The Journal of Clinical Endocrinology & Metabolism, 2022, Vol. 107, No. 1, e118–e129
https://doi.org/10.1210/clinem/dgab618
Clinical Research Article

Clinical Research Article

Association of Maternal Total Cholesterol With SGA or LGA Birth at Term: The Japan Environment and Children's Study

Kayo Kaneko,[1] Yuki Ito,[1] Takeshi Ebara,[1] Sayaka Kato,[1,2] Taro Matsuki,[1] Hazuki Tamada,[1] Hirotaka Sato,[1] Shinji Saitoh,[2] Mayumi Sugiura-Ogasawara,[3] Shin Yamazaki,[4] Yukihiro Ohya,[5] Reiko Kishi,[6] Nobuo Yaegashi,[7] Koichi Hashimoto,[8] Chisato Mori,[9] Shuichi Ito,[10] Zentaro Yamagata,[11] Hidekuni Inadera,[12] Takeo Nakayama,[13] Hiroyasu Iso,[14] Masayuki Shima,[15] Youichi Kurozawa,[16] Narufumi Suganuma,[17] Koichi Kusuhara,[18] Takahiko Katoh,[19] and Michihiro Kamijima[1]

[1]Department of Occupational and Environmental Health, Graduate School of Medical Sciences, Nagoya City University, Nagoya 467-8601, Japan; [2]Department of Pediatrics and Neonatology, Graduate School of Medical Sciences, Nagoya City University, Nagoya 467-8601, Japan; [3]Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Nagoya City University, Nagoya 467-8601, Japan; [4]National Institute for Environmental Studies, Tsukuba 305-8506, Japan; [5]National Center for Child Health and Development, Tokyo 157-0074, Japan; [6]Hokkaido University, Sapporo 060-0812, Japan; [7]Tohoku University, Sendai 980-8574, Japan; [8]Fukushima Medical University, Fukushima 960-1295, Japan; [9]Chiba University, Chiba 263-8522, Japan; [10]Yokohama City University, Yokohama 236-0004, Japan; [11]University of Yamanashi, Chuo 409-3898, Japan; [12]University of Toyama, Toyama 930-0194, Japan; [13]Kyoto University, Kyoto 606-8501, Japan; [14]Osaka University, Suita 565-0871, Japan; [15]Hyogo College of Medicine, Nishinomiya 663-8501, Japan; [16]Tottori University, Yonago 683-8503, Japan; [17]Kochi University, Nankoku 783-8505, Japan; [18]University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan; and [19]Kumamoto University, Kumamoto 860-8556, Japan

ORCiD numbers: 0000-0002-1580-9748 (K. Kaneko); 0000-0003-1617-1595 (Y. Ito); 0000-0003-3342-1058 (T. Ebara); 0000-0003-2527-3964 (S. Kato); 0000-0003-2935-3064 (T. Matsuki); 0000-0001-7410-0184 (H. Tamada); 0000-0001-8377-8464 (H. Sato); 0000-0001-6911-3351 (S. Saitoh); 0000-0002-2265-377X (M. Sugiura-Ogasawara); 0000-0002-9241-7289 (H. Iso); 0000-0003-0670-8790 (M. Kamijima).

Abbreviations: BMI, body mass index; CI, confidence intervals; HbA_{1c}, glycated hemoglobin A_{1c}; JECS, Japan Environment and Children's Study; LGA, large for gestation age; OR, odds ratio; SD, standard deviation; SGA, small for gestational age; TC, total cholesterol.

Received: 6 April 2021; Editorial Decision: 17 August 2021; First Published Online: 20 August 2021; Corrected and Typeset: 20 September 2021.

Abstract

Context: Maternal cholesterol is important for fetal development. Whether maternal serum total cholesterol (maternal TC) levels in midpregnancy are associated with small

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

(SGA) or large (LGA) for gestational age independent of prepregnancy body mass index (BMI) and weight gain during pregnancy is inconclusive.

Objective: This work aimed to prospectively investigate the association between maternal TC in midpregnancy and SGA or LGA.

Methods: The Japan Environment and Children's Study is a nationwide prospective birth cohort study in Japan. Participants in this study included 37 449 nondiabetic, nonhypertensive mothers with singleton birth at term without congenital abnormalities. Birth weight for gestational age less than the 10th percentile and greater than or equal to the 90th percentile were respectively defined as SGA and LGA by the Japanese neonatal anthropometric charts.

Results: The mean gestational age at blood sampling was 22.7 ± 4.0 weeks. After adjustment for maternal age, sex of child, parity, weight gain during pregnancy, prepregnancy BMI, smoking, alcohol drinking, blood glucose levels, household income, and study areas, 1-SD decrement of maternal TC was linearly associated with SGA (odds ratio [OR]: 1.20; 95% CI, 1.15-1.25). In contrast, 1-SD increment of maternal TC was linearly associated with LGA (OR: 1.13; 95% CI, 1.09-1.16). Associations did not differ according to prepregnancy BMI and gestational weight gain (*P* for interaction > .20).

Conclusion: Maternal TC levels in midpregnancy were associated with SGA or LGA in a Japanese cohort. It may help to predict SGA and LGA. Favorable maternal lipid profiles for fetal development must be explored.

Key Words: maternal total cholesterol, small for gestational age, large for gestational age, birth cohort

Small for gestational age (SGA) and large for gestational age (LGA) increase the long-term risk of hypertension, type 2 diabetes mellitus, cardiovascular diseases, and stroke in later life, leading to premature death worldwide (1-7). Additionally, undiagnosed SGA and LGA before delivery are related to adverse birth outcomes such as fetal asphyxia, still birth, and neonatal cerebral hemorrhage (8-11). Elucidating pathophysiological mechanisms underlying SGA and LGA can help explore potential prevention strategies.

Maternal cholesterol is important for membrane function and the development of the nervous and aortic systems of the fetus (12-14). Progressive elevation of maternal lipid levels according to gestational age is observed during normal pregnancy (15-17), and maternal total cholesterol (TC) level is considered one of the predictive markers for abnormal fetal development (18, 19). Lower maternal TC levels in midpregnancy reportedly increase the risk of SGA (20-22), whereas no associations have been observed in early (19, 23, 24) and late (25) pregnancy. In contrast, associations between higher maternal TC levels and adverse outcomes, including LGA, have been reported in early and midpregnancy (18, 19). However, whether maternal TC levels in midpregnancy are associated with SGA or LGA, independent of potential confounding factors such as weight gain during pregnancy (26), prepregnancy body mass index (BMI) (27, 28), and maternal blood glucose levels is still unclear (29). In addition, to our knowledge, no previous studies have examined the association between maternal TC in midpregnancy and adverse outcomes, such as SGA or LGA, among the same study population.

Thus, this study aimed to investigate the associations between maternal TC levels in midpregnancy (14th-27th gestational week) and SGA or LGA. We statistically controlled for potential confounding factors and conducted stratified analyses by important confounding factors, including prepregnancy BMI and weight gain during pregnancy.

Materials and Methods

The Japan Environment and Children's Study (JECS) is an ongoing nationwide prospective birth cohort study that examines the effect of environmental factors, lifestyle, medical, and psychosocial conditions during pregnancy and early childhood on the offspring's health and development at birth and in later life. Details of the JECS design have been described in a previous study (30). The JECS protocol was reviewed and approved by the Ministry of the Environment's Institutional Review Board on Epidemiological Studies and the ethics committees of all participating institutions. All participants in the present study provided written informed consent. Data for the present analyses were extracted from the data set released in October 2019 (data set jecs-ta-20190930-qsn).

