Ⅲ. 学会等発表実績

学 会 等 発 表 実 績

委託業務題目「低出生体重児の発症機序及び長期予後の解明に関する研究」
機関名　独立行政法人　国立成育医療研究センター

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
周産期データリンゲージによる研究の意義と可能性	森臨太郎	第50回日本周産期・新生児医学会	2014. 7	国内
周産期関連データベースの連結	森崎菜穂	第50回日本周産期・新生児医学会	2014. 7	国内
DOHaD (Developmental origins of health and Disease)からみた環境化学物及び栄養の次世代への影響	福岡秀興	第17回環境ホルモン学会（シンポジウム）	2014.12.9	国内
胎生期栄養環境と生活習慣病の形成機序	福岡秀興	第23回アジア栄養科学ワークショップ	2014.11.29	国内
胎生期エピゲノム変化と小児内分泌のUpdate	福岡秀興	第24回臨床内分泌代謝Update in Saitama（シンポジスト）	2014.11.28	国内
若い女性のやせ志向と危惧される次世代の生活習慣病リスク	福岡秀興	平成26年度食育健康サミット（基調講演）	2014.11.27	国内
胎生期の脂質代謝とエピジェネティクス	福岡秀興	脂質栄養学会第23回大会（特別講演）	2014.8.29	国内
将来母親となる女子の成長期における栄養管理の重要性について	福岡秀興	第61回日本栄養改善学会学術総会（シンポジスト）	2014.8.21	国内
エピジェネティックスとGWASからみたDOHaD研究の最近の動向」	福岡秀興	第３回日本DOHaD研究会年会（基調講演）	2014.7.25	国内
DOHaD研究の現状と今後	福岡秀興	第50回日本周産期・新生児医学会学術集会（シンポジウム）	2014.7.14	国内
危惧される若い女性の低栄養問題	福岡秀興	平成26年度日本フードスペシャリスト協会通常総会（特別講演）	2014.6.5	国内
胎生期・新生児期の環境の及ぼす精神疾患及び発達障害の素因形成	福岡秀興	第56回日本小児神経学会学術集会（シンポジウム）	2014.5.29	国内
「妊婦栄養のたいせつさ」～成人病・胎児期発症起源説より～	福岡秀興	第58回食品新素材研究会	2014.2.7	国内
Drug therapies for bronchopulmonary dysplasia in Japan: A questionnaire survey.	Ogawa R, Mori R, Sako M, Kageyama M, Tamura M, Namba F.	Pediatric Academic Societies and Asian Society for Pediatric Research Joint Meeting	2014.5	国外
わが国における新生児慢性肺疾患薬物療法：アンケート調査から	小川 亮、森臨太郎、佐古まゆみ、影山操、田村 正徳、難波 文彦	第50回日本周産期・新生児医学会	2014. 7	国内

動物モデルを用いた新生仔期高濃度酸素暴露の長期的影響と肺傷害修復過程における重要分子の同定	難波文彦、小川亮、伊藤誠人、新井幸男、郷勇人、加藤稲子、田村正徳	第59回日本未熟児新生児学会	2014. 11	国内
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胎盤における環境エピゲノム変化とその医療活用	秦健一郎	第３回日本DOHaD研究会学術集会	2014.7.26	国内
次世代シークエンサーの日常診療への応用～環境は遺伝するか？ 新型出生前診断の次は？	秦健一郎	第13回別府遺伝医学セミナー	2014.6.4	国内
生殖・周産期のエピジェネティクス.	秦健一郎	第87回日本内分泌学会学術総会	2014.4.27	国内
胎児発育異常のゲノム・エピゲノムの解析	秦健一郎	群馬大学生体調節研究所	2014.3.7	国内
環境は遺伝する？～周産期医療にかかわるジェネティクスとエピジェネティクス～	秦健一郎	第７回福岡胎児医療フォーラム	2014.1.31	国内
Does home-visit program for high-risk pregnant women reduce parenting stress and improve birth outcomes in Japan?	Ichikawa K, Fujiwara T, Nakayama T, Sekiguchi T, Takahashi Y.	International Congress on Child Abuse and Neglect (Poster Presentation)	2014.9.14-17	国内

２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Relationships between Birth Weight and Serum Cholesterol Levels in Healthy Japanese Late Adolescents.	Sanae I, Uenishi K, Fukuoka H et al.	J Nutr Sci Vitaminol	2014	国内
Metabolomics analysis of umbilical cord blood clarifies changes in saccharides associated with delivery method.	F Hashimoto, Nishiumi S, Fukuoka H, et al.	Early Hum Dev	2013	国外
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妊産婦のやせと胎児発育 DOHaD(Developmental Origins of Health and Disease)の視点から考える	福岡秀興, 伊藤早苗, 石田裕美	産婦人科の実際	2015	国内
胎内栄養環境と高血圧症—成人病胎児期発症起源説の視点から考える—	福岡秀興, 平野大志, 向井伸二	血圧	2014	国内

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がんおよび疾病予防の視点から見た周産期のエピゲノム変化	福岡秀興	栄養学レビュー（Nutrition Reviews日本語版）	2014	国内
成人病胎児期発症説とPIHの胎児栄養　成人病胎児期発症起源説の視点から	福岡秀興, 向井伸治	産婦人科の実際	2014	国内
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早産低出生体重児とnon-communicable diseases.	板橋家頭夫	新生児栄養学（板橋家頭夫編集）	2014	国内

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Ⅳ. 研究成果の刊行物・別刷

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Relationships between Birth Weight and Serum Cholesterol Levels in Healthy Japanese Late Adolescents

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Relationships between Birth Weight and Serum Cholesterol Levels in Healthy Japanese Late Adolescents

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Summary Poor growth in utero has been suggested to be associated with adverse levels of serum cholesterol concentrations in later life. In Asia, there have only been a limited number of studies examining the relationship between fetal status and serum lipids, especially in adolescents. The objective of this study was to examine the relationships between birth weight and serum high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels; adjusting for current physical status including percent body fat, physical activity and nutrient intake in healthy Japanese late adolescents. The data of 573 late adolescents with an average age of 17.6 (287 boys and 286 girls) who underwent physical examinations which included blood sampling and who had all the required data, were analyzed. Birth weight was obtained from their maternal and child health handbook. Multiple regression analysis showed that birth weight was positively associated with serum HDL in girls, independently of percent body fat or fat intake, when adjusted for current body height and weight. There were no associations between birth weight and serum HDL in boys, or serum LDL in either sex.

Key Words birth weight, serum cholesterol, body fat, physical activity, Japanese late adolescent

Poor growth in utero has been suggested to be associated with adverse levels of serum cholesterol concentrations in adult life (*1*). This could be because that impaired growth of the fetal liver in gestation may lead to permanent changes in lipid metabolism (*1*). In western countries, several studies have reported on the adverse association between size at birth and components of the lipid profile in later life (*2*, *3*), although there is variability in the degree of the associations. Meanwhile, there has only been limited number of studies conducted in Asia.

In previous studies, serum lipid levels had significant relationships with percentage body fat (*4*, *5*) and physical activity (*6*) in childhood and adolescence. However, few studies examined the association between birth weight and serum lipids in adolescence, adjusting for percent body fat and physical activity.

The objective of this study was to examine the relationships between birth weight and serum high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels adjusting for current physical status, including percent body fat, physical activity and nutrient intakes, in healthy Japanese late adolescents whose serum lipid levels were assumed to be in stable phase that approached adult levels.

