Brain & Development 44 (2022) 520–530

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Impact of maternal dyslipidemia on infant neurodevelopment: The Japan Environment and Children's Study

Noriko Motoki [a], Yuji Inaba [a,b,c,*], Takumi Shibazaki [d], Yuka Misawa [a,e], Satoshi Ohira [a], Makoto Kanai [a], Hiroshi Kurita [a], Teruomi Tsukahara [a,f], Tetsuo Nomiyama [a,f], the Japan Environment, Children's Study (JECS) Group

[a] *Center for Perinatal, Pediatric, and Environmental Epidemiology, Shinshu University School of Medicine, Matsumoto, Nagano, Japan*
[b] *Department of Neurology, Nagano Children's Hospital, Azumino, Nagano, Japan*
[c] *Life Science Research Center, Nagano Children's Hospital, Azumino, Nagano, Japan*
[d] *Department of Pediatrics, Shinshu University School of Medicine, Matsumoto, Nagano, Japan*
[e] *Department of Rehabilitation, Nagano Children's Hospital, Azumino, Nagano, Japan*
[f] *Department of Preventive Medicine and Public Health, Shinshu University School of Medicine, Matsumoto, Nagano, Japan*

Received 8 December 2021; received in revised form 8 May 2022; accepted 10 May 2022

Abstract

Background: Various genetic and environmental influences have been studied for developmental disorders; however, the precise cause remains unknown. This study assessed the impact of maternal serum total cholesterol (TC) level in early pregnancy on early childhood neurodevelopment.

Methods: The fixed data of 31,797 singleton births from a large national birth cohort study that commenced in 2011 were used to identify developmental disorders as estimated by Ages and Stages Questionnaire, third edition (ASQ-3) scores of less than −2 standard deviations at 12 months of age. Multiple logistic regression analysis was employed to search for correlations between possibility of developmental disorders and maternal TC levels in early pregnancy classified into 4 groups based on quartile (Q1–Q4) values.

Results: After controlling for potential confounding factors in 27,836 participants who ultimately underwent multivariate analysis, we observed that elevated TC levels were significantly associated with a higher risk of screen positive status for communication (Q4: adjusted odds ratio [aOR] 1.20, 95% confidence interval [CI] 1.05–1.37) and gross motor (aOR 1.13, 95% CI 1.03–1.25) ASQ-3 domain scores.

Conclusion: This large nationwide survey revealed a possible deleterious effect of hypercholesterolemia in early pregnancy on infant neurodevelopment and age-appropriate skill acquisition at 12 months age.

Keywords: Maternal serum cholesterol level; Infant; Neurodevelopment; Developmental disorder; Ages and Stages Questionnaire

1. Introduction

Developmental disorder is defined as impaired development in the areas of speech and language, motor skills, social interaction, and cognition [1]. The number of children with developmental disorder has increased

* Corresponding author at: Center for Perinatal, Pediatric, and Environmental Epidemiology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan.
E-mail address: inabay@shinshu-u.ac.jp (Y. Inaba).

https://doi.org/10.1016/j.braindev.2022.05.002
0387-7604/

dramatically in recent decades [2,3]. Although its estimated prevalence is generally 5–15% in pediatric populations [2–4], reported developmental disorder values vary with the socioeconomic characteristics of the study population, case definition, and age range [5]. Various genetic and environmental influences have been studied for developmental disorders [6–8]; however, the precise cause remains unknown.

Fetal development is sustained by metabolites crossing the placenta from the maternal circulation [9]. Glucose and amino acids are the most important nutrients traversing the placenta [10–12]. Although the transfer of lipids is limited [13], these also play a major role in fetal growth. Cholesterol is important in embryonic development as an essential component of cell membranes [14] and has functions in cell proliferation and differentiation [15,16] as well as cell-to-cell communication [17]. The neuronal myelin membrane contains a high level of cholesterol, which is known to be important for myelin production and maintenance [18]. In fetuses younger than 6 months, plasma cholesterol levels significantly correlate to maternal ones [19], suggesting that maternal cholesterol status influences fetal cholesterol in early gestation.

Several studies have revealed that maternal dyslipidemia could be associated with obstetric complications such as hypertension, eclampsia, macrosomnia, and preterm birth [20–23]. Catov et al. [20] reported that elevated serum total cholesterol (TC) level in early pregnancy was related to an increased risk of spontaneous preterm birth. Although relationships between maternal dyslipidemia and pregnancy complications are well documented, less is known on the longer-term neurodevelopmental outcomes in the offspring. To our knowledge, no studies have investigated if excess or insufficient maternal TC level increases the risk of offspring developmental disorder in Japan. Accordingly, we conducted a large birth cohort study with the specific objective of examining the impact of maternal TC levels on offspring neurodevelopment.

2. Materials and methods

2.1. Study design and participants

The data used in this study were obtained from the Japan Environment and Children's Study (JECS), an ongoing cohort study that began in January 2011 to determine the effect of environmental factors on children's health.

In the JECS, pregnant women were enrolled between January 2011 and March 2014. The inclusion criteria were: 1) having residence in the study area at the time of recruitment, 2) expected delivery after August 1, 2011, and 3) capable of comprehending the Japanese language and completing the self-administered structured questionnaire in Japanese. This study was registered in the UMIN Clinical Trials Registry (number: UMIN000030786). Details of the JECS project have been described previously [24–26]. The JECS protocol was reviewed and approved by the Institutional Review Board on Epidemiological Studies of the Ministry of the Environment (ethical number: No. 100910001) as well as by the Ethics Committees of all participating institutions. The JECS was conducted in accordance with the Helsinki Declaration and other nationally valid regulations and guidelines. Written informed consent was obtained from each participant.

The present study was based on the "jecs-an-20180131" dataset released in March 2018 containing information on 104,065 pregnancies, which included prospectively collected data on infants up to 12 months of age as well as their mothers. We excluded 3,921 cases of miscarriage/stillbirth/missing data on pregnancy and 1,889 cases of multiple births, leaving 98,255 mothers who had a singleton live birth, including 50,563 with the father's registration. Specifically, we focused on questionnaire data regarding the Ages and Stages Questionnaire, third edition (ASQ-3) developmental screening tool as self-described by mothers when their child was 12 months old [27]. Among the participants with the father's registration, we excluded 9,053 cases of insufficient or missing ASQ-3 data and 3,350 cases missing maternal serum TC level data in early pregnancy. Preterm births with a gestational age of less than 37 weeks were excluded since they would have needed to be adjusted to the appropriate age for the ASQ-3 [27]. Participants with obvious risk factors for developmental disorders, such as neonatal asphyxia, physical abnormality at birth including infection, respiratory distress, congenital abnormality, hearing disability, and chromosomal abnormalities, were excluded. Participants with missing information regarding the above exclusion criteria were also excluded, leaving 31,797 participants for analysis. Those with missing covariate data were not included in multiple logistic regression testing, resulting in 27,836 participants in the final analysis (Fig. 1).