Study Population

A total of 104 062 fetuses were registered in the JECS. Between January 2011 and March 2014, cooperating health care providers in 15 regional centers across Japan

recruited pregnant women in the first trimester in principle and/or at local government offices issuing home-based records for a pair of mother and child, namely maternal and child health handbooks (an official booklet issued to all expecting mothers in Japan to receive municipal services for pregnancy, delivery, and child care and keep related medical records at home). The eligibility criteria for participants were the following: (i) living in the study area at the time of recruitment (ie, any of the 15 regional centers located throughout Japan) and expected to continue to reside in Japan for the foreseeable future; (ii) an expected delivery between August 1, 2011, and mid-2014; and (iii) an understanding of the Japanese language to complete the self-administered questionnaire (30).

The present study was first restricted to 88 086 mother and infant pairs with singleton live births at term. Eligibility criteria aimed to exclude those who were at elevated risk of SGA and/or LGA and with missing values. In particular, the exclusion criteria were the following: 1) missing values for maternal TC in midpregnancy (14th-27th gestational week), blood glucose at pregnancy and/or fasting status, smoking status during pregnancy, alcohol drinking status during pregnancy, and household income; 2) gestational diabetes mellitus defined by medical records and/or glycated hemoglobin A_{1c} (HbA_{1c}) greater than or equal to 6.5% and/or fasting blood glucose greater than or equal to 92 mg/dL during pregnancy; 3) higher blood glucose level at pregnancy (nonfasting blood glucose during pregnancy ≥ 100 mg/dL, which is the first criterion to identify women with gestational diabetes mellitus) (31); 4) history of diabetes; 5) anemia, heart disease, hypertensive disorder of pregnancy, history of hypertension, polycystic ovary syndrome, thyroid or kidney disorders, and cancer during pregnancy; and 6) infants with congenital cerebral and/or heart abnormalities; and chromosomal abnormalities (32). Finally, a total of 37 449 mother and infant pairs of singleton births at term were eligible for the present analysis (Fig. 1).

We performed a sensitivity analysis of the exclusion procedure and found no significant difference in the participants' characteristics on all items of Table 1 except for blood glucose levels during pregnancy between the overall JECS participants (33) and eligible participants of the present study (results not shown). In addition, we previously reported that the characteristics of overall JECS participants were similar to those obtained from the Japanese Vital Statistics Survey in 2013 (33).

Measurement of Bioassay

Nonfasting blood samples were collected from pregnant women in early and midpregnancy. The blood samples were assayed by a contract clinical laboratory (SRL Inc, a commercial laboratory in Tokyo, Japan). Serum blood TC at midpregnancy was analyzed enzymatically using a 7700 clinical chemistry/immunoassay hybrid analyzer (Hitachi High-Technologies Co Ltd). HbA_{1c} at early pregnancy was measured using a high-performance liquid chromatographic method, with reference to the National Glycohemoglobin Standardization Program or Japan Diabetes Society. HbA_{1c} values identified in reference to the Japan Diabetes Society were adjusted by the following formula (34):

$$\text{Calculated } HbA_{1c} = 1.02 \times HbA_{1c} \text{ (Japan Diabetes Society)} + 0.25$$

Explanatory Variable

Raw maternal TC values had a significant positive correlation with gestational age (days) at the time of blood sampling. Therefore, we performed residual procedures (35) to adjust the maternal TC value to the gestational age at the time of blood sampling. Because of the normal distribution, gestational age-adjusted maternal TC was used as a continuous variable.

Ascertainment of Small for Gestational Age and Large for Gestational Age

Gestational age at birth and neonatal anthropometric measurements were transcribed from the medical records. Ultrasonography during early pregnancy and/or estimation from the last menstrual period were used to determine the gestational age in weeks and days in each facility. The *Z* score of the birth weight for gestational age was calculated based on the Japanese neonatal anthropometric charts. Then, *Z* scores of the birth weight for the gestational age less than –1.28 155 (< 10th percentile) and greater than or equal to 1.28 155 (≥ 90th percentile) were defined as SGA and LGA, respectively (36).

Covariates

Lifestyle-related data and socioeconomic status were self-reported during the early period of participants' pregnancies (37). Anthropometric measurements, medical history, and medical information during pregnancy and at delivery were transcribed from the medical records by cooperating health care providers. Because the Japan Society of Obstetrics and Gynecology recommends examination of random glucose levels in all pregnant women to screen for gestational diabetes mellitus (38), random and fasting blood glucose data during pregnancy are normally available in the medical records.

The age of the mother at delivery was used as a continuous variable. Parity (0, ≥ 1) and sex of the child (male,

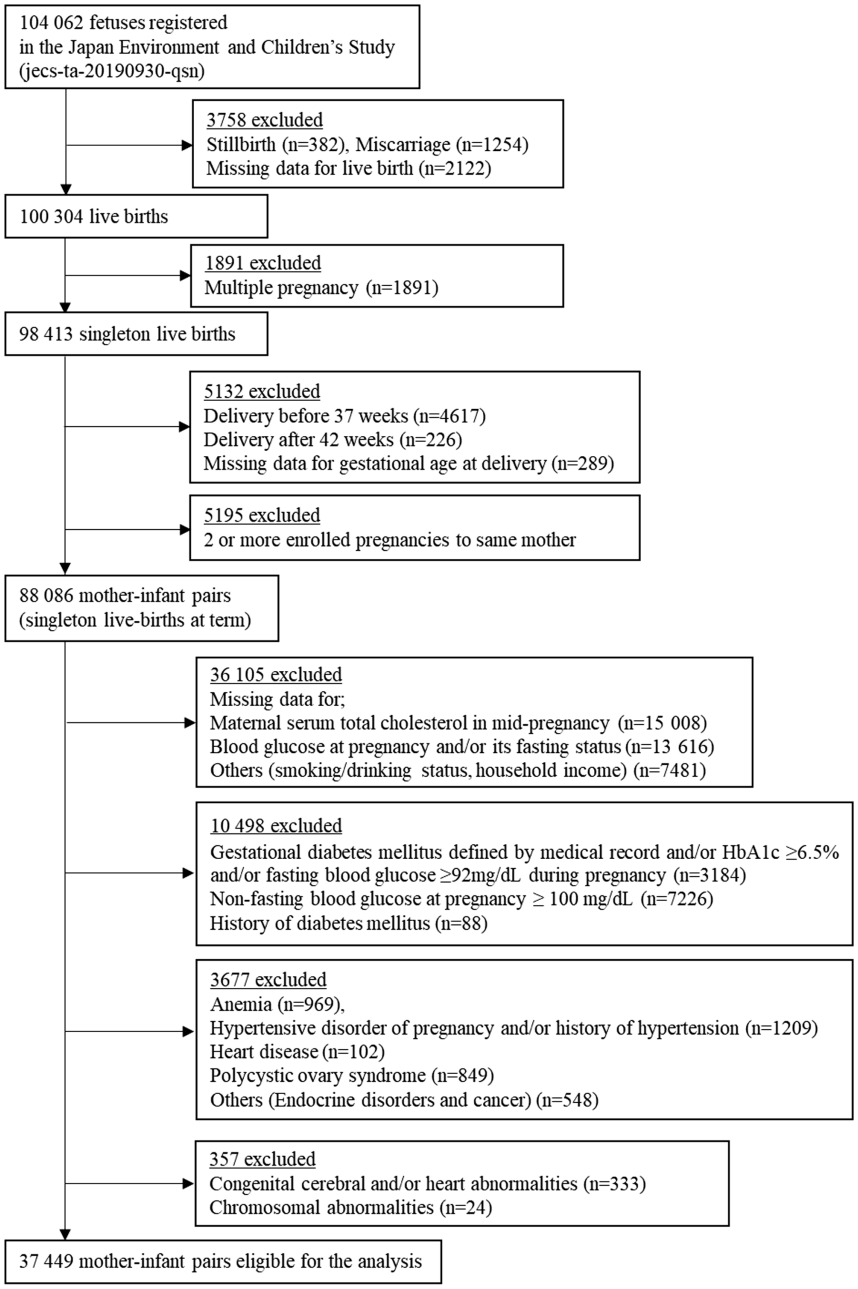

Figure 1. Study enrollment flowchart.

