ORIGINAL

Serum biotin level during pregnancy is associated with fetal growth and preterm delivery

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Abstract : Background : Biotin is a water-soluble vitamin that plays various biological roles through histone modification, such as immune functions and fetal growth. Mammalian maternal biotin deficiency during gestation induces fetal growth restriction. Preterm infants are known to be marginal biotin deficiency. However, studies on the biotin status of pregnant women under various conditions are lacking. Method : This was a retrospective case control study to analyze serum biotin concentration during pregnancy and cord blood in normal pregnancy, preterm delivery and small-for-gestational-age (SGA). Results : Twenty pregnant women with normal term delivery, 35 with preterm delivery, 24 with SGA, and 10 non-pregnant adult women were enrolled. Serum biotin concentrations of pregnant women remained low from first to third trimester. The levels of serum biotin in cord blood showed a significant positive correlation with gestational age, and that of pregnant women showed a weak positive correlation with gestational age. The maternal serum biotin levels during second and third trimester of SGA group were significantly lower than those of normal term delivery. Conclusion : This study suggests that maternal biotin deficiency during pregnancy might be the risk of preterm labor or fetal growth restriction. Further studies are required to clarify the roles of biotin in perinatal medicine. J. Med. Invest. 67 : 170-173, February, 2020

Keywords **:** *biotin, pregnancy, preterm birth, fetal growth restriction, small for gestational age*

INTRODUCTION

Biotin is a water-soluble vitamin (B_7) that acts as a coenzyme for multiple carboxylases involved in fatty acid metabolism, amino acid metabolism and gluconeogenesis (1). Furthermore, biotinylated histones participate in various biological mechanisms such as mitotic condensation of chromatin, cell differentiation and cell proliferation, all of which are important for germinal development (2,3). Fetuses from marginally biotin-deficient mouse dams show a > 50% incidence of intrauterine uterine growth retardation, cleft palate, micrognathia, brachymelia, and visceral organ malformations (4). However, whether human maternal marginal biotin deficiency during pregnancy is related to fetal growth has not yet been elucidated.

According to the previous reports, pregnant women are known to be marginally biotin-deficient (5). Because biotin deficiency cause immunodeficiency (6) and infection is regarded as a major risk of preterm birth (7), biotin deficiency of pregnant women might be associated with the risk of preterm. Whereas preterm infants are known to exhibit low serum biotin levels (8), the relationship between maternal biotin deficiency during pregnancy and preterm delivery has not yet been clarified.

Vitamin D deficiency during pregnancy has been attracting attention as a risk of premature birth and small for gestational age (SGA) recently (9), and many studies including ours have been conducted (10), but little has been done on biotin. This study aimed to investigate the association between maternal biotin concentration and the risks of preterm birth and fetal growth restriction.

Received for publication January 16, 2020 ; accepted February 3, 2020.

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MATERIALS AND METHODS

This is a retrospective case control study. Pregnant women and their newborns who were born in our perinatal medical center between 2012 and 2016 were randomly enrolled in this study. Written informed consents were obtained from all the participants and guardians. We had the approval of institutional review board (No. 2741, 2750). The remaining sera from the clinical specimen were stored frozen at -50℃ for this study. We analyzed serum biotin concentrations from stocked maternal sera during pregnancy and cord blood in normal pregnancy, preterm delivery, and fetal growth restriction. We collected clinical data from medical charts. Ten healthy, non-pregnant women between 22 and 40 years old were also recruited as controls. No participants were taking any supplements including biotin. The term-delivery group was defined as those mothers with : neonate born at 37-41 gestational weeks ; normal baseline body mass index for the mother ; appropriate-for-gestational-age (AGA) stature of the newborn ; and no complications in either mother or newborn. The preterm-delivery group was defined as those mothers with : neonate born earlier than 37 gestational weeks ; and no complications in either mother or newborn. The small-for-gestational-age (SGA) group was defined as those mothers with a neonate below the 10th percentile for both birth weight and height according to the neonatal growth chart (new Japanese neonatal anthropometric chart) used in general in Japan (11). Early term was defined as < 14 weeks, middle term was 14-27 weeks and late term was ≥ 28 weeks of gestational age.

