Association between Serum Biotin Levels and Cedar Pollinosis in Japanese Schoolchildren

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Summary Biotin is a water-soluble B complex vitamin and coenzyme of five types of carboxylase and plays crucial roles in fatty acid, glucose, and amino acid metabolism. Nutritional biotin deficiency and defective enzymes essential for biotin metabolism cause inflammatory diseases such as eczema-like dermatitis and Crohn’s disease; however, little is known about the pathophysiological roles of biotin. This study investigated the relationship between biotin metabolism and human allergic sensitization and diseases by measuring serum levels of biotin, total immunoglobulin E (IgE) and allergen-specific IgEs in more than 400 Japanese schoolchildren aged 6 to 12. The prevalence of allergic diseases, and environmental and life-style factors were also examined by a questionnaire. Like total IgE, serum biotin levels of children showed a log-normal distribution. Meanwhile, Spearman’s rank correlation analysis showed weak but significant positive associations between serum biotin levels and total IgE (rho=0.147, p=0.0029) as well as allergen-specific IgEs against egg whites (rho=0.215, p=0.00013), cedar pollen (rho=0.176, p=0.00036), and cat dander (rho=0.130, p=0.0085). Furthermore, mean serum biotin levels in children with cedar pollinosis, but not with other allergic diseases such as asthma and allergic rhinitis, were significantly higher than in those without (p=0.0015). These results suggest a correlation between serum biotin levels and the development of cedar pollinosis. Further prospective studies are needed to evaluate the causal relationship between biotin metabolism and cedar pollen sensitization and pollinosis development.

Key Words vitamin B7, cedar pollen-specific IgE, allergic sensitization, allergic diseases, pediatric allergies

Biotin is a water-soluble B complex vitamin and a cofactor of five carboxylases, including two types of acetyl-CoA carboxylase (ACC), methylcrotonyl-CoA carboxylase, pyruvate carboxylase, and propionyl-CoA carboxylase and is also essential for fatty acid, glucose, and amino acid metabolism (1). Biotinyl-AMP, which is synthesized by holocarboxylase synthase (HCS), plays a central role in the form of activated biotin. In addition, biothynyl-AMP regulates gene expression of biotin-cycle-related enzymes, including HCS, sodium-dependent multivitamin transporter (SMVT), biotin-dependent carboxylases, and asialoglycoprotein receptor through activation of the soluble form of the enzyme guanylate cyclase via the cGMP-dependent protein kinase signaling pathway (2).

The relationship between biotin and human immune responses has been reported previously. For instance, children with hereditary abnormalities in biotin metabolism are known to show defects in T-cell and B-cell immunity (3). Biotin deficiency causes alopecia and erythematous dermatitis (4). Nutritional deficiency in biotin and deficiency of enzymes essential for biotin metabolism are known to cause eczema-like dermatitis (5) and affect NK cell activity in Crohn’s disease (6).
The cellular and molecular mechanisms of immune system impairment under condition of biotin deficiency have also been examined using murine model systems and human lymphocytes (reviewed in Ref. 7). Biotin deficiency reduces antibody production (8), the number of spleen cells (9), and percentage of B lymphocytes in the spleen (10). Deterioration in nickel-induced allergy was also observed following the upregulation of IL-1β production from splenocytes in mice fed a biotin-deficient diet (11). Both intestine-specific SMVT knock-out mice and dietary biotin-deficient mice showed an increase in gut permeability and chronic active inflammation in the cecum, along with increased expression of proinflammatory cytokines such as IFN-γ and TNF-alpha (12). These effects of biotin on immune response have been partially explained by its role in the regulation of transcription factors such as specificity proteins 1 and 3 and nuclear factor kappa B (13, 14).

Thus, the relationship between biotin and various inflammatory responses and diseases is established in terms of nutritional biotin deficiency and deficiency of enzymes essential for biotin metabolism. However, little is known about the role of biotin metabolism in human allergic sensitization and diseases. In this study, to determine the relationship between allergies and biotin metabolism, we investigated levels of serum total/specific immunoglobulin E (IgE) and biotin and associated correlations in Japanese schoolchildren aged 6 to 12 y.

MATERIALS AND METHODS

Participants. Children attending an elementary school located in the central area of Chiba city were recruited for this study. We first asked all 843 children of the elementary school for participation in the survey. We then sent a detailed questionnaire to those who had a positive response. Children with congenital heart diseases and lung diseases caused by immaturebirth were excluded. A total of 473 school children aged 6 to 12 y were enrolled in this study. Blood samples were collected from 411 participants in 3rd or 12th, July 2006 (15). Written informed consent was obtained from parents of all participants prior to enrollment in the study.