MATERIALS AND METHODS

Subjects. We have conducted physical examinations including optional blood sampling of students at a junior and senior high school in Tokyo, Japan, annually in April of each year since 2000. A questionnaire about their birth records was mailed to parents of 2,056 students (1,054 boys and 1,002 girls) who were enrolled at the school in 2005. We obtained data at birth from 1,157 subjects (616 boys and 541 girls). In this study, we analyzed subjects who were born at full term pregnancy (gestational age: from 37 to 41 wk) from a single birth to avoid possible conflicts from pre- or postmaturity or multiple pregnancies. The following subjects were excluded from analysis: 31 subjects who were born before 37 wk or after 41 wk and 3 subjects who were born as twins. Results from the examination in the 3rd year of high school (aged 17 or 18) were used as the current physical status of late adolescents. Finally, 573 subjects (287 boys and 286 girls) who had all the required data were analyzed for this study. The purpose and protocol of this study were explained to the subjects in advance and written informed consent was obtained

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from each subject. This study was conducted in compliance with the Declaration of Helsinki. The Ethics Committee of Kagawa Nutrition University approved the procedures used in this study.

Measurements. A questionnaire about their birth record asked parents to provide measurements at birth from their maternal and child health handbook. In this study, birth weight was used as a measurement of fetal nutrition status. Body height and weight at the 3rd year of high school were measured to the nearest 0.1 cm and 0.1 kg, respectively, while subjects wore light clothes and no shoes. Body mass index (BMI, weight (kg)/height (m)^2) was calculated. Percent body fat was assessed using a multi-frequency impedance bioelectrical impedance analyzer (BIA, InBody, Biospace Japan Inc., Tokyo, Japan). Serum samples were obtained from blood drawn in the morning under nonfasting conditions. All samples were maintained at a temperature of −20°C while in transport for measurement at a laboratory in Tokyo (SRL Inc., Tokyo, Japan). Serum HDL and LDL cholesterol levels were measured directly. Dietary intakes during the preceding month were assessed using a self-administered food frequency questionnaire (FFQ) (*7*). Energy intake (kcal/d), and also protein and fat energy percent intake (% Energy) were calculated on the basis of the Standard Tables of Food Composition in Japan (*8*). Pearson's correlation coefficients between the FFQ and dietary records were 0.42 for energy ($p<0.001$), 0.32 for protein ($p<0.001$) and 0.31 for fat intake ($p<0.001$). Frequency of physical activity (d/wk) asked as "How many days do you usually exercise in a week besides for school classes?", and only for girls, their age at menarche were reported in a self-administered lifestyle questionnaire. Postmenarcheal age (age at the time of the survey minus age at menarche) was also calculated. All the measurements except the questionnaire about birth record were conducted on the same day.

Statistical analysis. Pearson's product-moment correlation coefficient was calculated to test for linear correlations between birth weight and serum HDL and LDL cholesterol levels with other measures. Multiple regression analysis was used to assess the relationships of serum HDL and LDL cholesterol levels with each measurement. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 19.0 (IBM Japan, Ltd., Tokyo, Japan).

RESULTS

The subjects' birth weight, and demographic and physical characteristics, serum HDL and LDL cholesterol level, frequency of physical activity and nutrient intakes in the 3rd year of high school are shown in Table 1. The mean±standard deviation (SD) of birth weights were 3.163±0.363 kg for boys and 3.069±0.347 kg for girls. The body height and weight of the subjects were equal to the subjects aged 17 to 18 y that participated in the National Nutrition Survey, Japan (*9*).

Table 2 shows the correlation coefficients for birth weight and serum HDL and LDL cholesterol levels with other measures. Birth weight correlated positively with body height ($r=0.27$, $p<0.001$ for boys and $r=0.26$, $p<0.001$ for girls), body weight ($r=0.24$, $p<0.001$ for boys and $r=0.28$, $p<0.001$ for girls) and BMI ($r=0.13$, $p<0.05$ for boys and $r=0.18$, $p<0.01$ for girls). Only in boys, birth weight also correlated positively with energy intake ($r=0.12$, $p<0.05$). Serum HDL correlated negatively with body weight ($r=-0.17$, $p<0.01$ for boys and $r=-0.18$, $p<0.01$ for girls), BMI ($r=-0.15$, $p<0.05$ for boys and $r=-0.14$, $p<0.05$ for girls), percent body fat ($r=-0.16$, $p<0.01$ for boys and $r=-0.21$, $p<0.001$ for girls) and positively with frequency of physical activity ($r=0.29$, $p<0.001$ for boys and $r=0.27$, $p<0.001$ for girls) in both sexes. In addition, only in girls, serum HDL also correlated negatively with body height ($r=-0.12$, $p<0.05$) and positively with fat intake ($r=0.17$, $p<0.01$). Serum LDL correlated positively with body weight ($r=0.22$, $p<0.001$), BMI ($r=0.26$, $p<0.001$) and percent body fat ($r=0.37$, $p<0.001$) and negatively with frequency of physical activity ($r=-0.17$, $p<0.01$) only in boys. There was no significant correlation between serum LDL and the other measurements in girls.

Table 3 shows the results of linear regression models used to examine the relationship between serum HDL or LDL cholesterol levels and other measurements. Predictor variables were birth weight, postmenarcheal age (only for girls), body height, body weight, percent body fat, physical activity, energy intake, protein intake and fat intake in model 1, and birth weight, postmenarcheal age (only for girls), percent body fat, physical activity, energy intake, protein intake and fat intake in model 2. BMI was not included in any of the models because of the high correlation between BMI and percent body fat. In boys, birth weight had no significant association with serum HDL, while a lower body weight

Table 1. Characteristics of study subjects.[1]

Variable	Boys (n=287)	Girls (n=286)
Birth weight (kg)	3.163±0.363	3.069±0.347
At 3rd grade of high school		
Age (y)	17.6±0.3	17.6±0.3
Postmenarcheal age (y)		5.6±1.3
Body height (cm)	171.9±5.4	158.4±5.0
Body weight (kg)	63.0±8.2	53.0±7.2
BMI[2] (kg/m^2)	21.3±2.5	21.1±2.5
Percent body fat (%)	14.6±4.4	26.0±5.0
Serum HDL[3](mg/dL)	59±11	67±13
Serum LDL[4] (mg/dL)	93±23	97±23
Physical activity (d/wk)	3.5±2.6	2.1±2.0
Energy intake (kcal/d)	2,034±486	1,674±343
Protein intake (% Energy)	17.7±2.7	18.0±2.8
Fat intake (% Energy)	31.4±5.0	34.7±4.7

[1] Values are mean±standard deviation.
[2] Body mass index.
[3] High-density lipoprotein.
[4] Low-density lipoprotein.

Table 2. Pearson's correlation coefficients for birth weight and serum HDL[1] and LDL[2] cholesterol levels with other measures.

	Boys (n=287)						Girls (n=286)					
	Birth weight (kg)		Serum HDL[1] (mg/dL)		Serum LDL[2] (mg/dL)		Birth weight (kg)		Serum HDL[1] (mg/dL)		Serum LDL[2] (mg/dL)	
	r[3]	p	r[3]	p	r[3]	p	r[3]	p	r[3]	p	r[3]	p
Birth weight (kg)	—	—	0.05	0.42	−0.04	0.50	—	—	0.10	0.10	−0.11	0.08
Postmenarcheal age (y)	—	—	—	—	—	—	−0.00	0.97	−0.02	0.74	−0.06	0.34
Body height (cm)	0.27	<0.001	−0.08	0.19	−0.05	0.42	0.26	<0.001	−0.12	<0.05	−0.11	0.06
Body weight (kg)	0.24	<0.001	−0.17	<0.01	0.22	<0.001	0.28	<0.001	−0.18	<0.01	0.01	0.82
BMI[4] (kg/m^2)	0.13	<0.05	−0.15	<0.05	0.26	<0.001	0.18	<0.01	−0.14	<0.05	0.07	0.21
Percent body fat (%)	−0.07	0.22	−0.16	<0.01	0.37	<0.001	−0.03	0.67	−0.21	<0.001	0.11	0.07
Serum HDL[1] (mg/dL)	0.05	0.42	—	—	−0.15	<0.05	0.10	0.10	—	—	0.05	0.42
Serum LDL[2] (mg/dL)	−0.04	0.50	−0.15	<0.05	—	—	−0.11	0.08	0.05	0.42	—	—
Physical activity (d/wk)	0.04	0.46	0.29	<0.001	−0.17	<0.01	0.10	0.09	0.27	<0.001	0.03	0.64
Energy intake (kcal/d)	0.12	<0.05	0.10	0.08	−0.05	0.37	0.00	0.95	0.02	0.78	0.10	0.10
Protein intake (% Energy)	0.06	0.35	−0.07	0.25	0.03	0.67	−0.05	0.38	−0.01	0.91	0.04	0.50
Fat intake (% Energy)	0.08	0.17	−0.09	0.13	0.08	0.16	−0.03	0.64	0.17	<0.01	0.09	0.15

[1] High-density lipoprotein.
[2] Low-density lipoprotein.
[3] Pearson's correlation coefficients.
[4] Body mass index.