Serum biotin was analyzed using a commercially available biotin enzyme-linked immunosorbent assay kit (Immundiagnostik, Bensheim, Germany) according to the instructions from the

manufacturer (12). Each serum sample (150 μL) was added to individual wells. Serum samples and standard biotin solutions were pre-incubated with 150 μL of streptavidin-enzyme conjugate solution for 15 min at 25°C. After stopping the reaction by adding 100 μL (0.12 mol/L) of hydrochloric acid to each well, absorbance at 450 nm was measured using a plate reader (SpectraMax 190 ; Molecular Devices, San Jose, CA). Absorbance was calculated by subtracting the reference absorbance at 620 nm from that at 450 nm. The normal range was defined as the 10% tile to 90% tile of serum biotin levels in healthy non-pregnant women.

Analysis of variance (ANOVA) and the Kruskal-Wallis test followed by a Bonferroni test or Tukey test were used for parametric and non-parametric multiple data, respectively. The χ^2 test was used to compare ratios of biotin deficiency in different groups. Pearson correlation coefficients were used as a statistical measure of the strength of a linear relationship between paired data. All data are presented as mean ± standard deviation (SD). A value of $P < 0.05$ was considered significant. All computations, including data management and statistical analyses, were performed using JMP software Version 12.01 (SAS Institute, Cary, NC) and GraphPad Prism Version 7e (GraphPad Software, La Jolla, CA).

RESULTS

A total of 79 pregnant women were enrolled in this study. Clinical background data are summarized in Table 1. Twenty pregnant women were recruited as the normal term-delivery group ; mean duration of gestation was 38.7 weeks (range, 37-41 weeks), and mean birth weight of the newborn was 3119 ± 246 g (range, 2714-3584 g). Thirty-five pregnant women were enrolled as the preterm-delivery group ; mean duration of gestation was 28.2 weeks (range, 24-35 weeks) and mean birth weight of newborns was 1243 ± 508 g (range, 574-2380 g). Twenty-four women were enrolled as the SGA group ; mean duration of gestation was 38.3 weeks (range, 37-41 weeks) ; and mean birth weight of the newborn was 2252 ± 296 g (-2.0 ± 0.75 SD ; range, 1600-2666 g). The 10 non-pregnant women enrolled as controls had a mean age of 31.1 ± 6.6 years (range, 22-40 years). Gestational age of sampling were as following, term 24.3 ± 9.9 week, preterm 20.5 ± 6.4 week, and SGA 29.4 ± 8.7 week, respectively. ANOVA test showed a significant difference between the three groups of sampling- gestational age ($P < 0.01$) and multiple comparisons by Tukey test revealed term vs preterm ($P = 0.23$), term vs SGA ($P = 0.08$), preterm vs SGA ($P < 0.01$), respectively.

Maternal serum biotin concentration in the term-delivery group sustained low throughout pregnancy, at 122.0 ± 86.2 ng/L (normal range ; 148.8-475.4 ng/L) in early-term pregnancy, 144.2 ± 93.9 ng/L in middle-term pregnancy, 220.9 ± 206.8 ng/L in late-term pregnancy (Fig. 1). Serum biotin levels revealed biotin deficiency (less than 100 ng/L) in about half of pregnant women. Maternal serum biotin increased in the late term but the difference was not significant compared with early and middle term ($P = 0.07$). In the term group, the serum biotin levels of cord blood were 412.9 ± 168.7 ng/L, significantly higher than maternal serum biotin during pregnancy ($P < 0.001$). The concentration of serum biotin was significantly lower among pregnant women of any three groups than among non-pregnant women ($p < 0.0001$) (Fig.2). The biotin concentration in cord