2.2. Data collection

Maternal non-fasting serum samples were collected in the first trimester to measure TC. Since the samples were obtained in a non-fasting state, triglyceride (TG) values were not included in the analysis.

Information on socioeconomic status, parental smoking habit, and maternal alcohol consumption was collected during mid/late pregnancy by means of self-reported questionnaires. Details on parental history of neurodevelopmental disorders, epilepsy, and mental disease were also gathered from questionnaires described by the mothers and their partners. Maternal

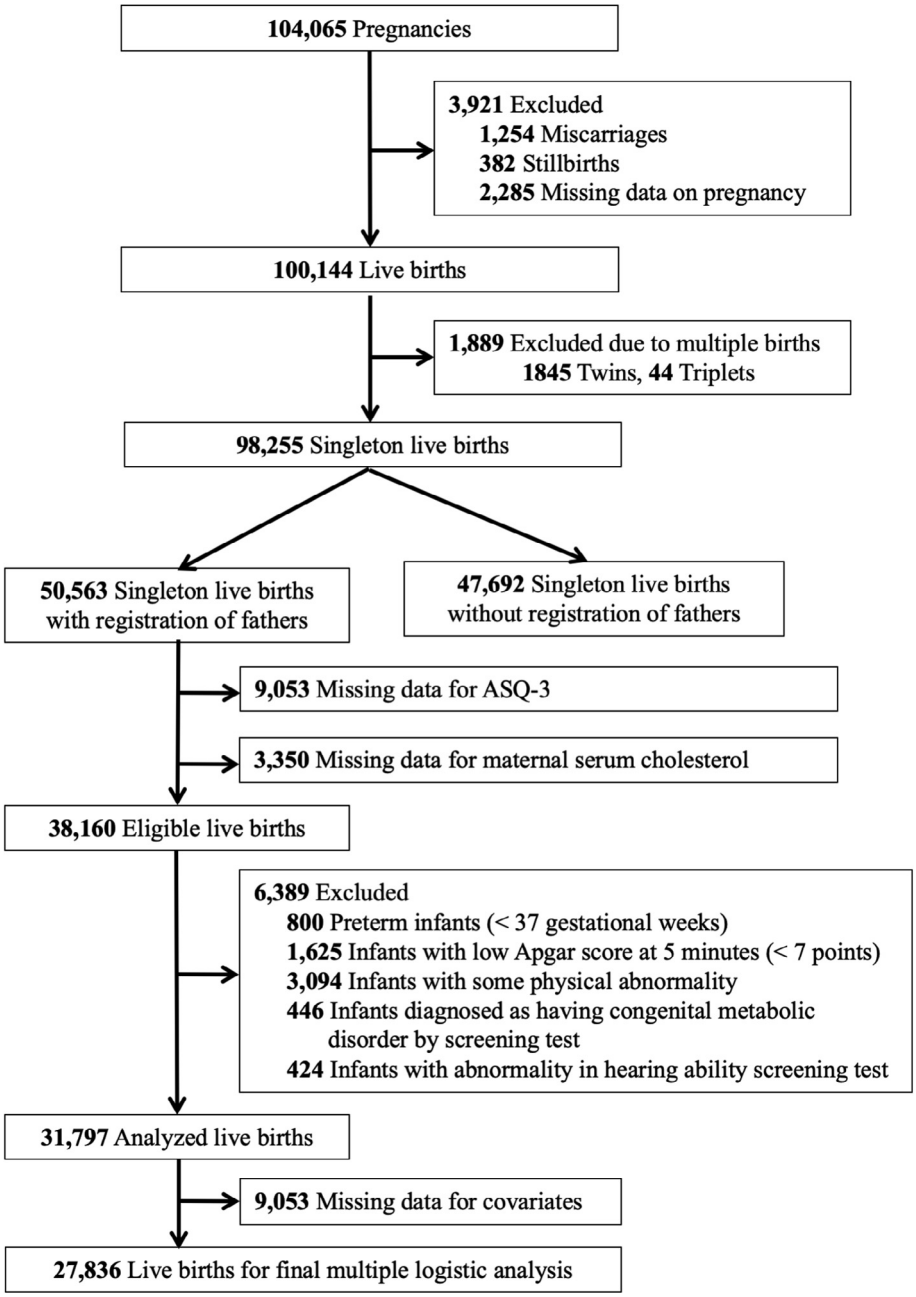

Fig. 1. Case selection flowchart.

anthropometric data before and during pregnancy, complications and medication during pregnancy related to placental abnormalities, hypertensive disorders of pregnancy (HDP), and diabetes mellitus/gestational diabetes mellitus (DM/GDM), and a history of previous pregnancy were obtained from subject medical record transcriptions performed by physicians, midwives/nurses, and/or research coordinators. Pre-pregnancy body mass index (BMI) to evaluate maternal weight status was calculated according to World Health Organization Standards as body weight (kg)/height $(m)^2$.

2.3. Outcomes

The main outcome of interest was ASQ-3 scores. We used the Japanese translation of the ASQ-3 [28]. The ASQ-3 is a parent-reported initial level developmental screening instrument for children aged 12 months with 30 items over 5 domains: communication, gross motor, fine motor, problem solving, and personal-social. Each item describes a skill, ability, or behavior to which a parent responds "yes" (10 points), "sometimes" (5 points), or "not yet" (0 points). Some parents omitted items

Table 1
Characteristics of participants according to maternal serum TC level quartile in first trimester.