Table 3. Multiple regression analysis of serum HDL[1] and LDL[2] against birth weight and other measurements.

Predictor variables	Boys (n=287)						Girls (n=286)					
	Serum HDL (mg/dL)			Serum LDL (mg/dL)			Serum HDL (mg/dL)			Serum LDL (mg/dL)		
	b (SE)[3]	β[4]	p	b (SE)[3]	β[4]	p	b (SE)[3]	β[4]	p	b (SE)[3]	β[4]	p
Model 1												
Birth weight (kg)	3.10 (1.88)	0.10	0.10	−1.61 (3.81)	−0.03	0.67	5.68 (2.19)	0.15	<0.05	−5.14 (4.10)	−0.08	0.21
Postmenarcheal age (y)	—	—	—	—	—	—	−0.21 (0.58)	−0.02	0.72	−1.67 (1.08)	−0.09	0.12
Body height (cm)	0.16 (0.17)	0.07	0.36	−0.12 (0.35)	−0.03	0.74	−0.22 (0.20)	−0.09	0.27	−0.44 (0.37)	−0.10	0.24
Body weight (kg)	−0.49 (0.14)	−0.35	<0.01	0.22 (0.29)	0.08	0.45	−0.36 (0.19)	−0.20	0.06	−0.01 (0.35)	−0.00	0.98
Percent body fat (%)	0.40 (0.25)	0.15	0.11	1.59 (0.50)	0.30	<0.01	−0.20 (0.24)	−0.01	0.93	0.65 (0.45)	0.14	0.15
Physical activity (d/wk)	1.53 (0.30)	0.34	<0.001	−0.81 (0.60)	−0.09	0.18	1.56 (0.35)	0.29	<0.001	0.67 (0.64)	0.07	0.30
Energy intake (kcal/d)	0.00 (0.00)	0.05	0.39	0.00 (0.00)	−0.01	0.94	0.00 (0.00)	−0.02	0.77	0.01 (0.00)	0.11	0.08
Protein intake (% Energy)	−0.19 (0.27)	−0.05	0.48	−0.09 (0.55)	−0.01	0.87	−0.07 (0.26)	−0.02	0.80	−0.01 (0.49)	−0.00	0.98
Fat intake (% Energy)	−0.02 (0.15)	−0.01	0.89	0.11 (0.30)	0.02	0.72	0.46 (0.16)	0.17	<0.01	0.39 (0.30)	0.08	0.20
R^2[5]	0.144			0.147			0.169			0.059		
Model 2												
Birth weight (kg)	0.79 (1.82)	0.03	0.67	−0.76 (3.58)	−0.01	0.83	2.93 (2.10)	0.08	0.17	−6.72 (3.84)	−0.10	0.08
Postmenarcheal age (y)	—	—	—	—	—	—	−0.22 (0.58)	−0.02	0.71	−1.48 (1.05)	−0.08	0.16
Percent body fat (%)	−0.22 (0.16)	−0.09	0.15	1.87 (0.31)	0.35	<0.001	−0.34 (0.16)	−0.13	<0.05	0.68 (0.28)	0.15	<0.05
Physical activity (d/wk)	1.18 (0.28)	0.26	<0.001	−0.65 (0.56)	−0.07	0.24	1.28 (0.33)	0.23	<0.001	0.64 (0.60)	0.07	0.29
Energy intake (kcal/d)	0.00 (0.00)	0.02	0.78	0.00 (0.00)	0.00	0.96	−0.00 (0.00)	−0.05	0.37	0.01 (0.00)	0.10	0.11
Protein intake (% Energy)	−0.23 (0.28)	−0.05	0.40	−0.08 (0.54)	−0.01	0.88	−0.07 (0.27)	−0.02	0.79	0.01 (0.49)	0.00	0.99
Fat intake (% Energy)	0.02 (0.15)	0.01	0.89	0.09 (0.30)	0.02	0.75	0.48 (0.16)	0.17	<0.01	0.42 (0.30)	0.09	0.16
R^2[5]	0.096			0.145			0.127			0.050		

[1] High-density lipoprotein.
[2] Low-density lipoprotein.
[3] Multiple regression coefficient (standard error).
[4] Standardized partial regression coefficient.
[5] Coefficient of determination.

(b=−0.49, p<0.01) and a higher frequency of physical activity (b=1.53, p<0.001) in model 1, and a higher frequency of physical activity (b=1.18, p<0.001) in model 2 individually were significantly associated with greater serum HDL. Also in boys, birth weight had no significant association with serum LDL, while a higher percent body fat (b=1.59, p<0.01 in model 1 and b=1.87, p<0.001 in model 2) was significantly associated with greater serum LDL. In girls, a higher birth weight (b=5.68, p<0.05) was significantly associated with greater serum HDL, while a higher physical activity (b=1.56, p<0.001) and fat intake (b=0.46, p<0.01) were significantly associated with greater serum HDL in model 1. However in girls, birth weight had no significant association with serum HDL, while a lower percent body fat (b=−0.34, p<0.05) and a higher physical activity (b=1.28, p<0.001) and fat intake (b=0.48, p<0.01) were significantly associated with greater serum HDL in model 2. There was no significant association with serum LDL in girls in model 1. In model 2, birth weight had no significant association with serum LDL, while a higher percent body fat (b=0.68, p<0.05) was significantly associated with greater serum LDL in girls. The results did not change when the subject's age was added into each model.

DISCUSSION

In this study, the relations between birth weight and serum HDL and LDL cholesterol levels were examined in Japanese late adolescents. Birth weight was positively associated only with serum HDL in girls independently of percent body fat and fat intake only when adjusted for current body height and weight. Most of the articles focusing on relationships between birth weight and serum cholesterol levels in later life used current body size as a correction factor (*2, 3*). However, current body size may have positive correlation with birth weight partly due to genetic effect. In fact, we found positive correlation between birth weight and body height and weight in both sexes in this study. Thus, we employed two types of models with or without body height and weight as predictor variables. In girls, significant correlation between birth weight and serum HDL was detected only when the model using body height and weight was applied; the relationship is still unclear. Another analysis method considering genetic effect would be required for the validation.

Serum lipid levels in adolescents change drastically with changes in sex steroid hormones as they develop (*10*). Thus, it should be considered that there is a range of serum lipid levels associated with the growing phase in individuals, even those of the same age, especially in early or middle adolescence. However, the relations between serum lipid levels and growing phase are not linear (*11*); therefore, it is not appropriate to adjust serum lipid levels linearly with the growing phase. Several studies have reported that total and LDL cholesterol levels decreased once during puberty, especially in males (*12–14*). We actually confirmed that in boys: LDL cholesterol decreased from the 1st to 2nd year of junior high school and thereafter increased in subjects of this study (data not shown). In this study, we analyzed the data in the 3rd year of high school where HDL and LDL cholesterol levels were assumed to be close to adult levels and stable.