Questionnaire. To assess the status of allergic disease, a questionnaire based on the International Study of Asthma and Allergies in Childhood (ISAAC) (16) was used. Food allergy was diagnosed when the parents answered “yes” to all three questions as follows: 1) Has your child ever showed either of skin lesion, bad feeling, or signs of illness within 2 h of taking a certain food? (exclude food poisoning); 2) Has your child ever diagnosed as food allergy?: 3) Is your child currently diagnosed as food allergy? Current and past (up to 2 y old) environmental and life-style factors were also examined in the questionnaire survey.

Serum measurements. Serum biotin levels were quantified using a Biotin ELISA kit (Immudiganostik, Bensheim, Germany) according to the manufacturer’s protocol. Total and specific serum IgE levels were measured using the CAP-radio-allergosorbent test (Pharmacia Diagnostics, Uppsala, Sweden) as previously described (17). IgE levels were then classified into one of seven classes as follows: class 0, <0.35 IU/mL; class 1, 0.35–0.69 IU/mL; class 2, 0.7–3.49 IU/mL; class 3, 3.5–17.49 IU/mL; class 4, 17.5–49.99 IU/mL; class 5, 50.00–99.99 IU/mL; and class 6, ≥100 IU/mL. Atopy was defined as the presence of at least one positive specific IgE (≥0.35 IU/mL) among the measured allergens. Participants showing symptoms of allergic rhinitis in any month from February to May plus a class 1 or higher cedar pollen-specific IgE level were defined as having cedar pollinosis.

Statistical analysis. SPSS software (version 21; SPSS Japan, Tokyo, Japan) was used to perform statistical analysis. Smirnov-Grubbs’s test in the R Package ‘outliers’ (http://cran.r-project.org/web/packages/outliers/outliers.pdf) was used to estimate outliers of serum biotin. Two-sided p-values of 0.05 were considered significant.

Ethical approval. This study was approved by the ethics committee of the Chiba University Graduate School of Medicine (approval no. 150, July 2006). All procedures were conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

RESULTS

Serum biotin levels were measured with competitive ELISA using streptavidin, giving results from 408 sam-

![Fig. 1. Distribution of serum biotin levels. The histogram represents serum biotin (ng/L) under logarithmic transformation (n=408). Two samples showing more than 600 ng/L were excluded from further analysis.](image1)

![Fig. 2. Distribution of serum total immunoglobulin E (IgE) levels. The histogram represents total IgE (IU/mL) under logarithmic transformation (n=411).](image2)
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The raw values showed log-normal distribution (mean ± SD, 142.3 ± 62.8 ng/L; median, 130.0; skewness, 4.08; kurtosis, 28.4; interquartile range (IQR), 109.0–157.8). As shown in Fig. 1, a histogram of the log-transformed serum biotin levels showed symmetrical normal distribution (mean ± SD, 2.13 ± 0.146; median, 2.11; skewness, 0.88; kurtosis, 2.77). To test for outliers, Smirnoff-Grubb’s test was performed, revealing two relevant values (2.85 [raw value: 705] and 2.82 [raw value: 660]). These values were therefore excluded from the following analyses. After elimination of the outliers, the statistics of the raw values were as follows: mean ± SD, 140.0 ± 50.1 ng/L; median, 129.9; skewness, 1.94; kurtosis, 6.28; and IQR, 109.0–157.8. The statistics of the log-transformed values were as follows: mean ± SD, 2.12 ± 0.138; median, 2.11; skewness, 0.46; and kurtosis, 0.89. Age (6 to 12 y old) and sex did not significantly affect the mean and SD of the serum biotin level. Thus, following analyses were carried out as single group of children.

Serum total IgE levels were also measured in 411 participants by using a radio-allergosorbent test. As shown in Fig. 2, the distribution of total IgE showed approximate normal distribution under logarithmic transformation, with a mean ± SD of 1.94 ± 0.67 IU/mL. Levels of allergen-specific IgEs, including antibodies against the DerP1 of dust mites, black mold, egg whites, cedar pollen, orchard grass, cat dander, dog dander, and hamster, were also measured using the same test. The results are shown in Fig. 3 as histograms on a scale of 7 from class 0 to 6, as described in the “Materials and Methods.” With all eight IgEs, class 0 (classified as negative) scored the highest, with relatively low scores in all remaining classes. Participants with class 2 scores

Table 1. Correlations between serum biotin levels and total/specific immunoglobulin E (IgEs).