female) were dichotomized. Prepregnancy BMI was computed as prepregnancy body weight (in kilograms) divided by the square of height (in meters) and used as a categorical variable (< 18.5, 18.5-24.9, ≥ 25.0) since its distribution was skewed. Smoking status was classified as "nonsmokers," "former smokers (quit before pregnancy)," "former smokers (quit during early pregnancy)," and "current smokers." Alcohol drinking status during pregnancy was categorized as "nondrinkers," "former drinkers," and "current drinkers" (39). Household income levels (million Japanese yen) were divided into less than 2, 2 to 5, 6 to 9, and 10 or greater. Blood glucose levels (in milligrams per deciliter; mg/dL) during pregnancy were categorized as less than 78, 78 to 84, 85 or greater for a nonfasting blood sample, and less than 76, 76 to 81, and 82 or greater for fasting blood samples according to the tertile of each distribution. Notably, we applied HbA_{1c} as an exclusion criterion but not as a covariate because HbA_{1c} at early pregnancy did not show any significant association with SGA, whereas blood glucose levels during pregnancy reported in medical records showed significant associations both with SGA and LGA. Study areas

Table 1. Characteristics of participants according to maternal total cholesterol level in midpregnancy (n = 37 449)

	No.	(%)	Maternal total cholesterol value[a] in midpregnancy		*P*
Sex of child					
Male	19 024	(50.8)	229.9	(229.4-230.4)	< .01
Female	18 425	(49.2)	231.2	(230.7-231.7)	
Age of mother at delivery, y					
< 35	27 600	(73.7)	229.5	(229.1-230.0)	< .01
≥ 35	9849	(26.3)	233.3	(232.6-234.0)	
Parity					
0	15 343	(41.0)	228.4	(227.9-229.0)	< .01
≥ 1	22 106	(59.0)	232.0	(231.5-232.5)	
Maternal weight gain during pregnancy, kg					
< 7.0	5600	(15.0)	228.0	(227.1-228.9)	< .05
7.0-11.9	19 590	(52.3)	230.2	(229.7-230.7)	
≥ 12.0	12 259	(32.7)	232.2	(231.6-232.8)	
Maternal body mass index before pregnancy					
< 18.5	6073	(16.2)	229.4	(228.6-230.3)	< .05
18.5-24.9	28 068	(74.9)	230.8	(230.4-231.3)	
≥ 25.0	3308	(8.8)	229.9	(228.7-231.2)	
Smoking status during pregnancy					
None	21 888	(58.4)	231.4	(230.9-231.9)	< .05
Former (quit smoking before pregnancy)	8885	(23.7)	230.6	(229.8-231.3)	
Former (quit smoking during early pregnancy)	5084	(13.6)	228.6	(227.6-229.5)	
Current smokers	1592	(4.3)	224.7	(223.0-226.5)	
Alcohol drinking status during pregnancy					
None	12 380	(33.1)	231.8	(231.2-232.5)	< .05
Former	24 020	(64.1)	230.1	(229.7-230.6)	
Current	1049	(2.8)	224.8	(222.7-226.9)	
Blood glucose levels[b] during pregnancy, mg/dL, tertile					
1	12 415	(33.2)	230.4	(229.8-231.0)	.29
2	11 501	(30.7)	231.1	(230.5-231.7)	
3	13 533	(36.1)	230.2	(229.6-230.8)	
Household income, million Japanese yen					
< 2	2066	(5.5)	227.2	(225.7-228.7)	< .05
2-5	25 456	(68.0)	230.9	(230.5-231.3)	
6-9	8359	(22.3)	230.2	(229.4-230.9)	
≥ 10	1568	(4.2)	230.5	(228.8-232.2)	

Maternal total cholesterol levels are reported in geometric mean and 95% CI. *P* values are from *t* test or analysis of variance for multiple measurements with Bonferroni correction.

[a]Maternal total cholesterol values were adjusted for gestational age (day) at blood sampling by the residual method.

[b]Blood glucose levels during pregnancy were categorized as < 78, 78-84, ≥ 85 mg/dL for a nonfasting blood sample and < 76, 76-81, ≥ 82 mg/dL for a fasting blood sample according to each tertile range.

were divided into the location of the 15 regional centers such as "Hokkaido", "Miyagi", "Fukushima", "Chiba", "Kanagawa", "Koshin", "Toyama", "Aichi", "Kyoto", "Osaka", "Hyogo", "Tottori", "Kochi", "Fukuoka", and "South Kyushu/Okinawa."

Statistical Analysis

The characteristics of participants were reported according to the geometric mean and 95% CI of the maternal TC levels. The differences in the geometric mean of the maternal TC were tested by *t* test or analysis of variance for multiple measurements with Bonferroni correction.

Multiple logistic regression models were used to estimate the adjusted odds ratios (ORs) and 95% CI of SGA or LGA by 1 SD (=35.33) of maternal TC. In addition, tests for linear and quadratic trends were performed.

The dose-response relationships between maternal TC and SGA or LGA were examined by restricted cubic spline models. In the models, maternal TC in midpregnancy was included as a restricted cubic spline using 6 knots at

prespecified locations according to the percentiles of the distribution, the 5th, 10th, 25th, 75th, 90th and 95th percentiles, and was adjusted for all covariates. Curves showed ORs compared with the median value of the maternal TC (231 mg/dL). The aforementioned dose-response analyses were carried out using R with package of "rms."

Additional sensitivity analyses included stratified analyses by prepregnancy BMI (< 18.5, 18.5-24.9, ≥ 25.0), maternal weight gain (< 7, 7-11.9, ≥ 12 kg), and multiplicative interactions test. We then analyzed the relationship between maternal TC and incidence of SGA or LGA in participants with normal prepregnancy BMI (18.5-24.9) and maternal weight gain during prepregnancy (7-11.9 kg) (40, 41).

All statistical analyses were performed using IBM SPSS Statistics for Windows software (version 27.0; IBM) and R (version 3.4.3) for Windows (http://cran.r-project.org/). Statistical significance was set at *P* less than .05.

Results

Study Population

The study participants had a mean (SD) age of 31.2 (4.9) years, 41.0% were nulliparous, and 16.2% had low BMI (< 18.5). The mean gestational age at the blood sampling of maternal TC was 22.7 ± 4.0 weeks.