In childhood and adolescence, serum low HDL or high LDL was shown to be associated with contemporary atherosclerosis (*15*). It may also indicate that risk factors such as serum total or LDL cholesterol levels in childhood or adolescence predict the risk associated with adult cardiovascular disease (*16, 17*). Thus, it may be necessary to examine serum lipid levels at a young age to predict current and future risk of cardiovascular events.

In a previous study of Japanese subjects, birth weight was inversely related to serum total cholesterol, but not to HDL cholesterol, at the middle adolescent age of 15 or 16 in both sexes (*18*). In another study, higher birth weight was significantly associated with lower serum total cholesterol in both sexes at age 20 (*19*). Furthermore, birth weight was inversely correlated with serum total cholesterol, but only in males at 22.5 y (*20*). We could not identify the reasons for the difference of the results among all of these studies, including this study. The differences of subject age and confounding factors may be part of the reason; moreover, we examined serum LDL cholesterol instead of total cholesterol. This may also have affected the differences in the results.

Increase in body fat among children and adolescents was suggested to be associated with adverse changes in serum lipids (*4, 5*). In this study, percent body fat had a positive association with serum LDL only in boys. Physical activity may also have a positive effect on serum lipids among children (*6*). In this study, physical activity had a positive association with serum HDL in both sexes.

Currently in Japan, the mean birth weight has been decreasing and the prevalence of low-birth weight infants (birth weight <2,500 g) has also been increasing, while an opposite trend has been shown in Western countries (*21*). Takimoto et al. indicated that the increase in preterm deliveries and multiple gestations were important factors with regard to the increase in low-birth weight infants in Japan (*21*). In addition, a decrease in BMI of young women with childbearing potential may be another important factor (*22*). Actually, the National Nutrition Survey showed a decreasing trend of BMI among young Japanese women in recent years (*23*). In this study, lower birth weight at full term and single births may be partly due to the mother's poor nutrition status.

Our study had several limitations; blood sampling was conducted under nonfasting conditions. However, it is known that the effects of meal intake on serum HDL and LDL cholesterol levels are insignificant (*24*). We were not able to estimate dietary cholesterol intake with a food frequency questionnaire. In addition, we did not examine the Tanner stage (*25*) as a growing phase out of consideration for the subjects' privacy. Thus, only for girls, were we able to verify the effects of growing phase by menarcheal age. Furthermore, we did not assess

dietary or physical activity habits of the subjects at an earlier age.

In conclusion, we found that in healthy Japanese late adolescents, birth weight had a significantly positive effect on serum HDL cholesterol levels independently of physical activity and fat intake in girls when adjusted for current body height and weight, but not in boys. Further studies would be required for the clarification of the relationship between birth size and serum lipids considering genetic effect and also the relationship in younger ages while appropriately adjusting for their growing phase.

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Metabolomics analysis of umbilical cord blood clarifies changes in saccharides associated with delivery method

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ABSTRACT

Background: A metabolomic approach using umbilical cord blood from infants at birth has not been studied widely yet.
Aim: We examined changes in metabolite levels in umbilical cord blood at birth via gas chromatography/mass spectrometry (GC/MS)-based metabolomics, with the aim of achieving a detailed understanding of fetal stress during labor.
Study design: All procedures were reviewed and approved by the Institutional Review Board of Kobe University School of Medicine. This was a cohort study of pregnant women based in Palmore Hospital, which is located in an urban area of Japan, and was carried out between December 2010 and May 2011.
Subject: Umbilical cord arterial blood samples were obtained from 41 infants immediately after delivery.
Outcome measures: Metabolites in the blood samples were measured using GC/MS to investigate whether the delivery method (spontaneous onset of labor, induction of labor or elective cesarean section) affected the metabolite profile in umbilical cord blood.
Results: Elective cesarean section without labor led to lower levels of isoleucine, fructose, mannose, glucose, allose, glucuronic acid, inositol and cysteine in comparison with vaginal delivery following spontaneous labor and without medication.
Conclusion: It is proposed that the stress associated with labor be involved in alterations in the levels of metabolites, particularly saccharides such as glucose, in umbilical cord blood.

1. Introduction

The mode of delivery during birth is strongly associated with fetal and maternal stress. In previous studies, it has been reported that fetal stress was decreased in newborns delivered by cesarean section compared with vaginal delivery, and cesarean section was associated with significantly lower maternal hormonal responses compared with vaginal delivery [1]. In addition, cortisol concentrations of second babies were significantly lower in comparison to first babies [1], and neonatal glucose concentration was lower in cesarean section infants than that in vaginal delivery infants [2]. Umbilical cord blood is the peripheral blood of the fetus, and various molecules derived from the maternal body are generally moved to the cord blood. To date, the low molecular weight metabolites in umbilical cord blood have not been comprehensively analyzed. Therefore, clarifying the metabolites in umbilical cord blood may be useful for the assessment of fetal nutrition or delivery stress for infants.

Metabolomics (metabolome analysis) provides comprehensive data about the metabolic processes of a cell or organism. Metabolomics has recently developed rapidly, and has been applied to various research fields including medicine. We have studied pancreatic [3], lung [4], and gastrointestinal [5] cancers, and gastroenterological diseases [6], and have revealed alterations in the serum metabolite profile associated with these diseases. In perinatology, analyses of neural tube defects [7] and intrauterine growth restriction [8,9] have been performed. Metabolomics analysis of human umbilical vein endothelial cells has demonstrated that activation of AMP-activated protein kinase induces metabolic effects in cell metabolism [10]. Here, we examined changes in metabolite levels in umbilical cord blood at birth via gas chromatography/mass spectrometry (GC/MS)-based metabolomics with the aim of achieving a detailed understanding of fetal stress during labor.

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2. Methods

2.1. Study design, participants, and location

All procedures were reviewed and approved by the Institutional Review Board of Kobe University School of Medicine. This was a cohort study of pregnant women based in Palmore Hospital, which is located in an urban area of Japan, and was carried out between December 2010 and May 2011. The study consisted of 60 pregnant women who volunteered to participate, or to have the study explained, after they had read our advertisement (Fig. 1). Informed consent was obtained from 54 of the 60 potential participants; the remaining six women declined. Thus, a total of 54 singleton pregnant women and their single infants participated. All of these pregnancies were full term. Forty-seven infants were delivered vaginally; the remaining seven infants were delivered by cesarean section prior to spontaneous labor owing to placenta previa or recurrent cesarean. The mother of one of these seven infants exhibited maternal hyperthyroidism, so this infant was excluded from the study. Among the 47 infants delivered vaginally, 19 were delivered without medication, and 16 were delivered vaginally with prostaglandin E2 and/or oxytocin independently. A total of 12 infants were excluded from the analysis because the mothers had been administered antibiotics, or because the infants had ventricular septal defects, low birth weight (birth weight <2500 g), or exhibited intrauterine growth retardation, or there was poor information available regarding the infants. All of the pregnant women were non-smokers and had no obesity, diabetes mellitus, or gestational diabetes mellitus. A total of 41 infants from 41 pregnant women were therefore analyzed. Our study also included 13 healthy adult volunteers, who had given a blood sample during a health examination at the hospital. Information about the participants is summarized in Supplemental Table 1.

2.2. Serum collection and preparation

Immediately after delivery of the newborn, a segment of the umbilical cord was doubly clamped, and an arterial blood sample was collected from this into a tube. The blood was centrifuged at 3000×g for 10 min at 4 °C, and the serum was transferred to a clean tube and stored at −80 °C until use. Previously described methods were used to extract low-molecular-weight metabolites from the sera [11], and to complete oximation and subsequent derivatization for GC/MS measurements [11].