Table 1. Clinical background data.
P values were calculated by ANOVA for parametric data or Kruskal-Wallis test for non-parametric data, respectively. SGA, small-for-gestational-age.

	term (n=20)	preterm (n=35)	SGA (n=24)	P value
age (year)	31.2±3.9 (24-38)	32.7±5.7 (21-42)	33.0±6.1 (20-44)	0.52
primiparity	9 (45%)	17 (48%)	12 (50%)	0.94
baseline BMI	20.6±1.2 (19.0-24.0)	21.6±4.0 (17.3-33.4)	20.2±2.4 (16.0-25.4)	0.18
gestational age (week)	38.7±1.3 (37-41)	28.2±3.3 (24-35)	38.3±1.1 (37-41)	<0.0001
female infant	7 (35%)	17 (48%)	12 (50%)	0.54
birth weight (g)	3119±246 (2714-3584)	1243±508 (574-2380)	2252±296 (1600-2666)	<0.0001
birth weight (SD)	+0.0±0.34 (-0.5～+0.9)	+0.0±0.48 (-1.0～+1.1)	-2.0±0.75 (-4.4～-1.3)	<0.0001
gestational age of sampling (week)	24.3±9.9 (9-36)	20.5±6.4 (9-27)	29.4±8.7 (9-36)	<0.01

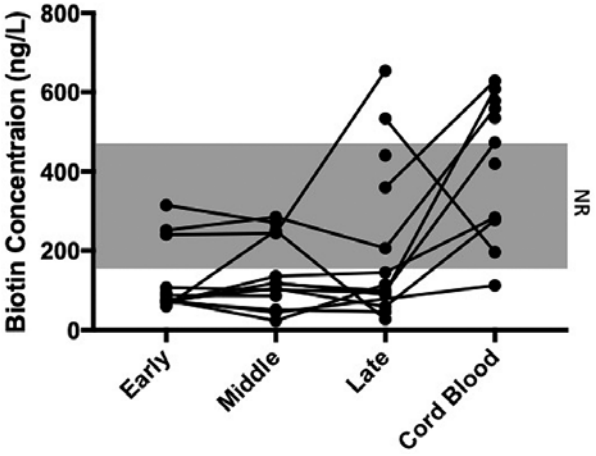

Fig 1. Serum biotin concentration in pregnant women and cord blood in the term-delivery group.
Individual values from the same subject are connected by each line. Whereas biotin concentration in cord blood was sufficient, maternal biotin concentration remained low from early to late term of pregnancy ($P < 0.05$). NR means normal range.

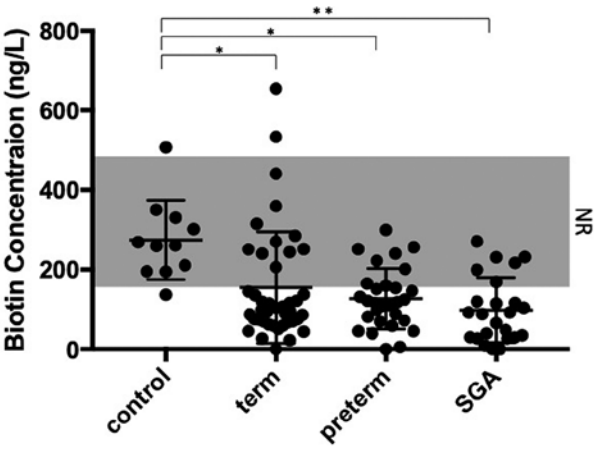

Fig 2. Serum biotin concentrations in pregnant women of term-, preterm-, and small for gestational age- group compared with non-pregnant women.
Maternal biotin concentrations during pregnancy of term, preterm, and small for gestational group were significantly lower than in non-pregnant women. *$p < 0.01$, **$p < 0.0001$. NR means normal range.

blood showed a significant positive correlation with gestational period ($r = 0.45$, $p < 0.01$) (Fig.3). Maternal biotin concentrations at middle term of pregnancy showed a weak positive correlation with gestational period at birth ($r = 0.22$, $p = 0.24$) (Fig. 4). In the middle to late term of pregnancy, the levels of maternal serum biotin was not increased in SGA group (93.9 ± 17.9 ng/L) in comparison of term-delivery group (230 ± 43.7 ng/L) ($P < 0.01$) (Fig.5).