Variable	All	Maternal serum TC level quartile				*P* value
		Q1 (≤ 175 mg/dL)	Q2 (176–196 mg/dL)	Q3 (197–219 mg/dL)	Q4 (≥ 220 mg/dL)	
Participants, n	31,797	8,288	7,854	7,743	7,912	
Maternal age at delivery (years)	31 (28, 35)	31 (28, 34)	31 (28, 35)	31 (28, 35)	32 (28, 35)	< 0.001 *
Maternal age group, n (%)						< 0.001
< 35 years	23,416 (73.6)	6,320 (76.3)	5,850 (74.5)	5,655 (73.1)	5,591 (70.7)	
≥ 35 years	8,379 (26.4)	1,968 (23.7)	2,004 (25.5)	2,086 (26.9)	2,321 (29.3)	
Pre-pregnancy BMI (kg/m^2)	20.5 (19.1, 22.5)	20.2 (18.9, 21.9)	20.5 (19.1, 22.4)	20.7 (19.2, 22.8)	20.8 (19.3, 23.0)	< 0.001 *
Pre-pregnancy BMI group, n (%)						< 0.001
Underweight (BMI < 18.5)	4,975 (15.6)	1,538 (18.6)	1,249 (15.9)	1,133 (14.6)	1,055 (13.3)	
Normal weight (BMI 18.5–24.9)	23,527 (74.0)	6,125 (73.9)	5,830 (74.2)	5,740 (74.1)	5,832 (73.7)	
Overweight (BMI 25.0–29.9)	2,573 (8.1)	489 (5.9)	606 (7.7)	678 (8.8)	800 (10.1)	
Obese (BMI ≥ 30.0)	720 (2.3)	135 (1.6)	168 (2.1)	192 (2.5)	225 (2.8)	
Body weight gain during pregnancy (kg)	10.2 (8.0, 12.6)	10.2 (8.0, 12.6)	10.2 (8.0, 12.6)	10.2 (8.0, 12.6)	10.2 (8.0, 12.6)	0.91 *
Standard daily calorie intake (kcal/day)						
First trimester	1,678 (1,366, 2,083)	1,664 (1,353, 2,073)	1,673 (1,369, 2,073)	1,681 (1,359, 2,080)	1,698 (1,379, 2,106)	0.007 *
Second/third trimester	1,602 (1,366, 1,989)	1,612 (1,306, 1,995)	1,592 (1,301, 1,984)	1,616 (1,309, 2,004)	1,593 (1,298, 1,974)	0.18 *
Highest level of maternal education, n (%)						0.054
Junior high school	1,066 (3.4)	291 (3.5)	240 (3.1)	270 (3.5)	265 (3.4)	
High school	9,438 (29.9)	2,369 (28.8)	2,349 (30.1)	2,296 (29.9)	2,424 (30.9)	
Vocational school/Junior college	13,756 (43.6)	3,659 (44.4)	3,340 (42.8)	3,354 (43.7)	3,403 (43.4)	
University/Graduate school	7,293 (23.1)	1,913 (23.2)	1,870 (24.0)	1,758 (24.1)	1,752 (22.3)	
Annual household income during pregnancy, n (%)						0.75
< 4,000,000 JPY	11,480 (38.5)	3,038 (38.6)	2,796 (38.0)	2,792 (38.4)	2,854 (38.7)	
4,000,000–7,999,999 JPY	15,107 (50.7)	3,924 (50.2)	3,781 (51.4)	3,659 (50.4)	3,743 (50.7)	
≥ 8,000,000 JPY	3,273 (10.9)	854 (10.9)	782 (10.6)	814 (11.2)	787 (10.7)	
Maternal smoking during pregnancy, n (%)	1,043 (3.3)	330 (4.0)	252 (3.2)	225 (2.9)	236 (3.0)	< 0.001
Partner's smoking during pregnancy, n (%)	13,166 (42.0)	3,403 (41.7)	3,318 (42.8)	3,261 (42.8)	3,184 (40.9)	0.045
Maternal drinking during pregnancy, n (%)	566 (1.8)	161 (2.0)	145 (1.9)	143 (1.9)	117 (1.5)	0.13
Maternal history of mental disease, n (%)	1,634 (5.2)	392 (4.7)	418 (5.3)	366 (4.7)	458 (5.8)	0.004
Maternal history of developmental disorder, n (%)	16 (0.1)	3 (0.0)	5 (0.1)	4 (0.1)	4 (0.1)	0.89
Maternal history of epilepsy, n (%)	161 (0.5)	38 (0.5)	45 (0.6)	35 (0.5)	43 (0.5)	0.63
Partner's history of mental disease, n (%)	775 (2.5)	185 (2.3)	184 (2.4)	202 (2.6)	204 (2.6)	0.34
Partner's history of developmental disorder, n (%)	22 (0.1)	8 (0.1)	2 (0.0)	4 (0.1)	8 (0.1)	0.21
Partner's history of epilepsy, n (%)	124 (0.4)	45 (0.5)	26 (0.3)	29 (0.4)	24 (0.3)	0.064
Parity, n (%)						< 0.001
Primiparous	13,814 (44.5)	3,827 (47.6)	3,509, (45.7)	3,334 (44.1)	3,144 (40.6)	
Multiparous	17,202 (55.5)	4,211 (52.4)	4,164 (54.3)	4,220 (55.9)	4,607 (59.4)	
Means of pregnancy for current birth, n (%)						< 0.001
Spontaneous	29,514 (93.2)	7,760 (94.0)	7,311 (93.4)	7,175 (93.0)	7,268 (92.3)	
Ovulation induction through medication	853 (2.7)	211 (2.6)	224 (2.9)	205 (2.7)	213 (2.7)	
Artificial insemination or in vitro fertilization	1,316 (4.1)	288 (3.5)	296 (3.8)	336 (4.4)	396 (5.0)	
Maternal use of folic acid supplements, n (%)	731 (2.3)	176 (2.1)	174 (2.2)	178 (2.3)	203 (2.6)	0.27
Diabetes mellitus/gestational diabetes mellitus, n (%)	894 (2.8)	197 (2.4)	200 (2.5)	231 (3.0)	266 (3.4)	< 0.001
Hypertensive disorder of pregnancy, n (%)	843 (2.7)	217 (2.6)	217 (2.8)	207 (2.7)	202 (2.6)	0.87
Intrauterine growth retardation, n (%)	529 (1.7)	167 (2.0)	136 (1.7)	122 (1.6)	104 (1.3)	0.005

(*continued on next page*)

Table 1 (*continued*)

