) levels (Spac-S FSH Kit, TFB, Inc., Tokyo Japan). Inhibin B levels were measured using an enzyme-linked immunosorbent assay (Inhibin B Gen II ELISA, Beckman Coulter, Inc., CA, USA). An enzyme immunoassay (Insulin-like 3 (INSL3) / RLF (Human)—EIA Kit, Phoenix Pharmaceuticals, Inc. CA, USA) was used to measure INSL3 levels. INSL3 was measured in males because it reflects Leydig cell function. It was also measured in 20 randomly selected samples from females. All sex hormone measurements were performed by Aska Pharma Medical Co., Ltd. (Kanagawa, Japan).

Statistical analyses

Data on the characteristics of participants, 2D/4D, and sex hormone levels were presented as a group mean ± standard deviation and were analyzed between groups using a one-way ANOVA. Sex hormones were converted to a log10 scale as these data did not fall into a normal distribution. A half of the detection limit was used when levels were below the detection limit for individual hormones. The relationship between 2D/4D and sex hormone levels in cord blood samples was calculated using a multiple linear regression analysis. The inclusion of covariates was based on biological considerations and adjustments were made for maternal age (continuous), birth weight (continuous), maternal smoking during pregnancy (yes or no), and maternal alcohol consumption during pregnancy (yes or no). All statistical analyses were performed using JMP pro 10 (SAS institute Inc., NC, USA), except for the intra-class correlation coefficient for right and left 2D/4D measurements, which was calculated using SPSS statistics version 19 (IBM, IL, USA). Significance levels were set to 0.05 for all comparisons.

Results

1) Patient characteristics

A total of 190 children from the 189 participants, including 88 males and 102 females, sent back photocopies of their palms. The characteristics of the participants and their children who

sent back photocopies for 2D/4D were compared to their children without 2D/4D. 2D/4D was derived from the following participants; older mothers, a higher annual household income, higher educational level, and fewer smokers among family members. No significant differences were observed in gender, birth weight, or gestational age (Table 1).

2) 2D/4D

In all right hand, left hand, and mean values, 2D/4D was significantly higher in females than in males (Fig. 1). 2D/4D fell into a normal distribution in all right hand, left hand, and mean values.

The intra-class correlation coefficient (1, 2) for right and left 2D/4D measurements was 0.720 (95% confidence interval: 0.627–0.789). The mean 2D/4D value in both hands was used to determine its relationship with sex hormones as a representative value of each participant.

3) Sex hormones in cord blood samples

T, E, P, and INSL3 were detected in all samples. INSL3 was only measured in 20 randomly selected samples from females. The detection percentages of LH in males and females were 25.7% and 0.7%, respectively, while those of FSH in males and females were 46.8% and 0%, respectively. Inhibin B was detected in 99.2% of males and 26% of females (Table 2). The mean intra-assay and inter-assay coefficients of variations in terms of sex hormone measurements were as follows; T: 1.4%–5.3%, E: 3.2%–11.3%, P: 2.7%–6.3%, LH: 4.8%–6.5%, FSH: 2.3%–3.7%, Inhibin B: < 3.8%, and INSL3: 1%–5% in the mean intra-assay coefficients of variations, and T: 3.4%–5.1%, E:

Table 1. Patient characteristics.

		2D/4D (+)		2D/4D (-)		
		n	Mean ± SD	n	Mean ± SD	
Maternal characteristics						
Age at delivery (years old)		189	31.4 ± 4.2	315	30.7 ± 5.2	**
Pre-pregnancy BMI (m^2/kg)		189	21.0 ± 3.1	315	21.6 ± 3.4	
Parity	Primiparous	92 (48.7)		148 (47.0)		
	Multiparous	97 (51.3)		167 (53.0)		
Annual house hold income (million yen per year)	<5	108 (57.1)		237 (75.2)		**
	≥5	81 (42.9)		78 (24.8)		
Educational level (years)	≤12	58 (30.7)		166 (52.7)		**
	≥13	131 (69.3)		149 (47.3)		
Smoking during pregnancy	Nonsmoker	174 (92.1)		232 (73.7)		**
	Smoker	12 (7.9)		83 (26.3)		
Alcohol consumption during pregnancy	Nondrinker	120 (63.5)		235 (74.6)		
	Drinker	69 (36.5)		80 (25.4)		
Infant characteristics						
Gender	Males	88 (46.3)		158 (49.2)		
	Females	102 (53.7)		163 (50.8)		
Birth weight (g)		190	3037.6 ± 379.7	321	3003.9 ± 444.5	
Gestational age (weeks)		190	38.9 ± 1.5	321	38.6 ± 1.6	

The values in brackets represent percentages.
**: p<0.01.