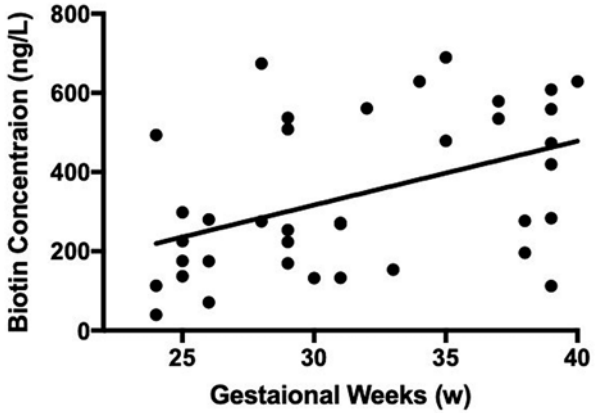

Fig 3. Correlation of gestational age and serum biotin concentration in cord blood of preterm and term groups.
Biotin concentration of cord blood showed a significant positive correlation with gestational age ($r = 0.45$, $p < 0.01$; Pearson correlation coefficient).

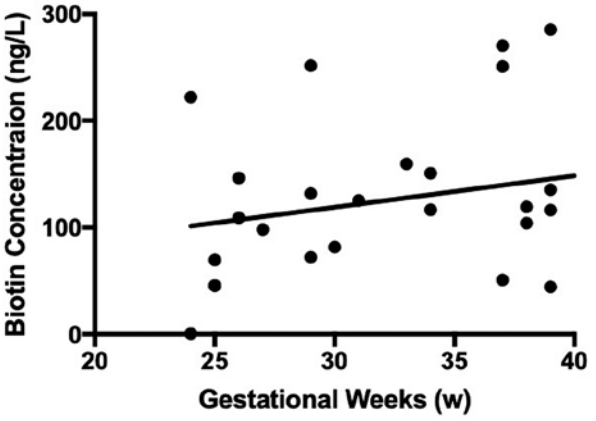

Fig 4. Correlation of gestational age and serum biotin concentration in maternal sera in middle term of pregnancy of term and preterm groups.
Maternal biotin concentration during pregnancy showed a weak correlation with gestational period ($r = 0.23$, $p = 0.24$)

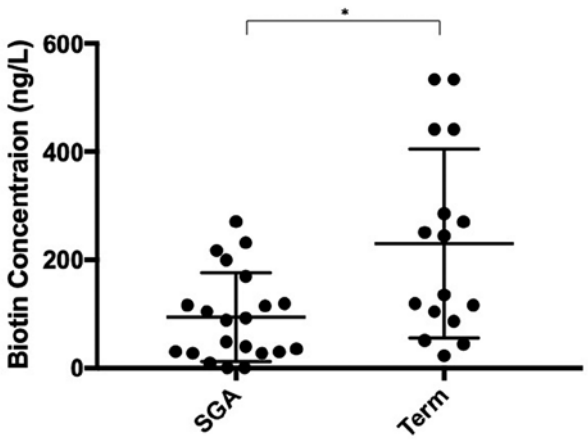

Fig 5. The serum biotin concentration of pregnant women with small for gestational group and term delivery group during middle to late term of pregnancy.
Maternal sera of small for gestational age group showed significant lower biotin concentration than normal term delivery group in the middle and late preterm of pregnancy ($p < 0.01$).