2.3. GC/MS analysis and data processing

As previously described [12], GC/MS analysis was performed using GCMS-QP2010 Ultra (Shimadzu Co., Kyoto, Japan) with a fused silica capillary column (CP-SIL 8 CB low bleed/MS; 30 m×0.25 mm (inner diameter), film thickness: 0.25 μm; Agilent Co., Palo Alto, CA). Data processing was performed using MetAlign software (Wageningen UR, The Netherlands) and in-house analytical software (Aloutput). The metabolite identification was performed according to previous reports [12,13]. In this analysis, the peak detection and alignment was performed by MetAlign software. In Aloutput, the retention time for the obtained data was also corrected using an internal standard. The metabolite database used in our study includes the information about retention time and EI spectrum for each metabolite, and the retention time of each metabolite in this database was also corrected on the basis of the results from n-alkane mix analysis. For semi-quantitative analysis, the peak height intensity of each ion was calculated and normalized using the peak height of 2-isopropylmalic acid as an internal standard. In GC/MS analysis, multiple peaks are sometimes detected for a particular metabolite owing to TMS-derivatization or isomeric form. In such cases, the peak that most closely reflected the level of the metabolite was adopted for semi-quantitative evaluation. The results obtained were also checked manually, and the low-trust data were excluded. Thus, the data analysis including database was strictly controlled.

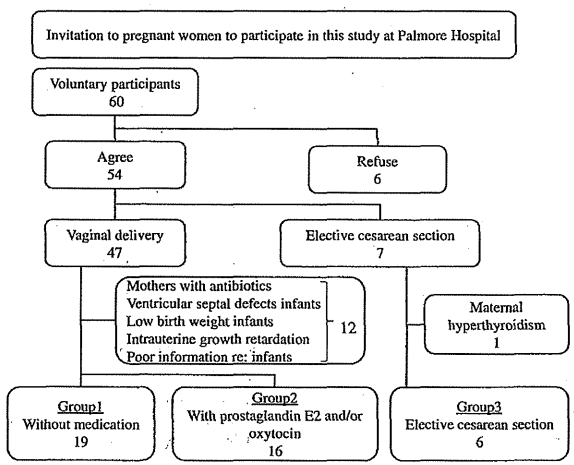

Fig. 1. Derivation of the cohort.

2.4. Measurement of glucose concentration

Serum glucose concentration was measured using a hexokinase method (UniCel DxC 600, Beckman Coulter) according to the manufacturer's instructions.

2.5. Statistical analysis

Data were analyzed using JMP9 software. Statistical significance was determined using the Steel–Dwass test or the median test, and $p<0.05$ was used as the criterion for significance.

3. Results

This study consisted of 60 pregnant women between the ages of 21 and 45 years old. Their infants were divided according to the method of delivery: the first group (group 1) included vaginal delivery without medication (n=19); the second group (group 2) included vaginal delivery with prostaglandin E2 and/or oxytocin treatment (n=16); and the third group (group 3) included cesarean section prior to spontaneous labor (n=6). Table 1 shows data from the three groups. There were no statistically significant differences in the birth weight among the three groups. The gestational age of group 3 was significantly lower than that of the other groups.

In our experimental conditions, a total of 81 metabolites were detected in the sera of umbilical cord blood (Supplemental Table 2). We compared the metabolites in sera obtained from umbilical cord blood with those from the venous blood of healthy adult controls. This comparison showed that the levels of 20 metabolites were significantly higher, and 18 metabolites were significantly lower, in the sera obtained from umbilical cord blood (Supplementary Table 3), suggesting that metabolites in umbilical cord blood may provide relevant information on differences compared with control venous blood, and that the metabolite profile of the umbilical cord blood may be useful for understanding the baby's status. Serum metabolites of the three groups (groups 1, 2, and 3) were therefore compared as described above. As shown in Table 2, the levels of nine metabolites were significantly different between groups 1 and 3 ($p<0.05$). 3-Hydroxybutyric acid ($p=0.038$) was higher in group 1, while the remaining eight significantly different metabolites (isoleucine ($p=0.027$), fructose ($p=0.003$), mannose ($p=0.023$), glucose ($p=0.003$), allose ($p=0.042$), glucuronic acid ($p=0.003$), inositol ($p=0.004$), and cystine ($p=0.002$)) were significantly higher in group 3 compared with those in group 1 (Table 2). In contrast, the levels of two metabolites (acetoacetic acid and 1-hexadecanol), were significantly higher ($p<0.05$) in group 2 compared with those in group 1; these differences may be due to the administration of prostaglandin E2 and/or oxytocin. As shown in Table 2, the levels of various saccharides (including glucose) in groups 1 and 2 were higher than those in group 3. To corroborate this observation using a non MS-based method, the serum glucose level in these groups was measured using an enzymatic (hexokinase) method (Fig. 2). In agreement with the results from GC/MS-based metabolomics analysis, serum glucose concentrations in group 1 ($p=0.002$) and group 2 ($p=0.001$) were significantly higher than those in group 3, suggesting that the results from the GC/MS-based metabolomics analysis are reliable.

Next, we compared the serum metabolites between primiparous and multiparous mothers who gave birth via vaginal delivery (Supplemental Tables 4 and 5): both the primiparous group (n=26) and the multiparous group included vaginal delivery with/without medication (n=9). Of the 81 metabolites identified, the serum levels of five metabolites were significantly higher in the primiparas compared with the multiparas (Table 3); fructose ($p=0.010$), mannose ($p=0.020$), glucose ($p=0.042$), allose ($p=0.043$), and glucuronic acid ($p=0.048$).

4. Discussion

In this study, a GC/MS-based metabolomic approach was performed using umbilical cord blood obtained from infants at birth. In the umbilical cord blood of infants born by vaginal delivery, serum levels of various saccharides and related molecules; e.g., fructose, mannose, glucose, allose, glucuronic acid, and inositol, were higher than those in the blood of infants delivered by cesarean section. In particular, the vaginal delivery group with prostaglandin E2 and/or oxytocin treatment had higher levels, suggesting a correlation between the stress associated with labor and alterations in metabolites.

Allose, which is a rare sugar, is known to be a potent inhibitor of ischemia/reperfusion injury [14]. Administration of allose has been shown to have protective effects against neutrophil-related post-ischemic injury of the liver [14]. There is little information on the effects of rare sugars in infants born by vaginal delivery following labor, and this is the first report to demonstrate that infants delivered by cesarean section had decreased levels of allose in umbilical cord blood sera (Table 2). This suggests that infants born via vaginal delivery have a tendency to produce more allose as protection against ischemia, although this hypothesis needs to be investigated in detail.

In a previous report, it was demonstrated that the glucose concentration in infants born by elective cesarean section without labor was lower than that in vaginally delivered term infants [2], which is consistent with our results. Marom et al. pointed out that fetal stress during labor may lead to an increase in the fetal glucose level [2]. The levels of hormones such as cortisol and progesterone, which cause fetal stress via the cord blood, were significantly higher in the normal vaginal delivery group compared with those in the cesarean group [15,16]. Increased cortisol associated with stress increases the level of blood sugar through gluconeogenesis. In this study, high levels of saccharides, including glucose, were observed in the vaginal delivery groups using GC/MS-based metabolomics (Table 2) and enzymatic (Fig. 2) methods. In addition, the level of glucuronic acid, which is derived from glucose via oxidation of the hydroxyl group, was also higher (Table 2). Therefore, elective cesarean section may be less stressful for the fetus in comparison to vaginal delivery, and labor may enhance stress via an up-regulation of cortisol and progesterone.

Table 1
Comparison of characteristics between groups.