Variable	All	Maternal serum TC level quartile				*P* value
		Q1 (≤ 175 mg/dL)	Q2 (176–196 mg/dL)	Q3 (197–219 mg/dL)	Q4 (≥ 220 mg/dL)	
Mode of delivery for current birth, n (%)						< 0.001
Spontaneous delivery	18,390 (57.9)	4,681 (56.6)	4,555 (58.1)	4,534 (58.6)	4,620 (58.5)	
Induced delivery	5,854 (18.4)	1,690 (20.4)	1,441 (18.4)	1,352 (17.5)	1,371 (17.4)	
Vacuum extraction/Forceps delivery	2,102 (6.6)	545 (6.6)	546 (7.0)	504 (6.5)	507 (6.4)	
Cesarean section	5,409 (17.0)	1,360 (16.4)	1.302 (16.6)	1,345 (17.4)	1,402 (17.7)	
Gestational age (weeks)	39 (38, 40)	39 (38, 40)	39 (38, 40)	39 (38, 40)	39 (38, 40)	< 0.001 *
Birth weight (g)	3,050 (2,820, 3,294)	3,018 (2,798, 3,270)	3,046 (2,820, 3,290)	3,060 (2,830, 3,300)	3,078 (2,846, 3,324)	< 0.001 *
Birth weight categories, n (%)						< 0.001
< 2500 g	1,609 (5.1)	486 (5.9)	407 (5.2)	355 (4.6)	361 (4.6)	
2500 to 3999 g	29,926 (94.1)	7,742 (93.4)	7,394 (94.2)	7,329 (94.7)	7,461 (94.3)	
≥ 4000 g	257 (0.8)	59 (0.7)	51 (0.6)	59 (0.8)	88 (1.1)	
Gender (male), n (%)	15,948 (50.2)	4,249 (51.3)	3,935 (50.1)	3,842 (49.6)	3,922 (49.6)	0.11
Method of feeding, n (%)						< 0.001
Breast feeding	17,415 (54.8)	4,683 (56.5)	4,400 (56.1)	4,133 (53.4)	4,199 (53.1)	
Mixed feeding	13,144 (41.3)	3,316 (40.0)	3,149 (40.1)	3.301 (42.6)	3,378 (42.7)	
Infant formula	1,017 (3.2)	241 (2.9)	241 (3.1)	253 (3.3)	282 (3.6)	
Other	221 (0.7)	48 (0.6)	64 (0.8)	56 (0.7)	53 (0.7)	
Neonatal jaundice, n (%)	4,209 (13.4)	1,152 (14.1)	1,087 (14.0)	1,064 (13.8)	906 (11.6)	< 0.001
Kaup index at 12 months of age	17.0 (16.1, 17.9)	16.9 (16.1, 17.9)	17.0 (16.1, 17.9)	17.0 (16.2, 17.9)	17.0 (16.1, 18.0)	0.089 *
Positive ASQ-3 screen ≥ 1 domain, n (%)	11,266 (35.4)	2,847 (34.4)	2,764 (35.2)	2,739 (35.4)	2,916 (36.9)	0.010
Total number of screen positive domains in ASQ-3 at 12 months of age, n (%)						0.19
1 domain	6,312 (19.9)	1,582 (19.1)	1,567 (20.0)	1,551 (20.0)	1,612 (20.4)	
2 domains	2,801 (8.8)	731 (8.8)	677 (8.6)	652 (8.4)	741 (9.4)	
3 domains	1,339 (4.2)	320 (3.9)	331 (4.2)	333 (4.3)	35 (4.5)	
4 domains	600 (1.9)	163 (2.0)	136 (1.7)	154 (2.0)	147 (1.9)	
5 domains	214 (0.7)	51 (0.6)	53 (0.7)	49 (0.6)	61 (0.8)	

TC, total cholesterol; BMI, body mass index; ASQ-3, Ages and Stages Questionnaire, third edition.

Continuous variables are expressed as the median (interquartile range).

* Kruskal–Wallis test among TC level categories.

Data were missing on maternal age (n = 2), pre-pregnancy BMI (n = 2), maternal education level (n = 244), household income (n = 1,973), maternal smoking habit (n = 304), partner's smoking habit (n = 470), maternal drinking habit (n = 267), maternal history of neurodevelopmental disorders (n = 69), partner's history of neurodevelopmental disorders (n = 357), parity (n = 781), mode of delivery (n = 42), birth weight (n = 5), and neonatal jaundice (n = 283).

when unsure of how to respond or if they had concerns about their child's performance. ASQ-3 scores were not calculated for domains with 3 or more omitted items. In the case of 1 or 2 omitted items, an adjusted total domain score was calculated by adding the averaged item score either once for a single omission or twice for 2 omissions. The scores calculated for each domain were classified as normal development (above the cut-off, i.e., on track) or referral zone (less than 2 standard deviations below the mean). The manual for the original ASQ recommends that a child be considered "screen positive" if his/her score falls below the referral cut-off in any 1 of the 5 domains [27].

2.4. Covariates

Maternal serum TC levels were divided into four quartile levels. The range of each quartile was as follows: first quartile (Q1) $\leq$ 175 mg/dL, second quartile (Q2) = 176–196 mg/dL, third quartile (Q3) = 197–219 mg/dL, and fourth quartile (Q4) $\geq$ 220 mg/dL.

The covariates in our models were selected *a priori* based on previously published literature [6–8,29–32] and biologic plausibility. Maternal pre-pregnancy BMI was calculated using the mothers' height and pre-pregnancy weight as listed in medical records and classified as underweight (<18.5 kg/m^2), normal weight (18.5–24.9), overweight (25.0–29.9), or obese ($\geq$30.0) [33]. Gestational weight gain (GWG) was categorized as below, within, or above reference values based on the guidelines of the Institute of Medicine (now known as the National Academy of Medicine) of 2009 [34]. Demographic covariates included maternal age, parental smoking habit, maternal alcohol consumption, socioeconomic status, and parental history of neurodevelopmental disorders, epilepsy, and mental disease. Socioeconomic status was evaluated by the highest level of education completed by the mother (junior high school, high school, vocational school/junior college, or university/graduate school) and annual household income (<4,000,000, 4,000,000–7,999,999, or $\geq$8,000,000 JPY) [35]. Obstetric and medical variables, such as parity, means of pregnancy, maternal infection and other complications, and medications during pregnancy, were also evaluated. The history of epilepsy, neurodevelopmental disorders, or mental disease of parents was obtained from the questionnaire at registration in early pregnancy (yes or no). Neurodevelopmental disorders included attention deficit and hyperactivity disorder, learning disability, autism, Asperger's syndrome, and pervasive developmental disorder. Mental disease included depression, schizophrenia, and anxiety disorder.

2.5. Statistical analysis

Distribution normality was confirmed by the Kolmogorov–Smirnov test. Data are expressed as the mean

Table 2
ASQ-3 domain scores at 12 months of age and proportions at risk of delay according to maternal serum TC level quartile in first trimester (n = 31,797).