Maternal TC levels were significantly lower in participants who had a male child or younger age (< 35 years) and who had a lower weight gain during pregnancy (< 7.0 kg) or a lower prepregnancy BMI (<18.5). Similarly, participants who were nulliparous, current smokers during pregnancy, current drinkers during pregnancy, and women from low-income households had significantly lower maternal TC levels. In contrast, maternal TC levels did not significantly differ according to the blood glucose levels during pregnancy (see Table 1).

Association Between Adjusted Maternal Total Cholesterol Levels and Small for Gestational Age or Large for Gestational Age

Among 37 499 mothers with a singleton birth, 2638 (7.0%) cases of SGA and 3709 (9.9%) of LGA were observed. After adjustment for all potential confounding variables, 1-SD decrement of maternal TC was linearly associated with SGA (OR = 1.20); 95% CI, 1.15-1.25) (*P* for linear trend < .01; *P* for quadratic trend = .48). In contrast, 1-SD increment of maternal TC was linearly associated with LGA (OR = 1.13; 95% CI, 1.09-1.16) (*P* for linear trend < .01; *P* for quadratic trend = .22) (Table 2). Furthermore, the restricted cubic spline models with multiple adjustment indicated the dose-response relationships between maternal TC and both SGA and LGA (Fig. 2).

Stratified Analyses By Prepregnancy Body Mass Index and Weight Gain During Pregnancy

Analysis that included only participants with normal BMI (18.5-24.9) in prepregnancy and appropriate weight gain during pregnancy (7-11.9 kg) did not materially change the results. This means that completely excluding the effect both of prepregnancy BMI and weight gain during pregnancy did not alter the significant association between maternal TC and both SGA and LGA (Table 3 and Fig. 3).

In addition, stratified analysis by prepregnancy BMI or weight gain during pregnancy showed an independent association between maternal TC and both SGA and LGA. The multiplicative interaction analysis confirmed that the associations between maternal TC and both SGA and LGA were not statistically affected by prepregnancy BMI levels and weight gain during pregnancy (*P* for interaction > .2) (Tables 4 and 5).

Discussion

This large-scale birth cohort study has shown that lower and higher maternal TC in midpregnancy is significantly associated with the development of SGA or LGA among Japanese mothers with singleton birth at term, independent of potential confounding factors, including prepregnancy BMI and weight gain during pregnancy. To our knowledge, this is the first study to show an association between maternal TC in midpregnancy and SGA and LGA in participants with normal prepregnancy BMI and normal weight gain during pregnancy.

The implications of the present findings are 3-fold. First, participants with low and high maternal TC levels in midpregnancy may have a higher risk of SGA and LGA, respectively. These results are consistent with and extend the findings of previous studies. Among Indian mother and child pairs, maternal TC in midpregnancy (at a gestational age of 18 weeks) was linearly associated with birth size (21). Similarly, our study showed a significant continuous OR by 1 SD of maternal TC for SGA and LGA, indicating a dose-response relationship between maternal TC and adverse weight for gestational age. Furthermore, a previous study in the United States reported that maternal TC less than the third percentile (< 138 mg/dL, mean gestational age of 17.6 weeks) in midpregnancy was associated with SGA among Black and White Americans (20). The present study indicated that lower maternal TC compared with the

Table 2. Odds ratios of small or large for gestational age by 1 SD of maternal total cholesterol[a] in midpregnancy (n = 37 449)

Outcome	n	(%)	Crude model	Multivariable-adjusted model[b]	*P* for linear trend	*P* for quadratic trend
Small for gestational age	2638	(7.0)	1.22 (1.18-1.28)	1.20 (1.15-1.25)	< .01	.48
Large for gestational age	3709	(9.9)	1.14 (1.10-1.18)	1.13 (1.09-1.16)	< .01	.22

Odds ratios of small for gestational age were reported by 1-SD decrement of maternal total cholesterol in midpregnancy. Odds ratios of large for gestational age were reported by 1-SD increment of maternal total cholesterol in midpregnancy. SD of maternal total cholesterol = 35.33 mg/dL.

[a]Maternal total cholesterol values were adjusted for gestational age (day) at blood sampling by the residual method.

[b]Adjustment for maternal age at delivery (year), sex of child (male, female), parity (0, ≥ 1), maternal weight gain during pregnancy (kg), maternal body mass index before pregnancy (< 18.5, 18.5-24.9, ≥ 25.0), smoking status during pregnancy (nonsmokers, quit smoking before pregnancy, quit smoking during early pregnancy, current smokers), alcohol drinking status during pregnancy (nondrinkers, former drinkers, current drinkers), blood glucose levels during pregnancy (< 78, 78-84, ≥ 85 mg/dL for a nonfasting blood sample and < 76, 76-81, ≥ 82 mg/dL for a fasting blood sample as each tertile value), household income (< 2, 2-5, 6-9, ≥ 10 million Japanese yen), and study areas (Hokkaido, Miyagi, Fukushima, Chiba, Kanagawa, Koshin, Toyama, Aichi, Kyoto, Osaka, Hyogo, Tottori, Kochi, Fukuoka, and South Kyushu/Okinawa).