	Group 1 (n=19)	Group 2 (n=16)	Group 3 (n=6)	p-Value
Gender (% male)	9/19 (47.3%)	4/16 (25.0%)	3/6 (50.0%)	0.946
Birth weight (g)	3060 (2620–3336)	3125 (2825–3285)	2894 (2594–2932)	0.435
Gestational age (weeks)	39.3 (37.1–40.4)	39.6 (39.6–41.4)	37.6 (37.0–40.0)	0.017 [a]
Primipara mothers (%)	12/19 (63.2%)	14/16 (87.5%)	1/6 (16.7%)	0.020 [a]
Apgar score (1 min)	8.7 (8–10)	8.8 (8–9)	9.2 (8–9)	0.591
Cord blood pH	7.31(7.24–7.36)	7.29(2.16–7.39)	7.33(7.25–7.37)	0.151
Cord blood base excess (mEq/L)	−3.1(−10.2–−0.3)	−5.2(−11.1–−2.7)	−1.8(−4.9–−0.3)	0.003
Cord blood CO_2 (mm Hg)	43(26–60)	45(18–58)	43(41–56)	0.128
Maternal BMI before pregnancy	19.3(17.8–24.6)	20.9(16.6–24.2)	18.7(16.9–22.5)	0.128
Maternal weight gain (kg)	11.4(5.2–15.7)	10.5(5.8–23.2)	12.1(6.6–16.7)	0.873

Data are represented as the mean followed by the range. P values were calculated according to the median-test, and superscript letters (*) indicate p values lower than 0.05.

Table 2
Metabolites with significantly different levels between groups.

No.	Compound name	Average				Fold induction			p-Value		
		All (n=41)	Group 1 (n=19)	Group 2 (n=16)	Group 3 (n=6)	Group 1	Group 2	Group 3	Group 1 vs. 2	Group 1 vs. 3	Group 2 vs. 3
7	3-Hydroxybutyric acid	0.0777	0.0670	0.0496	0.1864	0.36	0.27	1.00	0.058	0.038[a]	0.010[a]
18	Isoleucine	0.0118	0.0011	0.0122	0.0091	1.20	1.34	1.00	0.529	0.027[a]	0.016[a]
41	Acetoacetic acid	0.0010	0.0008	0.0011	0.0010	0.81	1.10	1.00	0.033[a]	0.467	0.719
63	Tagatose	0.0062	0.0065	0.0036	0.0121	0.54	0.30	1.00	0.896	0.127	0.029[a]
64	Fructose	0.128	0.1021	0.1983	0.0222	4.59	8.92	1.00	0.391	0.003[a]	<0.001[a]
65	Mannose	0.1097	0.1118	0.1307	0.0486	2.30	2.69	1.00	0.865	0.023[a]	0.020[a]
66	Glucose	2.2131	2.2154	2.7472	0.7814	2.84	3.52	1.00	0.203	0.003[a]	0.001[a]
67	Allose	0.0089	0.0091	0.0116	0.0011	8.56	10.59	1.00	0.737	0.042[a]	0.013[a]
71	Glucuronic acid	0.0017	0.0018	0.0020	0.0008	2.37	2.65	1.00	0.982	0.003[a]	0.004[a]
73	1-Hexadecanol	0.0010	0.0008	0.0014	0.0007	1.30	2.09	1.00	0.017[a]	0.666	0.024[a]
76	Inositol	0.2786	0.2990	0.2956	0.1683	1.78	1.76	1.00	0.988	0.004[a]	0.008[a]
81	Cystine	0.0193	0.0207	0.0219	0.0082	2.52	2.66	1.00	0.947	0.002[a]	0.044[a]

Values represented the fold-induction of the peak intensity of groups 1 and 2 compared with that of group 3. The *p* values were calculated according to the Steel–Dwass test, and superscript letters ([a]) indicate p values lower than 0.05. All data are shown in Supplemental Table 2, and those metabolites with significant differences between groups are represented in this table. 'No.' is described according to Supplemental Table 2.

The lower level of glucose in umbilical cord blood of infants born by cesarean section without labor may be due to shorter gestation. If the gestational age is lower, hepatic accumulation of glycogen in the fetus is small, leading to a lower level of serum glucose. The gestational age of babies delivered by the cesarean section group is generally less than that of babies born via the vaginal delivery group, and a significant difference between these groups was observed in this study (Table 1). Newborns delivered by cesarean section have relative hypoglycemia, because the mother is treated with dextrose infusion, leading to an increase of the blood insulin level in both mother and fetus, resulting in lower serum glucose in the fetus. Our observations may be explained by these phenomena. Furthermore, the stress to the mother also seems to increase the level of saccharides (including glucose) in the umbilical cord blood. In the case of vaginal delivery, prostaglandin E2 and/or oxytocin treatment tended to lead to higher levels of saccharides in the umbilical cord blood. Generally, a primipara mother is heavily stressed during delivery compared with a multipara, and the level of glucose in the umbilical cord blood of the baby of a primipara was higher than that of a multipara (Table 3). These results may therefore be related to stress of the mother.

Mannose is required for glycoprotein and glycophospholipid syntheses. The level of mannose is increased by fetal synthesis rather than by placental transport [17]. The umbilical uptake of mannose from the maternal circulation suggests the presence of a mannose transporter in human trophoblasts [17]. The characteristics of the mannose transporter

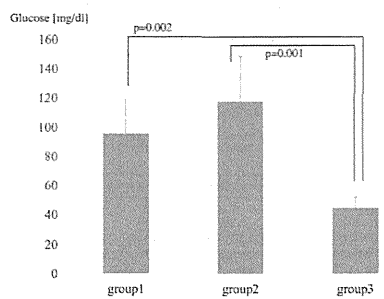

Fig. 2. Serum concentrations of glucose in infants. The serum concentration of glucose in the umbilical cord blood was measured using a hexokinase method. Data are the average value for each group, and error bars represent the standard deviation. Significant differences were evaluated using the Steel–Dwass test, and p values are as described in the graph.

are different from those of a glucose transporter; the mannose transporter has a high affinity for mannose and relatively low affinity for glucose [17]. The basement membranes of both the trophoblast layer and the endothelial layer have been observed to strongly and continuously express mannose [18]. Mannose glycans accumulate in fetal tissues during development, and the production of mannose glycans in human fibroblasts may require the presence of extra-cellular mannose, rather than relying on its intracellular production from glucose [19]. In this study, a higher level of mannose was observed in the vaginal delivery groups (Table 3). Placental function declines during labor, and therefore the accumulated mannose may be released into the umbilical cord blood during labor.

The production of fructose via the sorbitol pathway exists in the fetus and newborn, and is an alternative pathway in glucose metabolism that may maintain redox balance in the fetus [19]. The presence of fructose in the umbilical cord blood suggests fructose production by the fetus at term [17]. GLUT5, which is a fructose transporter into cells, has been identified in cells derived from trophoblasts, and the sorbitol pathway was also found to be active in macerated umbilical cords from human newborns [17]. Our findings suggest that the increased level of fructose in the umbilical cord blood during labor may contribute to its level in newborns. Fructose, glucose, and sucrose have been widely studied in human newborns, and were found to have equal analgesic effects [20]. Fructose may therefore reduce fetal pain and stress, in particular during labor.

Inositol is present in all tissues, and its level is particularly high in tissues whose cells do not divide rapidly in adult life [17]. The concentration of inositol in the fetus is higher than that in the mother, and is elevated during fetal life before decreasing postnatally [17]. Inositol may therefore have important biological roles during early development, but fetal inositol requirements are currently unknown. The level of inositol in the umbilical cord blood may be regulated in response to the stress associated with labor.