ASQ-3 domain (cut-off score)	Maternal serum TC level quartile				*P* value
	Q1 ($\leq$ 175 mg/dL) n = 8,288	Q2 (176–196 mg/dL) n = 7,854	Q3 (197–219 mg/dL) n = 7,743	Q4 ($\geq$ 220 mg/dL) n = 7,912	
Communication (15.64 points)					
Score (points)	38.0 ± 13.2	38.2 ± 13.2	38.4 ± 13.4	38.0 ± 13.5	0.22
On track, n (%)	7,752 (93.5)	7,357 (93.7)	7,238 (93.5)	7,353 (92.9)	
Referral, n (%)	536 (6.5)	497 (6.3)	505 (6.5)	559 (7.1)	0.26
Gross motor (21.49 points)					
Score (points)	43.5 ± 17.1 *	43.4 ± 17.1 *	43.1 ± 17.1	42.5 ± 17.7	**0.001**
On track, n (%)	7,211 (87.0)	6,839, (87.1)	6,708 (86.6)	6,744 (85.2)	
Referral, n (%)	1,077 (13.0)	1,015 (12.9)	1,035 (13.4)	1,168 (14.8)	**0.002**
Fine motor (34.50 points)					
Score (points)	48.5 ± 11.3	48.5 ± 11.2	48.6 ± 11.2	48.3 ± 11.4	0.59
On track, n (%)	7,507 (90.6)	7,093 (90.3)	7,023 (90.7)	7,122 (90.0)	
Referral, n (%)	781 (9.4)	761 (9.7)	720 (9.3)	790 (10.0)	0.47
Problem solving (27.32 points)					
Score (points)	42.7 ± 13.2	42.5 ± 13.4	42.8 ± 13.4	42.5 ± 13.4	0.38
On track, n (%)	7,098 (85.6)	6,646 (84.6)	6,565 (84.8)	6,701 (84.7)	
Referral, n (%)	1,190 (14.4)	1,208 (15.4)	1,178 (15.2)	1,211 (15.3)	0.23
Personal-social (21.73 points)					
Score (points)	37.6 ± 14.2	37.6 ± 14.3	37.5 ± 14.4	37.3 ± 14.4	0.48
On track, n (%)	6,961 (84.0)	6,612 (84.2)	6,466 (83.5)	6,588 (83.3)	
Referral, n (%)	1,327 (16.0)	1,242 (15.8)	1,277 (16.5)	1,324 (16.7)	0.37

ASQ-3, Ages and Stages Questionnaire, third edition; TC, total cholesterol. Plus-minus variables are the mean ± standard deviation.
Differences in scores of ASQ-3 domains were assessed with one-way repeated measures of variance followed by post hoc (Bonferroni) testing.
* $P < 0.01$ versus the Q4 group.

(standard deviation [SD]) or the median (interquartile range [IQR]) depending on whether they are normally distributed or not, respectively. We adopted multiple logistic regression models to investigate developmental disorder at 1 year as the dependent variable in association with maternal TC level in early pregnancy. Infants below and above the cut-off for each domain were judged as "screen positive" and "normal development", respectively. Maternal TC level was subdivided as low, normal (reference), or high. Possible differences in the scores of each domain among the TC level groups were evaluated by one-way repeated measures of analysis of variance (ANOVA) followed by *post hoc* (Bonferroni) testing. We adopted logistic regression models to calculate adjusted odds ratios (aORs) and their 95% confidence intervals (CIs) while controlling for covariates, as described above. We excluded participants with missing information on any of the covariates used in the multiple logistic regression analysis. Spearman's rank correlation coefficient was used to check for multicollinearity of covariates. Hosmer–Lemeshow testing was employed to assess the goodness-of-fit of the models. We also analyzed the subjects with incomplete ASQ-3 questionnaires as well as those without registered fathers to evaluate for possible selection bias. All statistical analyses were performed using SPSS statistical software version 24 (SPSS Inc., Chicago, Illinois). A P value of <0.05 was considered statistically significant.

3. Results

The characteristics regarding maternal biography, socioeconomic background, parents' past medication records, pregnancy and delivery history, feeding procedures, and perinatal records of the children are summarized according to maternal quartile serum TC levels in Table 1. There were significant differences among the quartile TC levels for maternal age, pre-pregnancy BMI, calorie intake in the first trimester, parental smoking habits, maternal history of mental disease, parity, means of pregnancy, diabetes mellitus, intrauterine growth retardation, mode of delivery, gestational age, birth weight, feeding procedure, and neonatal jaundice (all $P < 0.05$) (Table 1). Regarding the index of maternal and child physique, significant but weak correlations for maternal serum TC levels with maternal pre-pregnancy BMI ($P < 0.001$, $r = 0.102$) as well as with their child's physical growth (Kaup's index) at 12 months of age ($P = 0.011$, $r = 0.016$) were detected (Supplementary Fig. S1A and S1B). There were 11,266 screen positive participants (35.4%) who were outliers in at least 1 ASQ-3 domain (Table 1). We observed a significant difference among the maternal TC quartiles for the frequency of screen positives ($P = 0.010$). This incidence was greatest in the highest quartile (36.9% in the Q4 group).

The ASQ-3 domain classifications and proportions that were scored as a risk of referral zone at 12 months by maternal TC level are shown in Table 2. In chi-square analysis, there was a significant difference in the prevalence of referral zone in the gross motor domain among the maternal TC level groups. The distribution of prevalence in the referral zone for the communication domain was similar to that for the gross motor domain, although no significant difference was observed ($P = 0.26$). ANOVA showed that the score for the gross motor domain was significantly lower in the highest TC group (Q4) than in the Q1 and Q2 groups (both $P < 0.01$).

In multiple logistic regression analysis after adjustment for covariates, higher maternal serum TC levels were significantly associated with an increased risk of screen positive in the communication (aOR for Q4 serum TC level vs. Q1 1.20, 95% CI 1.05–1.37; P for trend = 0.010) and gross motor (aOR for Q4 serum TC level vs. Q1 1.13, 95% CI 1.03–1.25; P for trend = 0.014) ASQ-3 domains (Table 3).

Lastly, we evaluated the characteristics of 9,053 participants with missing ASQ-3 data (Supplementary Table S1) and 47,692 participants without registered fathers (Supplementary Table S2) to test for selection bias. Significant differences were detected for several maternal as well as offspring characteristics in both analyses. Moreover, participants with missing ASQ-3 data or without father registration showed significantly higher maternal TC levels ($P = 0.001$ and $P < 0.001$, respectively) (Supplementary Tables S1 and S2). Finally, the 38,160 participants with TC data were divided into those who were analyzed (n = 31,797) and those who were excluded by the exclusion criteria (n = 6,363). The distribution of maternal TC values was similar (Supplemental Fig. S2).

4. Discussion

We herein describe the first large-scale nationwide birth cohort study in Japan to determine the relationship of maternal TC in early pregnancy with offspring development. Our results indicate that maternal hypercholesterolemia may increase the risk of a screen positive ASQ-3 result at 12 months and offer a way to detect children who should be referred for further assessment.

Maternal cholesterol levels are physiologically elevated during early gestation, but it remains controversial whether maternal hypercholesterolemia can induce unfavorable effects on the fetus and pregnancy outcome [36,37]. However, high maternal TC levels during pregnancy have been linked to increased risks of preterm delivery, gestational diabetes, and preeclampsia [38–40]. Kaneko et al. [41] also demonstrated that higher maternal TC in mid-pregnancy was significantly associated with the development of large for gestational age

Table 3
Odds ratio and 95% confidence intervals for the association between maternal serum TC level quartile in first trimester and screen positive in ASQ-3 domains (n = 27,836).