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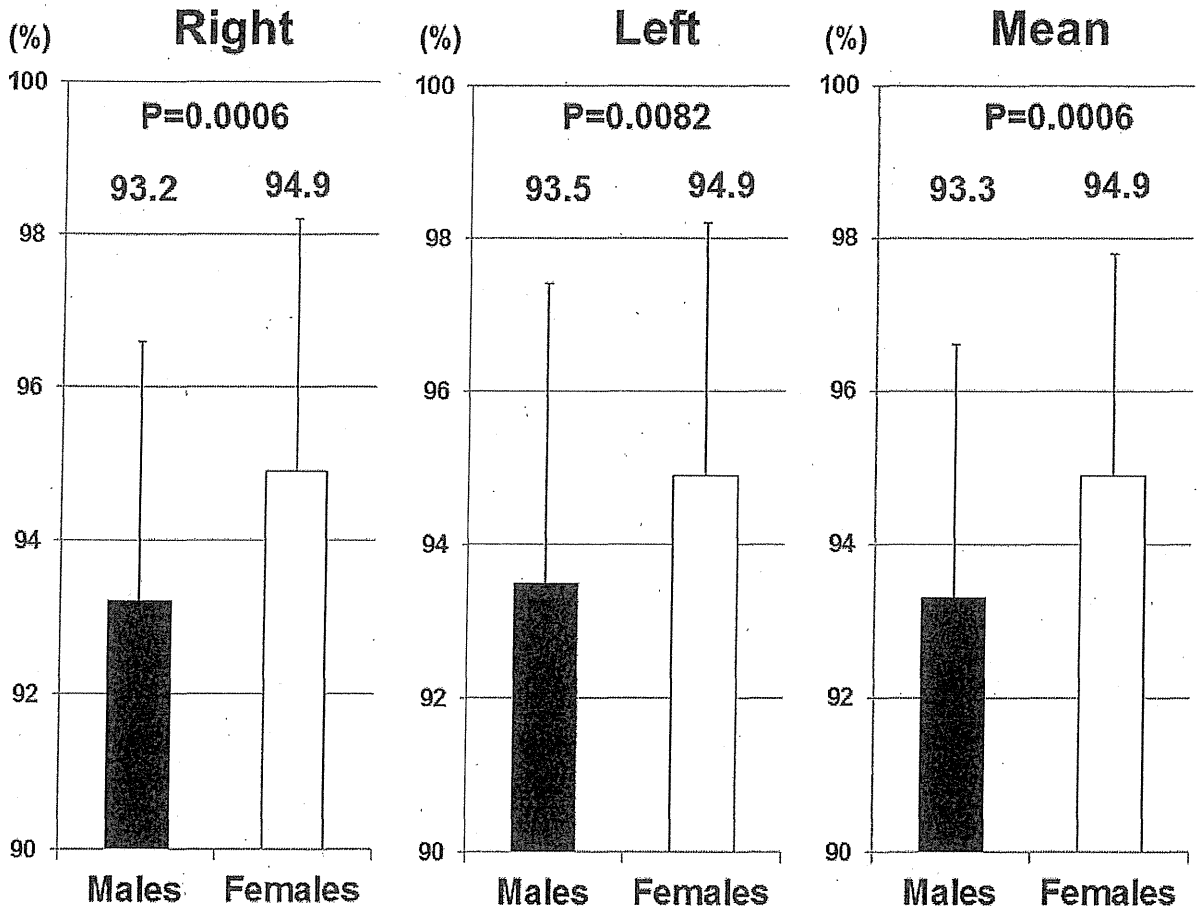

Fig 1. 2D/4D in right hands, left hands, and mean values. 2D/4D in right hands, left hands, and mean values were significantly higher in females than in males.

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Table 2. Sex hormone levels in cord blood in males and females.