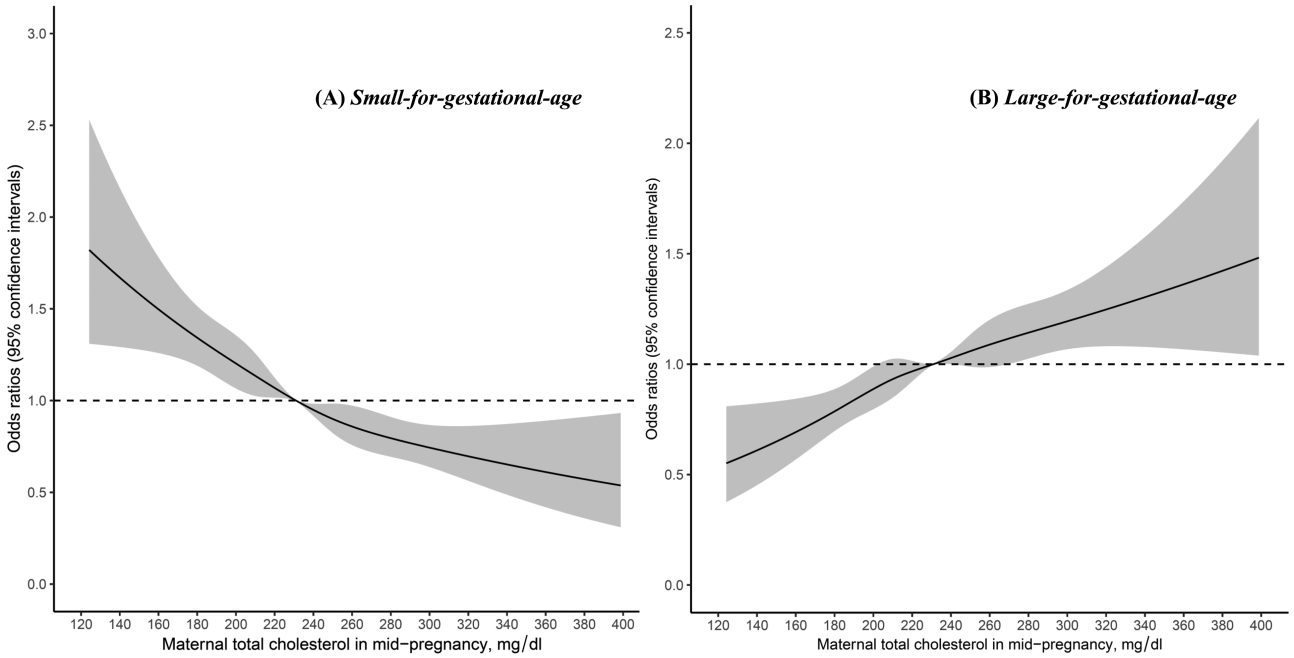

Figure 2. Multivariable-adjusted associations of maternal total cholesterol in midpregnancy with A, small for gestational age, and B, large for gestational age (n = 37 449). In the models, maternal total cholesterol in midpregnancy was included as a restricted cubic spline using 6 knots at prespecified locations according to the percentiles of the distribution, the 5th, 10th, 25th, 75th, 90th, and 95th percentiles, and was adjusted for maternal age at delivery (year), sex of child (male, female), parity (0, ≥ 1), maternal weight gain during pregnancy (kg), maternal body mass index before pregnancy (< 18.5, 18.5-24.9, ≥ 25.0), smoking status during pregnancy (nonsmokers, quit smoking before pregnancy, quit smoking during early pregnancy, current smokers), alcohol drinking status during pregnancy (nondrinkers, former drinkers, current drinkers), blood glucose levels during pregnancy (< 78, 78-84, ≥ 85 mg/dL for nonfasting blood sample and < 76, 76-81, ≥ 82 mg/dL for fasting blood sample as each tertile values), household income (< 2, 2-5, 6-9, ≥ 10 million Japanese yen), and study areas (Hokkaido, Miyagi, Fukushima, Chiba, Kanagawa, Koshin, Toyama, Aichi, Kyoto, Osaka, Hyogo, Tottori, Kochi, Fukuoka, and South Kyushu/Okinawa). The reference of maternal total cholesterol in midpregnancy (odds ratio fixed as 1.0) was 231 mg/dL.

median value of the maternal TC (231 mg/dL, mean gestational age of 22.2 weeks) showed a significantly higher OR of SGA among Japanese, even in women with normal prepregnancy BMI and weight gain during pregnancy. Conversely, compared with the median value, 1-SD increment of maternal TC indicated a significantly higher OR of LGA. These findings are consistent with those of a previous study in China, which indicated that maternal TC greater than or equal to the 95th percentile (≥ 290 mg/dL, at a gestational age of 14-27 weeks) was associated with LGA in midpregnancy (18).

Excessive maternal cholesterol may have adverse outcomes, even though an appropriate level of maternal cholesterol contributes to fetal development (42, 43). Low serum cholesterol level indicates undernutrition and may reflect several micronutrient deficiencies, such as

Table 3. Odds ratios of small or large for gestational age by 1 SD of maternal total cholesterol[a] in midpregnancy among mothers with normal body mass index (18.5-24.9) and weight gain (7-11.9 kg) (n = 14 917)

Outcome	n	(%)	Crude model	Multivariable-adjusted model[b]	*P* for linear trend	*P* for quadratic trend
Small for gestational age	1051	(7.0)	1.21 (1.13-1.29)	1.19 (1.12-1.27)	< .01	.38
Large for gestational age	1162	(7.8)	1.11 (1.05-1.18)	1.09 (1.03-1.16)	< .01	.99

Odds ratios of small for gestational age were reported by 1-SD decrement of maternal total cholesterol in midpregnancy. Odds ratios of large for gestational age were reported by 1-SD increment of maternal total cholesterol in midpregnancy. SD of maternal total cholesterol = 35.33 mg/dL.

[a]Maternal total cholesterol values were adjusted for gestational age (day) at blood sampling by the residual method.

[b]Adjustment for maternal age at delivery (year), sex of child (male, female), parity (0, ≥ 1), maternal weight gain during pregnancy (kg), maternal body mass index before pregnancy (< 18.5, 18.5-24.9, ≥ 25.0), smoking status during pregnancy (nonsmokers, quit smoking before pregnancy, quit smoking during early pregnancy, current smokers), alcohol drinking status during pregnancy (nondrinkers, former drinkers, current drinkers), blood glucose levels during pregnancy (< 78, 78-84, ≥ 85 mg/dL for a nonfasting blood sample and < 76, 76-81, ≥ 82 mg/dL for a fasting blood sample as each tertile value), household income (< 2, 2-5, 6-9, ≥ 10 million Japanese yen), and study areas (Hokkaido, Miyagi, Fukushima, Chiba, Kanagawa, Koshin, Toyama, Aichi, Kyoto, Osaka, Hyogo, Tottori, Kochi, Fukuoka, and South Kyushu/Okinawa).