ASQ-3 domain	Maternal serum TC level quartile				*P* for trend
	Q1 (≤ 175 mg/dL)	Q2 (176–196 mg/dL)	Q3 (197–219 mg/dL)	Q4 (≥ 220 mg/dL)	
Communication					
Crude OR (95% CI)	1.00 (Reference)	0.99 (0.87–1.13)	0.90 (0.90–1.16)	**1.14 (1.01–1.29)**	**0.040**
Adjusted* OR (95% CI)	1.00 (Reference)	1.01 (0.88–1.16)	1.03 (0.90–1.08)	**1.20 (1.05–1.37)**	**0.010**
Gross motor					
Crude OR (95% CI)	1.00 (Reference)	1.01 (0.92–1.11)	1.05 (0.95–1.15)	**1.18 (1.08–1.29)**	**< 0.001**
Adjusted* OR (95% CI)	1.00 (Reference)	0.98 (0.89–1.09)	1.00 (0.91–1.11)	**1.13 (1.03–1.25)**	**0.014**
Fine motor					
Crude OR (95% CI)	1.00 (Reference)	1.04 (0.94–1.16)	1.00 (0.90–1.12)	1.10 (0.99–1.23)	0.14
Adjusted* OR (95% CI)	1.00 (Reference)	1.08 (0.96–1.21)	0.99 (0.89–1.12)	1.09 (0.97–1.22)	0.38
Problem solving					
Crude OR (95% CI)	1.00 (Reference)	1.09 (0.99–1.19)	1.08 (0.98–1.18)	**1.11 (1.01–1.21)**	**0.035**
Adjusted* OR (95% CI)	1.00 (Reference)	1.10 (0.99–1.21)	1.07 (0.97–1.18)	1.09 (0.99–1.21)	0.14
Personal-social					
Crude OR (95% CI)	1.00 (Reference)	1.00 (0.92–1.09)	1.05 (0.96–1.14)	1.09 (0.99–1.18)	**0.034**
Adjusted* OR (95% CI)	1.00 (Reference)	0.99 (0.90–1.09)	1.01 (0.92–1.10)	1.06 (0.96–1.16)	0.24

ASQ-3, Ages and Stages Questionnaire, third edition; TC, total cholesterol; OR, odds ratio; CI, confidence interval. *Adjusted for maternal age, pre-pregnancy body mass index, parental smoking habit, maternal drinking habit, maternal highest level of education, annual household income, parental history of developmental disorders, epilepsy, and mental disease, means of pregnancy, use of folic acid supplements, maternal gestational weight gain, complications during pregnancy (including diabetes mellitus/gestational diabetes mellitus and hypertensive disorder of pregnancy), intrauterine growth restriction, gender, birth weight, method of feeding, and neonatal jaundice.

among Japanese mothers in a JECS cohort. However, the long-term outcome of offspring of mothers with hypercholesterolemia during pregnancy is still unknown.

It is uncertain why maternal hypercholesterolemia may affect offspring neurodevelopment. Among lipids, cholesterol plays a key role in embryo and fetal development as an essential component of cell membrane, and is also associated with cell proliferation, cell differentiation, and cell-to-cell communication in the embryo and fetus [14–17]. Cholesterol is also contained in neuronal myelin membranes at a level as high as 26% by weight and is known to be important for myelin production and maintenance during brain maturation [18]. Excess energy intake and insufficient energy expenditure are linked to an increased prevalence of dyslipidemia [42]. Several animal models have revealed relationships between a maternal high-fat diet and offspring neurodevelopment, whereby high-fat diet exposure during the perinatal period induced such mental disorders as anxiety behavior [43–45] and spatial cognitive function [46]. Maternal obesity has been associated with neurodevelopmental disorders, such as attention-deficit/hyperactivity disorder [47] and autism spectrum disorder [48]. However, the above studies used extremely high-fat diets for animals or did not measure serum TC levels among obese mothers. Further validation of the current investigation is desired.

It is important to ascertain whether neurodevelopmental evaluations at 12 months are clinically valid for subsequent diagnosis. In one study longitudinally comparing child ASQ-3 domain screening results based on cut-off scores, the vast majority (88.9–96.7%) received the same categorization results at 9, 18, and 24 months of age [49]. Other reports have provided evidence on the concurrent validity of the ASQ-3 and clinical diagnosis of developmental disorders, as well as on the reliability of the ASQ-3 in a multi-ethnic population [50–52]. The number of children who tested screen positive (i.e., failed at least 1 of the 5 domains) in this investigation was high at 35.4%. The majority of screen-positive children in the present study had a failure in 1 domain. One study evaluating the validity of the ASQ-3 in Japan suggested a revised deficit criterion of failure in at least 2 domains [28]. In addition, it was revealed that only communication and gross motor among the 5 domains were associated with maternal hypercholesterolemia in this study. They also reported that for the communication, the gross motor and personal-social domains of the 12 months questionnaire, the Japanese ASQ-3 cutoff score was lower than original ASQ-3 cut-off score by more than 10 points [28]. Compared with US children, Japanese children acquire the several skills including communication and gross motor skills >2 months later. The differences in score distribution between the Japanese and original ASQ-3 results found might reflect differences of lifestyle and culture, rather than a lack of validity. Although the results of this investigation could have been overestimated, analysis by TC quartile categories showed a dose–response pattern in 2 domains. Fetal exposure to hypercholesterolemia may have adversely affected neurodevelopment and

age-appropriate skill acquisition in the highest quartile group; the cohort will be followed until 13 years of age to verify this possibility.

A strength of this report was that both maternal and paternal history of neurodevelopmental problems were adjusted for as covariates. Indeed, genetic influences may outweigh those of a shared environment on the incidence of neurodevelopmental disorders [30,31]. Since selection bias might have been created by excluding participants without father registration, we performed an additional sub-analysis on the group without registered fathers to rule this possibility out.

This study had several limitations. First, the developmental score data measured by the ASQ-3 were self-reported and therefore subjective, and the diagnosis and severity of any potential developmental disorder could not be verified. Second, the data on neurodevelopment were collected between 1 month before and 1 month after 12 months of age; therefore, any disorders diagnosed afterwards were not considered. Third, the large attrition rate of unpaired participants or those not fully completing the ASQ-3 questionnaire might have been a source of selection bias (Supplementary Tables S1, S2). Thus, we could not conclusively discount the possibility of under-reporting the incidence of developmental disorders. Fourth, since the parental history of neurodevelopmental disorders, epilepsy, and mental disease were also obtained from self-reported questionnaires, those results might not have conformed to established diagnostic criteria or ICD coding. Another limitation was the non-fasting blood sampling in this study. Although cholesterol level is less affected by diet than that of TG, the maximum mean changes are reportedly +0.3 mmol/L (26 mg/dL) for TG and −0.2 mmol/L (8 mg/dL) for TC in non-fasting versus fasting blood samples [53]. Hence, there was a possibility that cholesterol values were slightly underestimated.