		Males				Females				
	DL	n	50th	25th-75th	>DL (%)	n	50th	25th-75th	>DL (%)	p-value
Testosterone (pg/mL)		135	98.9	76.5–126	100	156	69.9	51.9–96.3	100	<0.001
Estradiol (ng/mL)		135	4.86	3.33–7.42	100	159	4.67	3.15–6.48	100	0.227
Progesterone (ng/mL)		135	226	184–286	100	159	210	167–276	100	0.184
T/E		135	18.5	13.9–25.7	100	156	15.9	11.8–21.8	100	0.002
LH (mIU/mL)	0.5	132	<DL	<DL-0.82	25.7	155	<DL	<DL-<DL	0.7	<0.001
FSH (mIU/mL)	0.5	132	<DL	<DL-0.66	46.8	154	<DL	<DL-<DL	0.0	<0.001
Inhibin B (pg/mL)	11	134	44.0	33.9–58.3	99.2	159	<DL	<DL-11.8	26.0	<0.001
INSL3 (ng/mL)	0.01	132	0.29	0.25–0.34	100	20	0.18	0.17–0.23	100	<0.001

DL: detection limit.

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4.8%–9.5%, P: 4.7%–6.0%, LH: 7.2%–26.0%, FSH: 5.4%–6.7%, Inhibin B: < 5.6%, and INSL3: 6%–15.0% in the mean inter-assay coefficients of variations.

The median concentrations of T, LH, FSH, Inhibin B, and INSL3, which indicate androgen activity, were significantly higher in males than in females (Table 2).

4) Relationship between 2D/4D and sex hormones

No significant differences were observed in the hormone levels of children who sent back photocopies for 2D/4D and those who did not (Table 3).

A multivariate regression model showed that 2D/4D negatively correlated with INSL3 only in males. Regarding the other sex hormones in both males and females, no correlations were observed with 2D/4D (Table 4). The application of 0.32 ng/mL of INSL3 from the receiver operating characteristic curve as a cut-off value revealed that 2D/4D was significantly higher in males with <0.32 ng/mL of INSL3 ($p<0.01$) (Fig. 2). This result indicated that 2D/4D could be affected by prenatal Leydig cell function.

Table 3. Sex hormones in cord blood and 2D/4D.

	Males					Females				
	2D/4D (+)		2D/4D (-)			2D/4D (+)		2D/4D (-)		
	n	50th Min Max	n	50th Min Max	p-value	n	50th Min Max	n	50th Min Max	p-value
Testosterone (pg/mL)	45	90.9 12.2 483	90	101 5.45 620	0.240	69	64.9 12.3 457	87	71.3 6.25 168	0.255
Estradiol (ng/mL)	45	4.05 1.91 26.6	90	5.38 0.01 33.5	0.200	72	4.86 1.66 31.2	87	4.42 1.44 17.4	0.143
Progesterone (ng/mL)	45	183 13.7 455	90	234 0.43 471	0.378	72	201 6.25 467	87	216 8.86 514	0.457
T/E	45	21.7 2.05 52.1	90	17.5 2.73 21839	0.477	69	15.7 1.9 47.6	87	15.7 0.68 40.3	0.424
LH (mIU/mL)	45	<DL <DL 2.39	87	<DL <DL 3.37	0.986	70	<DL <DL 0.61	85	<DL <DL <DL	0.263
FSH (mIU/mL)	45	<DL <DL 1.43	87	<DL <DL 1.89	0.765	72	<DL <DL <DL	82	<DL <DL <DL	N/A
Inhibin B (pg/mL)	44	43.3 <DL 90.6	90	<DL <DL 104	0.957	72	<DL <DL 76.6	87	<DL <DL 65.7	0.947
INSL3 (ng/mL)	44	0.28 0.1 0.48	88	0.29 0.07 0.75	0.454		N/A N/A		N/A N/A	

N/A: not applicable.

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Table 4. Relationship between 2D/4D and sex hormones in cord blood.