DISCUSSION

This study revealed that maternal serum biotin remained low from early to late term during non-complicated normal pregnancy. The previous report hypothetically described such as maternal marginal biotin deficiency might be caused by transmission from mother to fetus (13). However, the low maternal levels of serum biotin in early-term gestation seen in the present study are inconsistent with this hypothesis because both the fetus and placenta are so small during early pregnancy that biotin-transfer from mother to fetus is considered very small amount. Because the injections of acute doses of biotin into maternal rats during early pregnancy cause resorption of fetuses and placentae (14), the maternal biotin deficiency might be physiological and contribute immune tolerance during pregnancy. This hypothesis is supported by the fact that biotin is associated with immunological function as following described. The size and cellularity of the thymus and the synthesis of antibodies are decreased in biotin-deficient rats (6). Biotin deficiency in mice decreases the number of spleen cells and the percentage of B lymphocytes in the spleen (15). Children with biotin deficiency due to hereditary abnormalities involving biotinidase develop immunocompromised conditions (16).

Our present study demonstrated that serum biotin levels of maternal sera showed a weak positive correlation with gestational age and those of the cord blood showed a significant positive correlation with gestational age. Based on the previous reports, the fetuses underwent blood sampling during the second trimester for prenatal diagnosis of thalassemia exhibited adequate serum biotin levels (mean, 780 ng/L) (17), suggesting that the low biotin levels are characteristic features of preterm birth. This suggests that preterm pregnant women themselves have marginal biotin deficiency, but that biotin is deficient in the uterus and fetus compared to full-term pregnant women. The *in vitro* biotin-deficient medium conditions not only decrease differentiation of CD4+ T cells toward $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs), but enhance differentiation toward the Th1 and Th17 lines that produce pro-inflammatory cytokines such as interleukin 17, interferon gamma, and tumor necrosis factor alpha (18). A transition from an anti-inflammatory state to a

pro-inflammatory state at the maternal-fetal interface has been implicated in the pathophysiology of microbial-induced preterm labor (7). Further researches, including animal experiments, are required to confirm the hypothesis that maternal biotin deficiency might be associated with preterm labor and the biotin supplementation can be a candidate for prevention of preterm delivery.

SGA is at higher risk of neonatal morbidity and mortality ; in adolescence and adulthood, they present worse neurodevelopment, metabolic and cardiovascular adverse outcomes (19). The present study showed significantly lower maternal biotin levels in SGA group than normal term delivery group during middle to late term, suggesting biotin deficiency might be fetal growth restriction. Pregnant mammals fed biotin-deficient diet also showed fetal growth restriction (4). Biotin is related with various biological mechanisms for fetal development and growth through histone modification (3). Our data suggest that biotin supplementation during pregnancy can be a candidate for prevention or treatment of fetal growth restriction, and further large studies and animal studies that biotin supplementation for mammalian models of fetal growth restriction are required.

This study includes several limitations from the study-design, a small retrospective case-control study that potential selection and/or information bias might affect the interpretation of the results. The gestational age of sampling showed a significant difference between preterm and SGA group. This is because many of preterm pregnant women delivered before the late term sampling, and that it was difficult to obtain the early term samples of the SGA group because most of pregnant women with fetal growth retardation were referred in the middle or late term. In addition, this study did not examine dietary intake, supplementation from the intestinal microbiota, urinary excretion or metabolism of biotin, which would explain why maternal serum biotin of SGA group was not increased in the late term of pregnancy, or why the preterm pregnant women showed significant lower biotin in the middle term. Based on these facts, larger and prospective researches are required.

In conclusion, serum biotin levels of pregnant women remained low from early- to late-term gestation of normal-term pregnancy. Biotin deficiency during pregnancy might be a risk of preterm and SGA birth, but this is a small retrospective study, so further large prospective studies are necessary.

ACKNOWLEDGEMENTS

The authors' contributions to the work are as follows : Ichihara collected and analyzed the data and drafted the manuscript ; Suga designed the study, analyzed and interpreted the data and drafted the manuscript ; Fukui and Yonetani collected and analyzed the data ; Shono collected the clinical data ; Nakagawa and Kagami supervised the work and critically revised the manuscript. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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