Despite the above limitations, this is the first study employing a large dataset from a Japanese nationwide birth cohort study to analyze the influence of maternal TC on apparently normal-born children after controlling for confounders identified by earlier reports. It provides important information on a possible adverse effect of maternal hypercholesterolemia on offspring neurodevelopment, particularly communication and gross motor ability, to suggest dietary consultation and monitoring for Japanese women desiring pregnancy.

Acknowledgments

The authors would like to thank all the participants of this study and all individuals involved in data collection, as well as Ms. Tomoko Kamijo for her assistance in data analysis and Mr. Trevor Ralph for his English editorial support.

Members of the JECS Group as of 2019: Michihiro Kamijima (principal investigator, Nagoya City University, Nagoya, Japan), Shin Yamazaki (National Institute for Environmental Studies, Tsukuba, Japan), Yukihiro Ohya (National Center for Child Health and Development, Tokyo, Japan), Reiko Kishi (Hokkaido University, Sapporo, Japan), Nobuo Yaegashi (Tohoku University, Sendai, Japan), Koichi Hashimoto (Fukushima Medical University, Fukushima, Japan), Chisato Mori (Chiba University, Chiba, Japan), Shuichi Ito (Yokohama City University, Yokohama, Japan), Zentaro Yamagata (University of Yamanashi, Chuo, Japan), Hidekuni Inadera (University of Toyama, Toyama, Japan), Takeo Nakayama (Kyoto University, Kyoto, Japan), Hiroyasu Iso (Osaka University, Suita, Japan), Masayuki Shima (Hyogo College of Medicine, Nishinomiya, Japan), Youichi Kurozawa (Tottori University, Yonago, Japan), Narufumi Suganuma (Kochi University, Nankoku, Japan), Koichi Kusuhara (University of Occupational and Environmental Health, Kitakyushu, Japan), and Takahiko Katoh (Kumamoto University, Kumamoto, Japan).

Author contributions

Study concept and design: NM and YI. Analysis of data: NM. Interpretation of data: NM, YI, TT, and TN. Drafting the manuscript: NM. Critical revision of the manuscript: YI, TS, YM, SO, MK, HK, TT, TN, and the JECS group.

Statement of financial support

The Japan Environment and Children's Study was funded by the Ministry of the Environment of the government of Japan. The finding and conclusions of this study are solely the response of the authors and do not represent the official views of the above government.

Disclosure

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2022.05.002.

References

[1] Oberklaid F, Efron D. Developmental delay–identification and management. Aust Fam Physician 2005;34:739–42.
[2] Blumberg SJ, Bramlett MD, Kogan MD, Schieve LA, Jones JR, Lu MC, et al. Changes in prevalence of parent-reported autism spectrum disorder in school-aged U.S. children: 2007 to 2011-2012. Natl Health Stat Report 2013 2007;65:1–11.

[3] Boyle CA, Boulet S, Schieve LA, Cohen RA, Blumberg SJ, Yeargin-Allsopp M, et al. Trends in the prevalence of developmental disabilities in US children, 1997–2008. Pediatrics 2011;127:1034–42.
[4] Simeonsson RJ, Sharp MC. Developmental delays. In: Hoekelman RA, Friedman SB, Nelson NM, Seidel HM eds. Primary pediatric care. St Louis; Mosby-Year Book; 1992. p 867-870.
[5] Gottlieb CA, Maenner MJ, Cappa C, Durkin MS. Child disability screening, nutrition, and early learning in 18 countries with low and middle incomes: data from the third round of UNICEF's Multiple Indicator Cluster Survey (2005–06). Lancet 2009;374:1831–9.
[6] Deciphering Developmental Disorders Study. Large-scale discovery of novel genetic causes of developmental disorders. Nature 2015;519:223–8.
[7] Tran NQV, Miyake K. Neurodevelopmental disorders and environmental toxicants: Epigenetics as an underlying mechanism. Int J Genomics 2017;2017:7526592.
[8] Bölte S, Girdler S, Marschik PB. The contribution of environmental exposure to the etiology of autism spectrum disorder. Cell Mol Life Sci 2019;76:1275–97.
[9] Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. Endocrine 2002;19:32–55.
[10] Herrera E, Palacin M, Martin A, Lasunción MA. Relationship between maternal and fetal fuels and placental glucose transfer in rats with maternal diabetes of varying severity. Diabetes 1985;34:42–6.
[11] Knipp GT, Audus KL, Soares MJ. Nutrient transport across the placenta. Adv Drug Deliv Rev 1999;38:41–58.
[12] Sibley C, Glazier J, D'Souza S. Placental transporter activity and expression in relation to fetal growth. Exp Physiol 1997;82:389–402.
[13] Herrera E. Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development–a review. Placenta 2002;23:S9–S19.
[14] Ohvo-Rekilä H, Ramstedt B, Leppimäki P, Slotte JP. Cholesterol interactions with phospholipids in membranes. Prog Lipid Res 2002;41:66–97.
[15] Martínez-Botas J, Suárez Y, Ferruelo AJ, Gómez-Coronado D, Lasunció MA. Cholesterol starvation decreases p34 (cdc2) kinase activity and arrests the cell cycle at G2. FASEB J 1999;13:1359–70.
[16] Suárez Y, Fernández C, Ledo B, Ferruelo AJ, Martín M, Vega MA, et al. Differential effects of ergosterol and cholesterol on Cdk1 activation and SRE-driven transcription. Eur J Biochem 2002;269:1761–71.
[17] Mauch DH, Nägler K, Schumacher S, Göritz C, Müller E-C, Otto A, et al. CNS synaptogenesis promoted by glia-derived cholesterol. Science 2001;294:1354–7.
[18] Chrast R, Saher G, Nave K-A, Verheijen MHG. Lipid metabolism in myelinating glial cells: lessons from human inherited disorders and mouse models. J Lipid Res 2011;52:419–34.
[19] Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, et al. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. J Clin Invest 1997;100:2680–90.
[20] Catov JM, Bodnar LM, Ness RB, Barron SJ, Roberts JM. Inflammation and dyslipidemia related to risk of spontaneous preterm birth. Am J Epidemiol 2007;166:1312–9.
[21] Jin WY, Lin SL, Hou RL, Chen XY, Han T, Jin Y, et al. Associations between maternal lipid profile and pregnancy complications and perinatal outcomes: a population-based study from China. BMC Pregnancy Childbirth 2016;16:60.
[22] Spracklen CN, Smith CJ, Saftlas AF, Robinson JG, Ryckman KK. Maternal hyperlipidemia and the risk of preeclampsia: a meta-analysis. Am J Epidemiol 2014;180:346–58.
[23] Wang X, Guan Q, Zhao J, Yang F, Yuan Z, Yin Y, et al. Association of maternal serum lipids at late gestation with the risk of neonatal macrosomia in women without diabetes mellitus. Lipids Health Dis 2018;17:78.
[24] Michikawa T, Nitta H, Nakayama SF, Yamazaki S, Isobe T, Tamura K, et al. Baseline profile of participants in the Japan Environment and Children's Study (JECS). J Epidemiol 2018;28:99–104.
[25] Ishitsuka K, Nakayama SF, Kishi R, Mori C, Yamagata Z, Ohya Y, et al. Japan Environment and Children's Study: backgrounds, activities, and future directions in global perspectives. Environ Health Prev Med 2017;22:61.
[26] Kawamoto T, Nitta H, Murata K, Toda E, Tsukamoto N, Hasegawa M, et al. Rationale and study design of the Japan environment and children's study (JECS). BMC Public Health 2014;14.
[27] Squires J, Bricker D. Ages and Stages Questionnaires. Third Edition (ASQ-3^{TM}). Baltimore: Paul H. Brookes Publishing Co.;2009.
[28] Mezawa H, Aoki S, Nakayama SF, Nitta H, Ikeda N, Kato K, et al. Psychometric profile of the Ages and Stages Questionnaires, Japanese translation. Pediatr Int 2019;61:1086–95.
[29] McDonald S, Kehler H, Bayrampour H, Fraser-Lee N, Tough S. Risk and protective factors in early child development: results from the All Our Babies (AOB) pregnancy cohort. Res Dev Disabil 2016;58:20–30.
[30] Demirci A, Kartal M. Sociocultural risk factors for developmental delay in children aged 3–60 months: a nested case-control study. Eur J Pediatr 2018;177:691–7.
[31] Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A. The familial risk of autism. JAMA 2014;311:1770–7.
[32] Colvert E, Tick B, McEwen F, Stewart C, Curran SR, Woodhouse E, et al. Heritability of autism spectrum disorder in a UK population-based twin sample. Psychiatry 2015;72:415.
[33] Morisaki N, Piedvache A, Morokuma S, Nakahara K, Ogawa M, Kato K, et al. Gestational weight gain growth charts adapted to Japanese pregnancies using a Bayesian approach in a longitudinal study: The Japan Environment and Children's Study. J Epidemiol 2021. doi: 10.2188/jea. JE20210049. Online ahead of print.
[34] Rasmussen K, Yaktine AL, editors. Institute of Medicine and National Research Council Committee to reexamine IOM pregnancy weight guidelines. Weight gain during pregnancy: reexamining the guidelines. Washington DC: National Academic Press; 2009.
[35] Mitsuda N, N Awn JP, Eitoku M, Maeda N, Fujieda M, Suganuma N, et al. Association between maternal active smoking during pregnancy and placental weight: The Japan environment and Children's study. Placenta 2020;94:48–53.
[36] Bartels Ä, O'Donoghue K. Cholesterol in pregnancy: a review of knowns and unknowns. Obstet Med 2011;4:147–51.
[37] Vrijkotte TG, Krukziener N, Hutten BA, Vollebregt KC, van Eijsden M, Twickler MB. Maternal lipid profile during early pregnancy and pregnancy complications and outcomes: the ABCD study. J Clin Endocrinol Metab 2012;97:3917–25.
[38] Edison RJ, Berg K, Remaley A, Kelley R, Rotimi C, Stevenson RE, et al. Adverse birth outcome among mothers with low serum cholesterol. Pediatrics 2007;120:723–33.
[39] Gunderson EP, Quesenberry Jr CP, Jacobs Jr DR, Feng J, Lewis CE, Sidney S. Longitudinal study of prepregnancy cardiometabolic risk factors and subsequent risk of gestational diabetes mellitus: The CARDIA study. Am J Epidemiol 2010;172:1131–43.