Hormone levels	Total			Males			Females		
	n	B (95%CI)	R^2	n	B (95%CI)	R^2	n	B (95%CI)	R^2
T (pg/mL)	114	-0.021 (-2.449, 1.956)	0.113	45	-0.209 (-8.080, 1.754)	0.060	69	0.151 (-0.835, 3.909)	0.214
E (ng/mL)	117	-0.070 (-2.893, 1.257)	0.111	45	-0.051 (-4.956, 3.625)	0.022	72	-0.104 (-3.346, 1.219)	0.180
P (ng/mL)	117	0.036 (-1.323, 1.977)	0.107	45	-0.020 (-4.461, 3.971)	0.020	72	0.078 (-1.114, 3.647)	0.175
T/E	114	0.010 (-2.259, 2.514)	0.113	45	-0.138 (-6.331, 2.650)	0.036	69	0.200 (-0.440, 5.190)	0.228
LH (mIU/mL)	115	0.017 (-2.167, 2.610)	0.104	45	0.207 (-1.335, 5.346)	0.055	70	0.126 (-6.313, 21.64)	0.180
FSH (mIU/mL)	117	-0.038 (-3.696, 2.448)	0.105	45	0.180 (-2.162, 7.177)	0.048		N/A	N/A
INSL3 (ng/mL)		N/A N/A	N/A	44	-0.377* (-30.17, -2.318)	0.145		N/A N/A	N/A
Inhibin B (pg/mL)	116	-0.139 (-2.238, 0.331)	0.124	44	-0.068 (-5.877, 3.891)	0.024	72	-0.082 (-1.387, 2.732)	0.172

*: $p<0.05$,
N/A: not applicable.

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Discussion

In the present study, the ratio of the digit length of the 2nd finger to that of the 4th finger, which has been used as an easily measurable and stable anthropometric index of prenatal exposure to androgens, was calculated in school-aged children, and sex hormone levels in cord blood samples were then measured. The levels of sex hormones indicating androgen activity in cord blood were significantly higher in males than in females. 2D/4D was significantly higher in females than in males, and negatively correlated with INSL3 only in males.

The biosynthesis of testosterone hypothetically occurs at a gestational age of 9 weeks, whereas 2D/4D dimorphism appears as early as at 14 weeks of gestation [7, 8], which indicated that early levels of sex hormones can influence 2D/4D. A previous study reported that 2D/4D reflected a genetic background subjected to a given level of exposure to prenatal androgens [1]. A gestational peak in testosterone production due to the development of Leydig cells occurred between 14 and 18 weeks. Thus, compelling evidence currently shows that 2D/4D is affected by prenatal exposure to androgens in humans.

In the present study, we used the mean 2D/4D value in both hands as a representative value of each participant, as previously reported, because the influence of the stronger side of the hands in 2D/4D on correlations with any factors has not yet been established and the intraclass correlation coefficient (1, 2) for right and left 2D/4D measurements was 0.720 (95% confidence interval: 0.627–0.789). 2D/4D in the left hand negatively correlated with INSL3 (β = -0.414, p = 0.0125), whereas 2D/4D in the right hand was not correlated with INSL3 (β = -0.268, p = 0.1093). We attributed these differences in 2D/4D between the right and left hands to various factors including measurement errors, the relatively small sample size, and the