Tagatose is a stereoisomer of fructose, and is a rare sugar present in nature products. It has a minimal effect on blood glucose and insulin levels. Supplemental D-tagatose could lower the plasma glucose level in previous animal and clinical studies [21]. Therefore, the alteration in the fetal tagatose level caused by fetal stress during labor may be associated with the alteration in the glucose level.

In the umbilical cord blood of infants born by vaginal delivery, serum levels of various saccharides and related molecules were higher than those in the blood of infants delivered by cesarean section, but the molecules except saccharides and related molecules also had the distinctive alterations. In humans, 3-hydroxybutyric acid, which is one of the ketone bodies, is synthesized in the liver from acetyl-CoA in a reaction catalyzed by the enzyme 3-hydroxybutyrate dehydrogenase, and can be

Table 3
Metabolites with significantly different levels between primiparous and multiparous mothers.

No.	Compound name	Average			Fold induction primi/multi	*p*-Value
		All (n=35)	Primiparous (n=26)	Multiparous (n=9)		
64	Fructose	0.1047	0.1852	0.0365	5.07	0.010[a]
65	Mannose	0.1201	0.1350	0.0772	1.75	0.020[a]
66	Glucose	2.4585	2.6571	1.8848	1.41	0.042[a]
67	Allose	0.0103	0.0121	0.0049	2.45	0.043[a]
71	Glucuronic acid	0.0019	0.0020	0.0014	1.41	0.048[a]

Values represented the fold-induction of the peak intensity of primiparous mothers compared with that of multiparous mothers. The *p* values were calculated according to the Steel–Dwass test, and superscript letters (*) indicate p values lower than 0.05. All data are shown in Supplemental Table 5, with significantly different metabolites shown in this table. 'No.' is described according to Supplemental Table 5.

used as an energy source in the brain when the blood glucose level is low. Little is known whether 3-hydrocybutyric acid can act as a potential central signal in maintaining energy homeostasis [22], but the high nutritional supply is needed during suckling, possibly showing that its level is increased during early development and decreased after weaning. Actually, in this study, the level of 3-hydroxybutyric acid was significantly higher in the infants' group compared with that in the normal adult controls (Supplemental Table 3). In addition, the level of 3-hydroxybutyric acid in the vaginal delivery group was significantly lower than that in the cesarean group (Table 2). Fetal stress during labor increased the fetal glucose level, and so its increase may lead to the decreased level of 3-hydroxybutyric acid.

Acetoacetic acid is also one of the ketone bodies. The level of acetoacetic acid was not significantly different between the infants' group and the normal adult controls (Supplemental Table 3), and the significant difference between group 1 and group 2 was observed (Table 2). Therefore, the treatment with prostaglandin E2 and/or oxytocin may lead to its significant alteration, although we could discuss the reason.

1-Hexadecanol, which is one of the fatty alcohols, had the higher level in group 2 compared with other groups (Table 2). However, we could not obtain the insight into the relationship between the fetal level of 1-hexadecanol and the labor/delivery method. Therefore, the alteration in the level of 1-hexadecanol might be false-positive, although detailed research may be needed.

In conclusion, in the umbilical cord blood of infants born by vaginal delivery, serum levels of various saccharides and related molecules were higher than those in infants delivered by cesarean section, and we propose that the stress associated with labor would be involved in the alterations of these metabolite levels, although it is unknown whether the alterations are the cause or effect of the stress. Interestingly, the levels of various metabolites are different between the umbilical cord blood obtained from the fetus and the venous blood obtained from healthy adult controls, and metabolome analysis may be capable of representing the degree of stress during labor. Our findings raise the possibility that metabolite profiling may be useful for evaluating infants at birth. In the future, more comprehensive studies using a larger number of infants are required to verify the detailed metabolomic profiles, and further investigations monitoring these at birth may improve the quality of life of neonates and their parents.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.earlhumdev.2012.10.010.

Conflict of interest

The authors declare no conflicts of interest.

Role of the funding source

The study sponsors had no involvement.

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妊産婦のやせと胎児発育
DOHaD(Developmental Origins of Health and Disease)の視点から考える

福岡秀興*1　伊藤早苗*2　石田裕美*2

受精した後の約2週間はインプリント現象という著しい遺伝子の再構築が起こり，エピジェネティクスが大きく変化していく。今日本では妊孕年代のやせが進展しており注意する必要がある。やせた状態での妊娠は，胎児・胎仔への影響として，生活習慣病素因の形成，出生体重の低下，早産・切迫早産のリスクが高くなる。ストレス耐性の低下，薬物中毒，閉塞性肺疾患，凝固線溶系の異常なども起こしやすいとの報告がある。また妊娠初期であればあるほど，それは世代を超えて伝達されていく。栄養の重要性を理解して，若年時から望ましい食習慣を確立することが重要である。

はじめに

西欧では，古くより出生体重と疾病罹患との関連について疫学研究が行われてきた。これはバーカー説，さらに発展してDOHaD(Developmental Origins of Health and Disease)学説として展開している[1]。胎生期に形成される疾病リスクの素因はエピジェネティックス変化である。これら疾病群は，non communicative disease(NCD：WHO)とする新たに統一した疾患概念で見られている。経済的発展が期待されている国で特に増加していく。胎生期，新生児期にその疾病素因が形成されるので，栄養状態が必ずしも良くない国が経済的な発展を遂げると著しくNCDが増加することが想定されている。母体の栄養状態，胎内栄養環境が次世代の健康を大きく決めていく。その背景を考えると今の日本では，受精時のやせ，低栄養状態が拡がっており，いかなる影響を児に及ぼすのかを検討していく必要がある。体格指数〔BMI〕18.5以下をやせと定義するが，世界的に見ても日本のやせ女性の頻度は著しく高い。やせ女性は増えており，やせは本人自身の健康へ，また妊娠した場合は子供や孫にまで，望ましくない影響が及ぶ可能性がある。経済的に豊かでありながらやせ女性の頻度の高いのは，女性自ら意図して栄養を摂らずやせているのか，それとも栄養を摂りたくても摂れない状況が背景にあるのかは，十分検討するべきである。日本社会の階層化が進んでいる結果でないことを祈るばかりである。

1．やせ(妊娠前からの低栄養)の影響

日本では若年女性の著しいやせ傾向が顕著になってきている(図1)。特に，20代女性のやせが増加しており，22～29%(平成23年：22.1%)がやせであり，この頻度は次第に上昇している。「やせ」は，当然その女性本人の一生の健康・QOL・寿命に影響する。本人への影響を考えと，卵巣機能の障害，さらに無月経でも重症の第二度になると卵巣機能が回復できるのは約50～70%に過ぎないと報告されており難治性疾患と言ってよい。回復できない場合は，低エストロゲン状態が持続することで，骨量の減少，骨粗鬆症，動脈硬化などが起こっていく。また，この栄養摂取量の低下は寿命へも影響する。日本では各年代で寿命が延びているが，20代女性群は，寿命は短縮すると予想されている。

日本で「やせ」女性が増えている1つの要因として，摂取エネルギー量が少ないことが挙げられている。日本では1995年から2012年まで約15年間で，20代の女性の栄養摂取量は1日1,900 kcalから1,669 kcalと著しく減少してきている(図2)。摂取エネルギーが不足することは，

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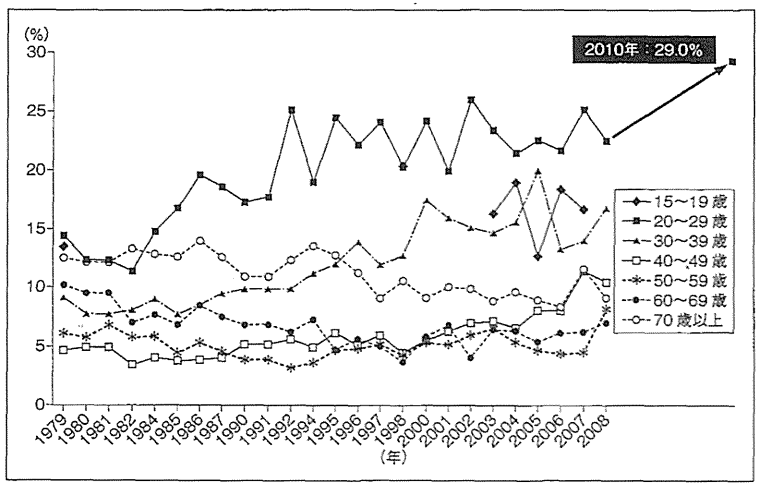