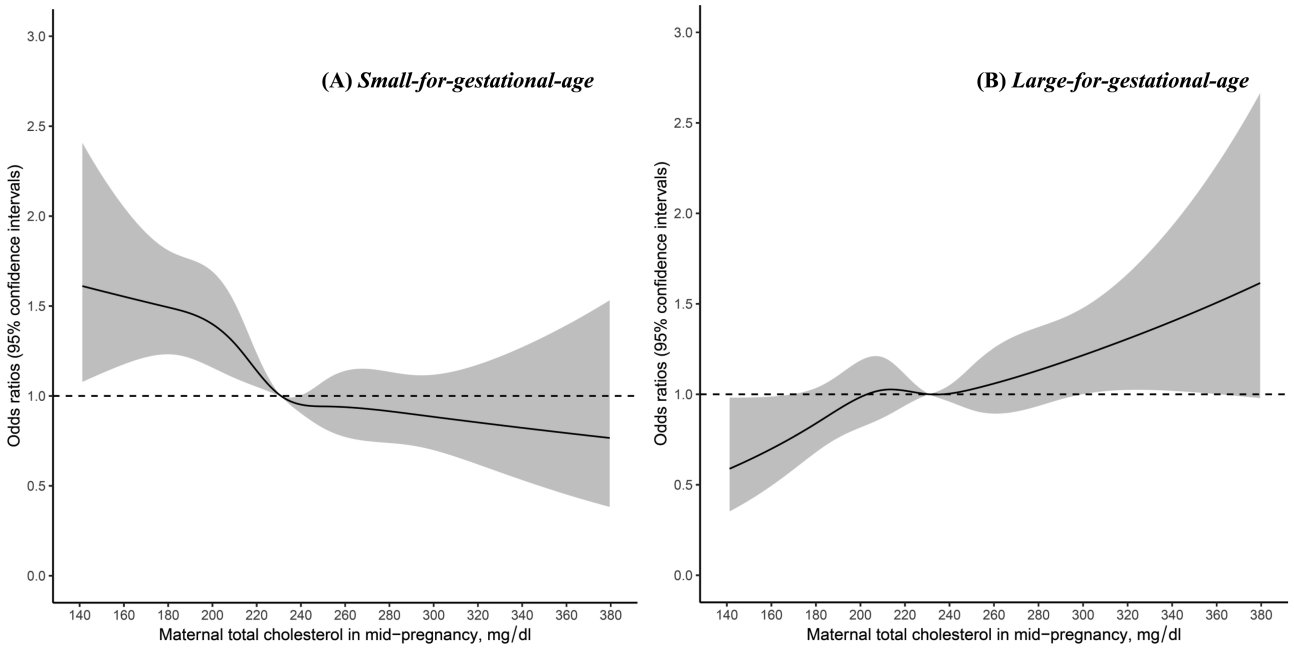

Figure 3. Multivariable-adjusted associations of maternal total cholesterol in midpregnancy with A, small for gestational age, and B, large for gestational age among mothers with normal body mass index (18.5-24.9) and weight gain (7-11.9 kg) (n = 14 917). In the models, maternal total cholesterol in midpregnancy was included as a restricted cubic spline using 6 knots at prespecified locations according to the percentiles of the distribution, the 5th, 10th, 25th, 75th, 90th and 95th percentiles, and was adjusted for maternal age at delivery (year), sex of child (male, female), parity (0, ≥ 1), maternal weight gain during pregnancy (kg), maternal body mass index before pregnancy, smoking status during pregnancy (nonsmokers, quit smoking before pregnancy, quit smoking during early pregnancy, current smokers), alcohol drinking status during pregnancy (nondrinkers, former drinkers, current drinkers), blood glucose levels during pregnancy (< 78, 78-84, ≥ 85 mg/dL for nonfasting blood sample and < 76, 76-81, ≥ 82 mg/dL for fasting blood sample as each tertile values), household income (< 2, 2-5, 6-9, ≥ 10 million Japanese yen), and study areas (Hokkaido, Miyagi, Fukushima, Chiba, Kanagawa, Koshin, Toyama, Aichi, Kyoto, Osaka, Hyogo, Tottori, Kochi, Fukuoka, and South Kyushu/Okinawa). The reference of maternal total cholesterol in midpregnancy (odds ratio fixed as 1.0) was 231 mg/dL.

high-density lipoprotein cholesterol (44, 45) and essential fatty acids (46), necessary for fetal development. Although the present study did not examine the effect of energy intake on maternal TC levels, our results suggest that low energy intake or undernutrition during pregnancy implied by extremely low maternal TC in midpregnancy should be avoided to prevent SGA, even in mothers with normal prepregnancy BMI and weight gain during pregnancy. In contrast, previous animal studies have shown that maternal high-fat feeding during pregnancy might affect adipocyte differentiation, adipose tissue development, and hepatic steatosis in offspring, which leads to a lifelong risk of adiposity and atherosclerosis (47, 48). Future studies should evaluate the effect of maternal TC concentration on adiposity and development of offspring and the incidence of noncommunicable diseases in adulthood.

Table 4. Odds ratios of small for gestational age (SGA) by 1 SD of maternal total cholesterol[a] in midpregnancy by stratified analyses (n = 37 449)

		SGA	(%)	Crude model	Multivariable-adjusted model[b]	*P* for linear trend	*P* for quadratic trend	*P* for interaction
Stratified by prepregnancy body mass index, kg/m^2								
< 18.5	(n = 6073)	649	(10.7)	1.15 (1.05-1.25)	1.12 (1.03-1.22)	< .01	.66	.20
18.5-24.9	(n = 28 068)	1850	(6.6)	1.25 (1.19-1.31)	1.22 (1.16-1.28)	< .01	.26	
≥ 25.0	(n = 3308)	139	(4.2)	1.28 (1.07-1.52)	1.26 (1.05-1.51)	< .05	.47	
Stratified by weight gain during pregnancy, kg								
< 7.0	(n = 5600)	615	(11.0)	1.22 (1.12-1.33)	1.21 (1.11-1.33)	< .01	.19	.99
7.0-11.9	(n = 19 590)	1473	(7.5)	1.19 (1.13-1.26)	1.18 (1.11-1.24)	< .01	.78	
≥ 12.0	(n = 12 259)	550	(4.5)	1.24 (1.14-1.36)	1.23 (1.12-1.35)	< .01	.95	

Odds ratios of small for gestational age were reported by 1-SD decrement of maternal total cholesterol in midpregnancy. Odds ratios of large for gestational age were reported by 1-SD increment of maternal total cholesterol in midpregnancy. SD of maternal total cholesterol = 35.33 mg/dL.

[a]Maternal total cholesterol values were adjusted for gestational age (day) at blood sampling by the residual method.

[b]Adjustment for maternal age at delivery (year), sex of child (male, female), parity (0, ≥ 1), maternal weight gain during pregnancy (kg), maternal body mass index before pregnancy (< 18.5, 18.5-24.9, ≥ 25.0), smoking status during pregnancy (nonsmokers, quit smoking before pregnancy, quit smoking during early pregnancy, current smokers), alcohol drinking status during pregnancy (nondrinkers, former drinkers, current drinkers), blood glucose levels during pregnancy (< 78, 78-84, ≥ 85 mg/dL for a nonfasting blood sample and < 76, 76-81, ≥ 82 mg/dL for a fasting blood sample as each tertile value), household income (< 2, 2-5, 6-9, ≥ 10 million Japanese yen), and study areas (Hokkaido, Miyagi, Fukushima, Chiba, Kanagawa, Koshin, Toyama, Aichi, Kyoto, Osaka, Hyogo, Tottori, Kochi, Fukuoka, and South Kyushu/Okinawa).

Table 5. Odds ratios of large for gestational age by 1 SD of maternal total cholesterol[a] in midpregnancy by stratified analyses (n = 37 449)

		LGA (%)	Crude model	Multivariable-adjusted model[b]	*P* for linear trend	*P* for quadratic trend	*P* for interaction
Stratified by prepregnancy body mass index, kg/m^2							
< 18.5	(n = 6073)	340 (5.6)	1.20 (1.07-1.33)	1.17 (1.05-1.31)	< .01	.14	.29
18.5-24.9	(n = 28 068)	2772 (9.9)	1.14 (1.10-1.19)	1.14 (1.08-1.17)	< .01	.41	
≥ 25.0	(n = 3308)	597 (18.0)	1.10 (1.01-1.20)	1.09 (0.99-1.04)	.06	.61	
Stratified by weight gain during pregnancy, kg							
< 7.0	(n = 5600)	332 (5.9)	1.19 (1.07-1.33)	1.20 (1.07-1.34)	< .01	.45	.95
7.0-11.9	(n = 19 590)	1561 (8.0)	1.11 (1.05-1.16)	1.09 (1.03-1.15)	< .01	.98	
≥ 12.0	(n = 12 259)	1816 (14.8)	1.14 (1.09-1.20)	1.13 (1.08-1.19)	< .01	.13	

Odds ratios of small for gestational age were reported by 1-SD decrement of maternal total cholesterol in midpregnancy. Odds ratios of large for gestational age were reported by 1-SD increment of maternal total cholesterol in midpregnancy. SD of maternal total cholesterol = 35.33 mg/dL.

[a]Maternal total cholesterol values were adjusted for gestational age (day) at blood sampling by the residual method.

[b]Adjustment for maternal age at delivery (year), sex of child (male, female), parity (0, ≥ 1), maternal weight gain during pregnancy (kg), maternal body mass index before pregnancy (< 18.5, 18.5-24.9, ≥ 25.0), smoking status during pregnancy (nonsmokers, quit smoking before pregnancy, quit smoking during early pregnancy, current smokers), alcohol drinking status during pregnancy (nondrinkers, former drinkers, current drinkers), blood glucose levels during pregnancy (< 78, 78-84, ≥ 85 mg/dL for a nonfasting blood sample and < 76, 76-81, ≥ 82 mg/dL for a fasting blood sample as each tertile value), household income (< 2, 2-5, 6-9, ≥ 10 million Japanese yen), and study areas (Hokkaido, Miyagi, Fukushima, Chiba, Kanagawa, Koshin, Toyama, Aichi, Kyoto, Osaka, Hyogo, Tottori, Kochi, Fukuoka, and South Kyushu/Okinawa).