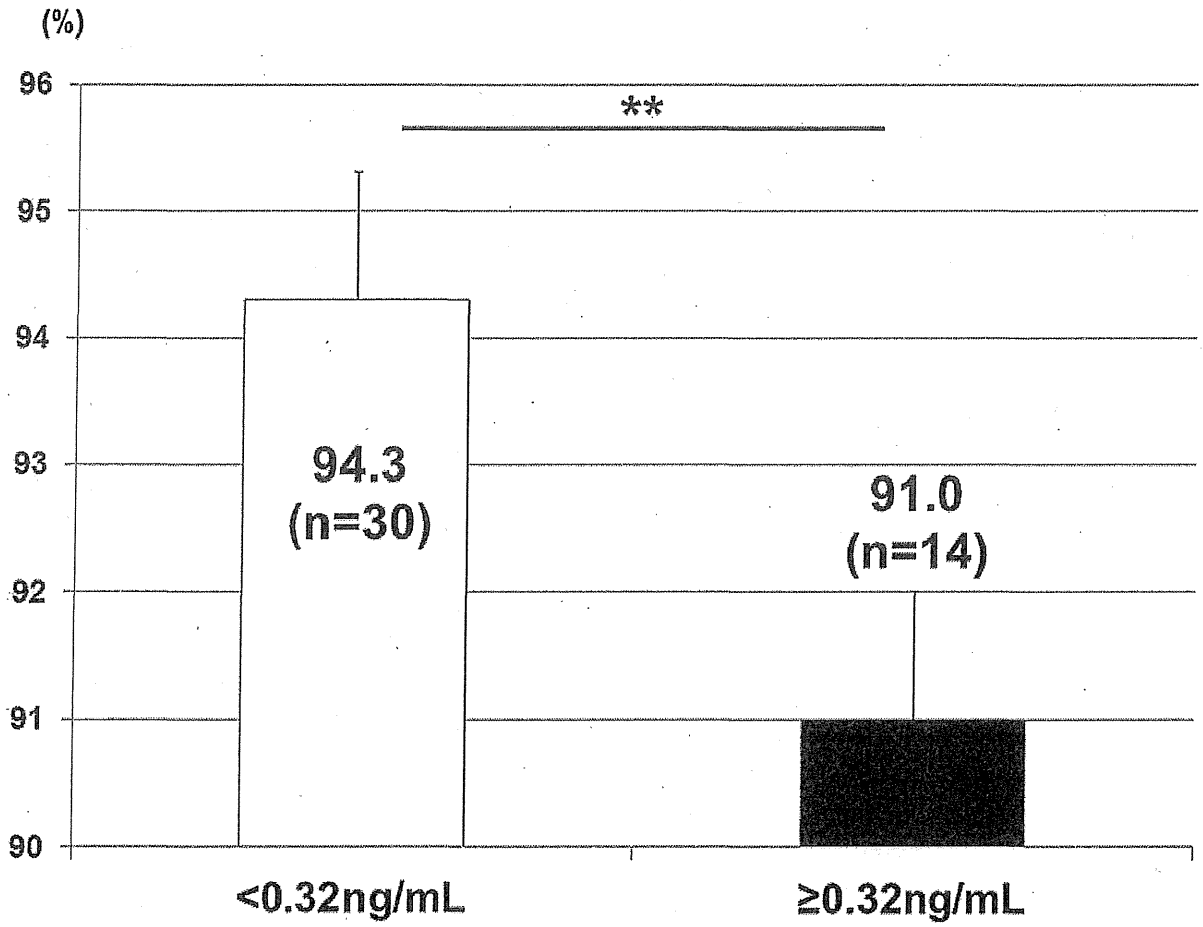

Fig 2. 2D/4D and INSL3. 2D/4D was significantly higher in males with <0.32 ng/mL of INSL3 in cord blood ($p<0.01$). **: $p<0.01$.

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limitations associated with physical measurements. Thus, we considered it reasonable to use the mean value of 2D/4D as a representative value of each participant.

In the present study, no correlation was observed between the level of testosterone in cord blood and 2D/4D. This result was compatible with previous findings, which demonstrated that the concentration of testosterone in cord blood could not predict 2D/4D[18]. Furthermore, a previous study suggested that amniotic fluid, but not cord blood, was the best candidate for investigating the effects of early fetal exposure to androgens [19]. These findings taken together with our results indicated that testosterone in cord blood did not influence 2D:4D or reflect fetal exposure during the critical period of digit development at approximately 14 weeks of gestation. The measurement of sex hormones in cord blood may be affected by obstetric and maternal factors, such as prematurity, labor onset, placental weight, intrauterine infection, and preeclampsia, which have not yet been established in detail [9].

INSL3 levels in cord blood samples correlated with 2D/4D in males. INSL3 is constitutively produced by Leydig cells in the fetal testis, not by other organs, after sex determination [20], and is a gender-specific fetal hormone. The fetal testis is established at approximately 7 weeks of pregnancy and the *INSL3* gene in fetal Leydig cells is detectable by 8–10 weeks of pregnancy

in humans [21]. This period of transition from the first to the second trimester is important for development, and is very vulnerable to a range of endocrine-disrupting insults to male reproductive development. Thus, the detection of INSL3 in fetal blood during mid-gestation reliably indicates a male fetal gender [21]. INSL3 in cord blood reflects prenatal Leydig cell function, which serves in the production of testosterone, and may also reflect androgen exposure during the important developmental window of earlier pregnancy for the digits as well as male reproductive development. In the present study, a correlation was observed between INSL3, but not testosterone, in cord blood and 2D/4D, and a previous study also demonstrated that 2D/4D was significantly related to adult testosterone levels and the presence of testosterone deficiency syndrome [22].