[40] Gratacós E, Casals E, Gómez O, Llurba E, Mercader I, Cararach V, et al. Increased susceptibility to low density lipoprotein oxidation in women with a history of pre-eclampsia. BJOG 2003;110:400–4.
[41] Kaneko K, Ito Y, Ebara T, Kato S, Matsuki T, Tamada H, et al. Association of Maternal Total Cholesterol With SGA or LGA Birth at Term: the Japan Environment and Children's Study. J Clin Endocrinol Metab 2022;107:e118–29.
[42] Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. Int J Mol Sci 2014;15:6184–223.
[43] Janthakhin Y, Rincel M, Costa AM, Darnaudéry M, Ferreira G. Maternal high-fat diet leads to hippocampal and amygdala dendritic remodeling in adult male offspring. Psychoneuroendocrinology 2017;83:49–57.
[44] Winther G, Elfving B, Müller HK, Lund S, Wegener G. Maternal high-fat diet programs offspring emotional behavior in adulthood. Neuroscience 2018;388:87–101.
[45] Sasaki A, de Vega WC, St-Cyr S, Pan P, McGowan PO. Perinatal high fat diet alters glucocorticoid signaling and anxiety behavior in adulthood. Neuroscience 2013;240:1–12.
[46] Tozuka Y, Kumon M, Wada E, Onodera M, Mochizuki H, Wada K. Maternal obesity impairs hippocampal BDNF production and spatial learning performance in young mouse offspring. Neurochem Int 2010;57:235–47.
[47] Rodriguez A, Miettunen J, Henriksen TB, Olsen J, Obel C, Taanila A, et al. Maternal adiposity prior to pregnancy is associated with ADHD symptoms in offspring: Evidence from three prospective pregnancy cohorts. Int J Obes (Lond) 2008;32:550–7.
[48] Krakowiak P, Walker CK, Bremer AA, Baker AS, Ozonoff S, Hansen RL, et al. Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics 2012;129:e1121–8.
[49] Agarwal PK, Xie H, Sathyapalan Rema AS, Rajadurai VS, Lim SB, Meaney M, et al. Evaluation of Ages and Stages Questionnaire (ASQ 3) as a developmental screener at 9, 18, and 24 months. Early Hum Dev 2020;147 105081.
[50] Fauls JR, Thompson BL, Johnston LM. Validity of the Ages and Stages Questionnaire to identify young children with gross motor difficulties who require physiotherapy assessment. Dev Med Child Neurol 2020;62:837–44.
[51] Ga HY, Kwon JY. A comparison of the Korean ages and stages questionnaires and Denver developmental delay screening test. Ann Rehabil Med 2011;35:369–74.
[52] Romero Otalvaro AM, Grañana N, Gaeto N, Torres MLÁ, Zamblera MN, Vasconez MA, et al. ASQ-3: Validation of the Ages and Stages Questionnaire for the detection of neurodevelopmental disorders in Argentine children. Arch Argent Pediatr 2018;116:7–13.
[53] Nordestgaard BG. A test in context: lipid profile, fasting versus nonfasting. J Am Coll Cardiol 2017;70:1637–46.