Second, maternal TC may help identify high-risk pregnancies for SGA and LGA in Japanese women. Since total cholesterol is not greatly influenced by fasting states at blood sampling, it is often measured concurrently with other blood examination components during pregnancy. It would be worth carefully observing lower and higher maternal TC in clinical settings.

However, we must also focus on the inconsistent findings regarding the association between maternal TC and offspring's birth size in previous studies as no significant associations in early pregnancy were reported (23-25). This might be explained by different timing of maternal blood sampling, such as early or midpregnancy, or differences in the adjusted confounders. As our present study strictly excluded and controlled major potential confounding factors, the present findings may be meaningful in demonstrating the risk of abnormal birth size in Japanese women. In addition, recent studies reported that ethnic differences in genetic

factors related to lipid metabolism regulation may affect the associations between maternal TC and birth size and risk of cardiovascular diseases in adulthood (49). Inconsistent findings between studies from European countries (23, 24) and ours might be due to the different ethnic background. Future multiple-ethnic studies must investigate the genetic differences that may influence maternal lipid metabolism and its relationship with fetal and child development.

Finally, the molecular pathway underlying the disturbance in lipid metabolism during pregnancy must be elucidated. Although the association between maternal TC in midpregnancy and SGA or LGA may be partially explained by maternal fat or energy intake, our study showed that the associations were independent of prepregnancy BMI and weight gain during pregnancy. This implies that the alteration of maternal TC biosynthesis or degradation would be a consequence of factors other than maternal food intake, and maternal TC might be a mediator of other causal factors and background diseases. We previously reported that exposure to environmental chemicals such as di (2-ethylhexyl) phthalate induces stunting of fetal development mediated by maternal lipid malnutrition in animals (50-52). Further studies must investigate the effect of environmental chemicals on fetal development, which is mediated by a disturbance in lipid metabolism during pregnancy.

The strengths of the present study are the diverse sample from a nationwide, large-scale birth cohort and the availability of important covariates such as prepregnancy BMI and weight gain during pregnancy. In addition, stratified analyses by important variables were performed while conducting multiplicative interaction analysis. In addition, we applied the residual method to control the influence of blood sampling timing on the maternal TC. However, there are several limitations in the present study. First, we did not measure sexual hormone levels, which can influence the offspring's birth size via maternal lipid metabolism perturbation (53). Second, we did not investigate maternal triglyceride and lipid profiles such as low- and high-density lipoprotein cholesterol and fatty acids. Further studies exploring favorable lipid profiles during pregnancy for appropriate fetal development are warranted.

In conclusion, lower and higher maternal TC in midpregnancy were associated with the development of SGA and LGA, respectively, in Japanese mothers with a singleton birth at term, independent of potential confounding factors, including prepregnancy BMI and weight gain during pregnancy. Maternal TC in midpregnancy may help predict SGA and LGA before delivery, which can prevent adverse birth outcomes among SGA and LGA infants. Further studies must explore favorable lipid profiles during pregnancy for fetal development.

Acknowledgments

The authors are grateful to all the participants in the study. We thank all staff members of the JECS.

Financial Support: This work was supported by the Ministry of the Environment, Japan. The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of this government agency.

Additional Information

Correspondence: Yuki Ito, PhD, Department of Occupational and Environmental Health, Graduate School of Medical Sciences, Nagoya City University, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan. Email: yukey@med.nagoya-cu.ac.jp.

Disclosures: All authors had no conflict of interest to disclose related to this work.

Data Availability: Data are unsuitable for public deposition because of ethical restrictions and the legal framework of Japan. It is prohibited by the Act on the Protection of Personal Information (Act No. 57 of May 30, 2003, amendment September 9, 2015) to publicly deposit the data containing personal information. The ethical guidelines for medical and health research involving human subjects enforced by the Japan Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare also restricts the open sharing of the epidemiologic data. All inquiries about access to data should be sent to Dr Shoji F. Nakayama, JECS Programme Office, National Institute for Environmental Studies (jecs-en@nies.go.jp).

References

1. Barker DJ. Maternal and fetal origins of coronary heart disease. *J R Coll Physicians Lond*. 1994;**28**(6):544-551.
2. Yiu V, Buka S, Zurakowski D, McCormick M, Brenner B, Jabs K. Relationship between birthweight and blood pressure in childhood. *Am J Kidney Dis*. 1999;**33**(2):253-260.
3. Godfrey KM, Barker DJ. Fetal nutrition and adult disease. *Am J Clin Nutr*. 2000;**71**(5 Suppl):1344S-1352S.
4. Gluckman PD, Hanson MA, Pinal C. The developmental origins of adult disease. *Matern Child Nutr*. 2005;**1**(3):130-141.
5. Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. *J Clin Endocrinol Metab*. 2007;**92**(3):804-810.
6. Varvarigou AA. Intrauterine growth restriction as a potential risk factor for disease onset in adulthood. *J Pediatr Endocrinol Metab*. 2010;**23**(3):215-224.
7. Knop MR, Geng TT, Gorny AW, et al. Birth weight and risk of type 2 diabetes mellitus, cardiovascular disease, and hypertension in adults: a meta-analysis of 7 646 267 participants from 135 studies. *J Am Heart Assoc*. 2018;**7**(23):e008870.
8. Lindqvist PG, Molin J. Does antenatal identification of small-for-gestational age fetuses significantly improve their outcome? *Ultrasound Obstet Gynecol*. 2005;**25**(3):258-264.
9. Callec R, Lamy C, Perdriolle-Galet E, Patte C, Heude B, Morel O; EDEN Mother-Child Cohort Study Group. Impact on obstetric