No correlation was noted between other hormones with androgen activity, such as LH, FSH, and Inhibin B, and 2D/4D. This may have been due to more than 50% of the stored cord blood samples being below the detection limit for LH and FSH. Therefore, more sensitive kits are needed to measure LH and FSH. Since Inhibin B reflects Sertoli cell function, its levels may not directly indicate androgen exposure *in utero* for digit development. Furthermore, a previous study using mice showed that receptors for androgen and estrogen were particularly located in the 4^{th} digit and the growth of this digit was stimulated by androgen, but arrested by estrogen[23]. Although it has already been reported that 2D/4D cannot be determined by prenatal testosterone alone and the balance between prenatal testosterone and prenatal estrogen is another important factor in fetal digit development [24], our results showed that T/E in cord blood did not correlate with 2D/4D. Thus, the present study revealed that only prenatal Leydig cell function, indicating early exposure during gestation to androgens, could be implicated in 2D/4D.

As one of factors that affects sex hormones during gestation, endocrine-disrupting chemicals, e.g. phthalates, dioxins, polychlorinated biphenyls (PCBs), and perfluorinated alkyl acids (PFAAs), have been shown to induce a broad spectrum of toxic effects on the reproductive system and genital development in the prenatal period in humans. Our cohort study already demonstrated that maternal exposure to phthalates reduced the levels of T/E, P, inhibin B, and INSL3 in cord blood, suggesting that exposure to DEHP *in utero* may have adverse effects on both Sertoli and Leydig cell development in males [25]. Previous studies also revealed that other endocrine-disrupting chemicals affected the hormonal environment during the prenatal period in humans. Cao et al. demonstrated that maternal exposure to dioxins decreased T and E in cord blood [26]. Furthermore, Hsu et al. showed that maternal exposure to PCBs decreased T/E in boys at puberty [27]. Regarding PFAAs, Vested et al. reported that maternal exposure to perfluorooctane sulfonate (PFOS) during gestation decreased the concentration and counts of sperm and increased LH and FSH levels in males after puberty, suggesting that maternal exposure to PFOS may affect semen quality and reproductive hormone levels in adult human males. Thus, maternal exposure to endocrine-disrupting chemicals influences sex hormones during gestation, as demonstrated by anti-androgen activity in males. These findings indicate that maternal exposure to endocrine-disrupting chemicals affects sex hormone levels during gestation and induces physical changes to the digits of children. An animal study has already showed that prenatal exposure to low doses of endocrine-disrupting chemicals induced feminized digit ratios in male rats[28]. Further studies are warranted to confirm this in humans.

Polymorphisms in androgen receptors (AR) may also affect sensitivity to androgen exposure in 2D/4D. AR are produced by the AR gene, which is located on the X-chromosome and repeats the nucleotide sequence CAG on exon 1. Furthermore, the number of CAG repeats varies in length among individuals and code for the length of a polyglutamine stretch on the N-terminal domain of AR. Although previous studies revealed that there was no evidence for a

clear association between CAG repeats and 2D/4D [29, 30], the synergic effects of polymorphisms in AR and sex hormones in cord blood on 2D/4D remain unclear. Therefore, further investigations are needed in our cohort study.

The first limitation of this study was that we performed multiple analyses, which are associated with the risk of false positives in the main result of a correlation between 2D:4D and INSL3. The second limitation of this study was the relatively small cohort of school-aged children for whom we had data on both 2D:4D and sex hormones because only 190 (54.3%) of 350 children sent photocopies of their palms for the measurement of 2D/4D. Larger studies are needed to reveal the effects of sex hormone levels *in utero* on physical changes to children.

Conclusions

The levels of sex hormones indicating androgen activity in cord blood were significantly higher in males than in females. 2D/4D in school-aged children, which was significantly lower in boys than in girls, was affected by prenatal Leydig cell function in males.

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Author Contributions

Conceived and designed the experiments: TM AA RK KN. Performed the experiments: TM AA AI S. Sato CM SI TK K. Moriya KC K. Morioka RK KN. Analyzed the data: TM AA AI S. Sato CM SI TK K. Moriya KC K. Morioka RK KN. Contributed reagents/materials/analysis tools: TM AA AI S. Sato CM SI TK K. Moriya KC K. Morioka RK KN. Wrote the paper: TM AA AI S. Sato CM SI S. Sasaki TK K. Moriya KC K. Morioka RK KN.