図1「やせ女性」頻度の推移　(国民健康・栄養調査より)

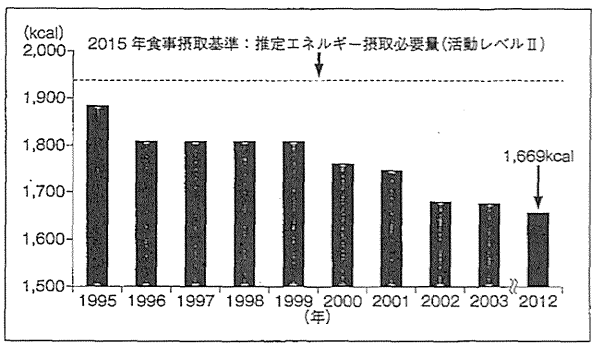

図2 20代女性の栄養摂取量の推移(エネルギー摂取量の推移)
(国民健康・栄養調査より)

表1 妊娠前・受精時「やせ」のリスク (peri-conceptional malnutrition)

1) 成人病素因の形成リスク
2) 低出生体重児のリスク
3) 早産，切迫早産のリスク

他の栄養素の不足状態をも意味している。日本および世界では，この神経管閉鎖障害の予防のために葉酸の摂取を強く勧めている。穀類に添加している国もあり，その発症頻度は減少している。しかし日本は二分脊椎症の発症頻度は少しずつであるが増加している。これはやせ女性の増加，エネルギー摂取量の低下と併せ考えると，葉酸を含めて十分なほかの栄養素を摂取していないことを示唆する1つの現象とみるべきかもしれない。

2．やせて妊娠した場合の子どもへの影響

やせた状態で受精・妊娠した場合(peri-conceptional and pre-conceptional period)は，生まれてくる子どもへの影響が心配される。やせて妊娠した場合に生ずるリスクの概略を表1に掲げた。受精してから2週間の期間に父親・母親からのDNAが結合して大きく変化するインプリンティング現象が生じている。受精時の低栄養は，当然エピジェネティクス変化を起こす。この時期に低栄養であっても，妊娠中に十分な栄養を摂取した場合は出生体重が必ずしも小さくないことがある。しかし，この時期の低栄養は成人病発症リスクを高くするのである。早産の原因の1つには子宮頸管部の感染があるが，非感染性の切迫早産・早産が相当に存在していることも事実である。受精周辺期の低栄養がその原因の1つである。

オランダの飢餓事件に遭遇して生まれた人々の長期にわたるコホート研究がなされており，受精周辺期の低栄養曝露は，表2に示す疾患群の発症リスクの高いことが明らかとなっている[2]。なお初期に飢餓に曝露された母親から生まれたこれら人々の出生体重は必ずしも小さくないことは理解しておくべきである。

表2 受精前後の低栄養曝露により発症リスクが高くなる疾患

1) 糖代謝異常・耐糖能の低下
2) 脂質異常症
3) 凝固系の異常
4) ストレス耐性の低下
6) 肥満(女性)
7) 動脈疾患
8) 統合失調症
9) 薬物中毒
10) 乳癌
11) 他

受精周辺期，その後の妊娠中，新生児，乳児期の低栄養曝露は異なった疾病を引き起こす。オランダの飢餓事件の調査からは，初期の低栄養曝露は多様な疾患の発症リスクを示している。
(文献2より引用)

3．受精周辺期の母体低栄養の及ぼすエピジェネティクス変化

ヒツジを対象とした低栄養実験の1つ[3]を紹介する。ヒツジに受精8週間前から受精後6日までの間，エピジェネティクス代謝に関連する栄養素である葉酸，ビタミンB12，メチオニンを完全に欠如した飼料を与えて，その後は通常食に戻す実験である。そして胎生50日目に胎仔肝臓組織を採取し，RLGS(restriction landmark genome scanning)でメチル基の付加状態をみた(図3)。低栄養曝露群と対照群とではメチル基の付加状態が大きく変わっているのが見られる。主として低メチル化であるが，メチル基が結合している部位もある。総合的にみると約4.1%に変化がみられている。さらに出生仔の生後23カ月でアンギオテンシンⅡを投与して昇圧反応を検討した。その結果，図4に示すごとく，昇圧物質に対する過剰な血圧上昇がみられている。これは将来的には高血圧の発症を示す現象である。さらに雄仔ヒツジの22週齢で，ブドウ糖負荷試験を行った(表3)。0.5 g/kgのブドウ糖を静脈内投与すると血糖のピーク値はほぼ同じであるが，インスリンのピーク値はまったく異なっていた。この結果は，インスリン感受性の著しい低下が生じていることを示し

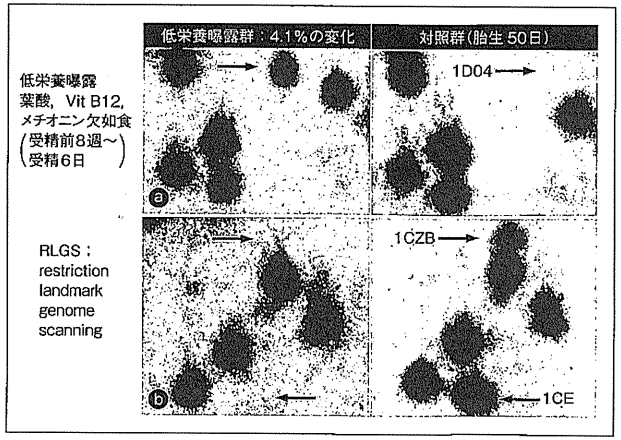

図3 RLGSでみた胎仔90日肝臓のCpGのメチル化プロファイル
一部を拡大したもので右側の上下が対照群，左側上下が低栄養群。右上の欠如している1D04スポットが左では出現している。これは低栄養群でこのCpG部位にメチル化が起こっていることを示す。また下段で低栄養群で出現している1CZB，1CEが低栄養群では消失しており，脱メチル化が低栄養群で生じていることが示される。
(文献3より引用改変)

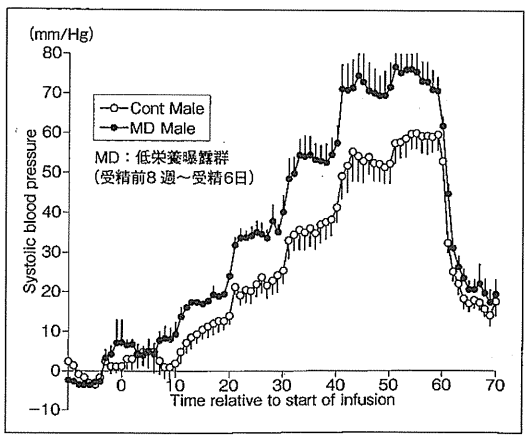

図4 受精時低栄養曝露のアンギオテンシンⅡ投与に対する仔ヒツジの血圧上昇反応
受精時にメチオニン，葉酸，ビタミンB12を欠如した飼料を与えて，出生後23カ月後に，仔ヒツジにアンギオテンシンⅡを血管内投与して収縮期血圧の変化をみたもの。低栄養群で血圧上昇が著しい。
(文献3より引用改変)