outcome of third-trimester screening for small-for-gestational-age fetuses. *Ultrasound Obstet Gynecol*. 2015;**46**(2):216-220.
10. Yamamoto JM, Kallas-Koeman MM, Butalia S, Lodha AK, Donovan LE. Large-for-gestational-age (LGA) neonate predicts a 2.5-fold increased odds of neonatal hypoglycaemia in women with type 1 diabetes. *Diabetes Metab Res Rev*. 2017;**33**(1):e2824.
11. Flenady V, Koopmans L, Middleton P, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. *Lancet*. 2011;**377**(9774):1331-1340.
12. Crume TL, Shapiro AL, Brinton JT, et al. Maternal fuels and metabolic measures during pregnancy and neonatal body composition: the Healthy Start Study. *J Clin Endocrinol Metab*. 2015;**100**(4):1672-1680.
13. Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine*. 2002;**19**(1):43-55.
14. Woollett LA. The origins and roles of cholesterol and fatty acids in the fetus. *Curr Opin Lipidol*. 2001;**12**(3):305-312.
15. Jimenez DM, Pocovi M, Ramon-Cajal J, Romero MA, Martinez H, Grande F. Longitudinal study of plasma lipids and lipoprotein cholesterol in normal pregnancy and puerperium. *Gynecol Obstet Invest*. 1988;**25**(3):158-164.
16. Mazurkiewicz JC, Watts GF, Warburton FG, Slavin BM, Lowy C, Koukkou E. Serum lipids, lipoproteins and apolipoproteins in pregnant non-diabetic patients. *J Clin Pathol*. 1994;**47**(8):728-731.
17. Oliver MF, Boyd GS. Plasma lipid and serum lipoprotein patterns during pregnancy and puerperium. *Clin Sci*. 1955;**14**(1):15-23.
18. Wang C, Kong L, Yang Y, et al. Recommended reference values for serum lipids during early and middle pregnancy: a retrospective study from China. *Lipids Health Dis*. 2018;**17**(1):246.
19. Wang C, Zhu W, Wei Y, et al. The associations between early pregnancy lipid profiles and pregnancy outcomes. *J Perinatol*. 2017;**37**(2):127-133.
20. Edison RJ, Berg K, Remaley A, et al. Adverse birth outcome among mothers with low serum cholesterol. *Pediatrics*. 2007;**120**(4):723-733.
21. Kulkarni SR, Kumaran K, Rao SR, et al. Maternal lipids are as important as glucose for fetal growth: findings from the Pune Maternal Nutrition Study. *Diabetes Care*. 2013;**36**(9):2706-2713.
22. Sattar N, Greer IA, Galloway PJ, et al. Lipid and lipoprotein concentrations in pregnancies complicated by intrauterine growth restriction. *J Clin Endocrinol Metab*. 1999;**84**(1):128-130.
23. Vrijkotte TGM, 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**(11):3917-3925.
24. Vrijkotte TG, Algera SJ, Brouwer IA, van Eijsden M, Twickler MB. Maternal triglyceride levels during early pregnancy are associated with birth weight and postnatal growth. *J Pediatr*. 2011;**159**(5):736-742.e1.
25. Jin WY, Lin SL, Hou RL, 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.
26. Goldstein RF, Abell SK, Ranasinha S, et al. Association of gestational weight gain with maternal and infant outcomes: a systematic review and meta-analysis. *JAMA*. 2017;**317**(21):2207-2225.
27. Enomoto K, Aoki S, Toma R, Fujiwara K, Sakamaki K, Hirahara F. Pregnancy outcomes based on pre-pregnancy body mass index in Japanese women. *PLoS One*. 2016;**11**(6):e0157081.
28. Fujiwara K, Aoki S, Kurasawa K, Okuda M, Takahashi T, Hirahara F. Associations of maternal pre-pregnancy underweight with small-for-gestational-age and spontaneous preterm birth, and optimal gestational weight gain in Japanese women. *J Obstet Gynaecol Res*. 2014;**40**(4):988-994.
29. Metzger BE, Lowe LP, Dyer AR, et al; HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med*. 2008;**358**(19):1991-2002.
30. Kawamoto T, Nitta H, Murata K, et al; Working Group of the Epidemiological Research for Children's Environmental Health. Rationale and study design of the Japan Environment and Children's Study (JECS). *BMC Public Health*. 2014;**14**:25.
31. The Japan Diabetes Society. *Treatment Guide for Diabetes 2020-2021* [in Japanese]. Bunkodo, 2020;101.
32. Mezawa H, Tomotaki A, Yamamoto-Hanada K, et al. Prevalence of congenital anomalies in the Japan Environment and Children's Study. *J Epidemiol*. 2019;**29**(7):247-256.
33. Michikawa T, Nitta H, Nakayama SF, et al; Japan Environment and Children's Study Group. Baseline profile of participants in the Japan Environment and Children's Study (JECS). *J Epidemiol*. 2018;**28**(2):99-104.
34. Sasaki H, Arata N, Tomotaki A, et al; Japan Environment and Children's Study (JECS) Group. Time course of metabolic status in pregnant women: the Japan Environment and Children's Study. *J Diabetes Investig*. 2020;**11**(5):1318-1325.
35. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol*. 1986;**124**(1):17-27.
36. Itabashi K, Miura F, Uehara R, Nakamura Y. New Japanese neonatal anthropometric charts for gestational age at birth. *Pediatr Int*. 2014;**56**(5):702-708.
37. Iwai-Shimada M, Nakayama SF, Isobe T, et al; Japan Environment and Children's Study Group. Questionnaire results on exposure characteristics of pregnant women participating in the Japan Environment and Children Study (JECS). *Environ Health Prev Med*. 2018;**23**(1):45.
38. Minakami H, Maeda T, Fujii T, et al. Guidelines for obstetrical practice in Japan: Japan Society of Obstetrics and Gynecology (JSOG) and Japan Association of Obstetricians and Gynecologists (JAOG) 2014 edition. *J Obstet Gynaecol Res*. 2014;**40**(6):1469-1499.
39. Yokoyama Y, Takachi R, Ishihara J, et al. Validity of short and long self-administered food frequency questionnaires in ranking dietary intake in middle-aged and elderly Japanese in the Japan Public Health Center-Based Prospective Study for the Next Generation (JPHC-NEXT) Protocol Area. *J Epidemiol*. 2016;**26**(8):420-432.
40. Japanese Ministry of Health, Labour and Welfare. *Promotion Council for Healthy Parents and Children 21*. 2nd ed. [in Japanese]. Ministry of Health, Labour and Welfare; 2015.
41. Suzuki S. Optimal weight gain during pregnancy in Japanese women. *J Clin Med Res*. 2016;**8**(11):787-792.
42. Woollett LA. Review: Transport of maternal cholesterol to the fetal circulation. *Placenta*. 2011;**32**(Suppl 2):S218-S221.

43. Woollett LA. Maternal cholesterol in fetal development: transport of cholesterol from the maternal to the fetal circulation. *Am J Clin Nutr*. 2005;**82**(6):1155-1161.
44. Kramer MS, Kahn SR, Dahhou M, et al. Maternal lipids and small for gestational age birth at term. *J Pediatr*. 2013;**163**(4):983-988.
45. Misra VK, Trudeau S, Perni U. Maternal serum lipids during pregnancy and infant birth weight: the influence of prepregnancy BMI. *Obesity (Silver Spring)*. 2011;**19**(7):1476-1481.
46. Jia X, Tagawa M, Yatsuya H, et al. Association of maternal whole blood fatty acid status during the prenatal period with term birth dimensions: a cross-sectional study. *J Perinat Med*. 2015;**43**(5):565-575.
47. Elahi MM, Cagampang FR, Mukhtar D, Anthony FW, Ohri SK, Hanson MA. Long-term maternal high-fat feeding from weaning through pregnancy and lactation predisposes offspring to hypertension, raised plasma lipids and fatty liver in mice. *Br J Nutr*. 2009;**102**(4):514-519.
48. Llopis M, Sánchez J, Priego T, Palou A, Picó C. Maternal fat supplementation during late pregnancy and lactation influences the development of hepatic steatosis in offspring depending on the fat source. *J Agric Food Chem*. 2014;**62**(7):1590-1601.
49. Gazzola K, Reeskamp L, van den Born BJ. Ethnicity, lipids and cardiovascular disease. *Curr Opin Lipidol*. 2017;**28**(3):225-230.
50. Nakashima R, Hayashi Y, Md K, et al. Exposure to DEHP decreased four fatty acid levels in plasma of prepartum mice. *Toxicology*. 2013;**309**:52-60.
51. Ito Y, Kamijima M, Nakajima T. Di(2-ethylhexyl) phthalate-induced toxicity and peroxisome proliferator-activated receptor alpha: a review. *Environ Health Prev Med*. 2019;**24**(1):47.
52. Hayashi Y, Ito Y, Naito H, et al. In utero exposure to di(2-ethylhexyl)phthalate suppresses blood glucose and leptin levels in the offspring of wild-type mice. *Toxicology*. 2019;**415**:49-55.
53. Christ JP, Gunning MN, Meun C, et al. Pre-conception characteristics predict obstetrical and neonatal outcomes in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2019;**104**(3):809-818.