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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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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/m²)[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

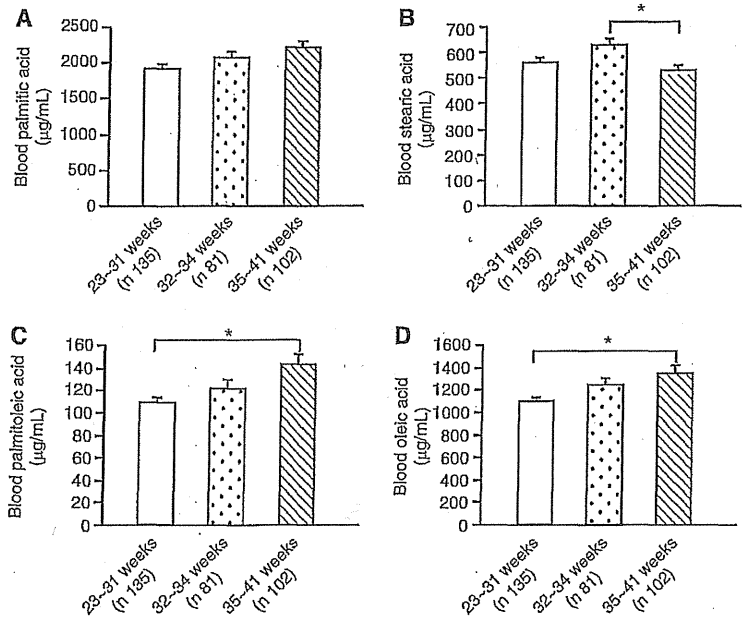

Figure 1 Concentrations of SFAs and MUFAs in maternal blood according to gestational age at blood sampling. Palmitic (A), stearic (B), palmitoleic (C) and oleic (D) acids were measured. *P<0.05. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids.

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,

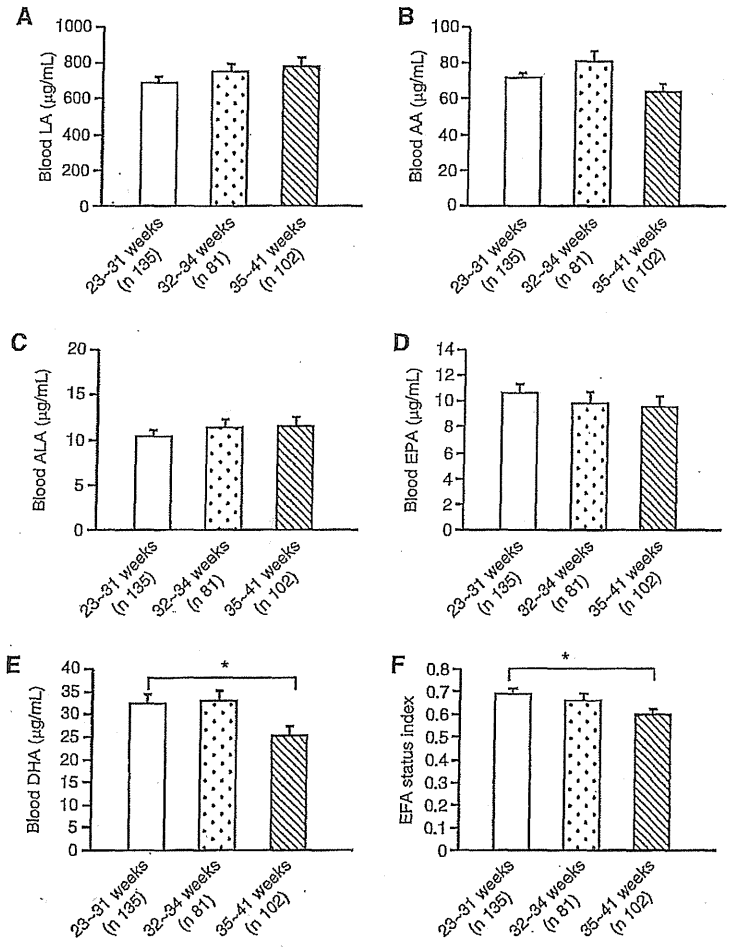

Figure 2 N-6 and n-3 PUFA concentrations in maternal blood according to gestational age at blood sampling. LA (A), AA (B), ALA (C), EPA (D) and DHA (E), and EFA status index (F) were included. *P<0.05. LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic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

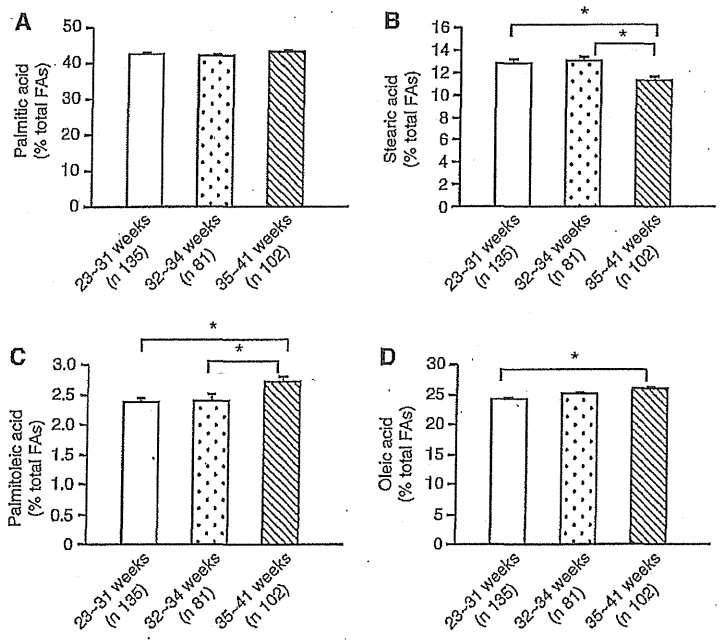

Figure 3 Compositions of SFAs and MUFAs in maternal blood according to gestational age at blood sampling. Palmitic (A), stearic (B), palmitoleic (C) and oleic (D) acids were included. *P<0.05. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids.

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

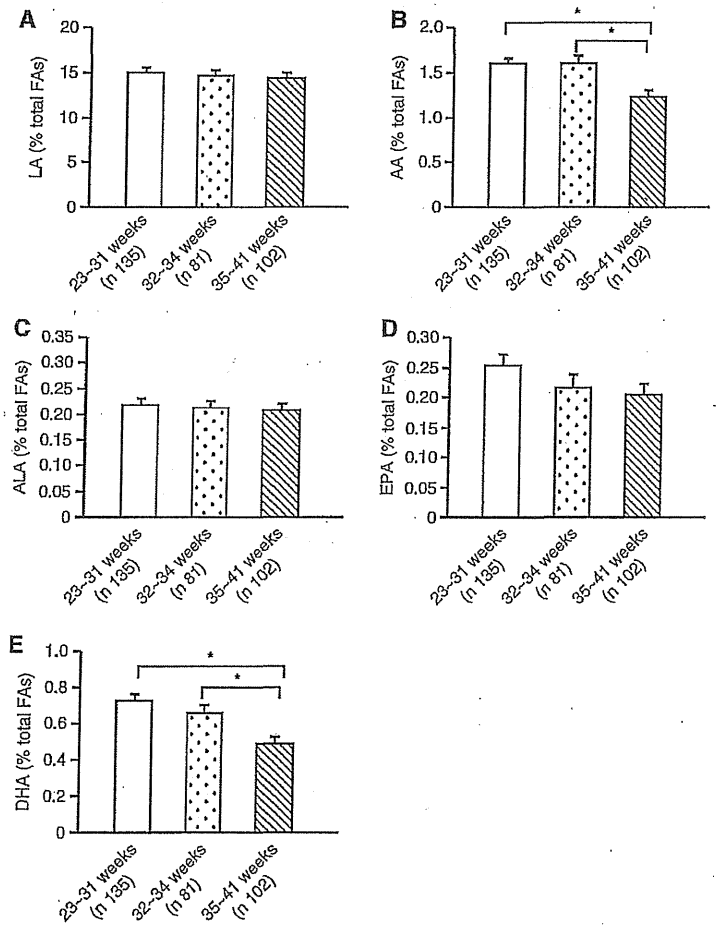

Figure 4 N-6 and n-3 PUFA Compositions in maternal blood according to gestational age at blood sampling. LA (A), AA (B), ALA (C), EPA (D) and DHA (E), were included. *P<0.05. LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FAs, fatty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