| Serum IgE     | Correlation coefficient | p-value      |
|---------------|-------------------------|--------------|
| Total IgE     | 0.147                   | 0.0029       |
| Dust mites    | 0.096                   | 0.053        |
| Cedar pollen  | 0.176                   | 0.00036      |
| Orchard grass | 0.087                   | 0.079        |
| Black mold    | −0.021                  | 0.68         |
| Cat dander    | 0.130                   | 0.0085       |
| Dog dander    | 0.088                   | 0.076        |
| Hamster       | −0.022                  | 0.66         |
| Egg whites    | 0.215                   | 0.00013      |
| Atopy         | 0.124                   | 0.013        |

1 Spearman’s rho.
2 Defined as at least one positive allergen-specific IgE (≥0.35 IU/mL) among the measured allergens.
and higher showed more abundant in DerP1 and cedar pollen-specific IgEs compared with the remaining allergen-specific IgEs.

The correlations between serum levels of biotin and total IgE, the abovementioned eight allergen-specific IgEs and atopy were subsequently determined using Spearman’s rank correlation coefficient (rho). As shown in Table 1, a weak but significant positive correlation was observed between serum biotin and total IgE levels (rho=0.147, p=0.0029) (Supplemental Online Material, Fig. S1). Serum biotin also showed weak correlations with egg whites (rho=0.215, p=0.00013), cedar pollen (rho=0.176, p=0.00036), cat dander (rho=0.130, p=0.0085), and atopy (rho=0.124, p=0.013) in descending order of rho (Table 1 and Supplemental Online Material, Fig. S2). These results suggest that serum biotin levels are positively correlated with a predisposition for allergic sensitization against various allergens.

Using analysis of variance, we also investigated the associations between serum biotin levels and the presence of allergic diseases, namely, atopic dermatitis, asthma, allergic rhinitis, cedar pollinosis, and food allergy (Table 2). Children with cedar pollinosis showed significantly higher biotin levels than those without (p=0.0015), in line with the correlation between serum biotin and cedar pollen-specific IgE shown in Table 1.

The correlations between serum biotin and various physical, biochemical, environmental, and life-style factors were also examined using Spearman’s rank correlation coefficient. Accordingly, only yogurt consumption and snack intake habits (unrestricted/guided by parents/no) showed significant correlations with biotin levels (Supplemental Online Material, Table S1). Next, a multivariate general linear model was constructed by starting with factors that showed a significant correlation with serum biotin levels in single-variant analyses (total and specific IgEs, cedar pollinosis, yogurt consumption, and snack intake habits). After eliminating non-significant (p>0.05) factors, three remaining factors (egg white-specific IgE, cedar-pollen-specific IgE, and snack intake habits) remained significant (Supplemental Online Material Table S2). These results suggest that dietary habits reflecting frequent intake of certain foods are correlated with higher serum biotin levels.

**DISCUSSION**

In this study, we investigated serum biotin levels, total and specific IgE levels, disease status, and various epidemiologic factors in more than 400 elementary schoolchildren. To our knowledge, this is the largest epidemiologic study to focus on serum biotin levels in people. Our results show that higher levels of serum biotin are associated with higher levels of cedar pollen-specific IgE and the presence of cedar pollinosis. We carried out blood sampling from participants in the first half of July, about a month after the end of cedar pollinosis season in Japan. Since cedar pollen-specific IgE changes seasonally (18), collecting blood samples in early summer, when the IgE is still maintained at relatively high levels in a population, is favorable for the evaluation of the IgE.

Competitive ELISA was used to measure serum biotin levels. The concentration of serum biotin obtained in this study was comparable to that obtained previously (47–220 ng/L, calculated using a two-sided 95%CI, n=188) in healthy Japanese children using the same kit (19). Raw values of serum biotin levels were slightly higher than the normal distribution, as reflected by the difference between the mean and median values, and by the large skewness values of the raw biotin levels. Meanwhile, the distribution of log-transformed biotin was more closely matched to the normal distribution; the mean and median became almost the same, and skewness was much reduced. To our best knowledge, this is the first study to reveal the log-normal distribution of human serum biotin levels. The larger number of samples examined in this study may explain why previous studies overlooked this fact. Meanwhile, Smirnoff-Grubb’s test revealed only the two highest values as outliers. Because we did not determine the biotin supplementation status or obtain detailed food consumption data in the questionnaire, the reason for these higher values is not known. However, it is possible that they were related to regular intake of biotin supplements.

In addition to total IgE and atopy, the allergen-specific IgE levels to cedar pollen, egg white and cat dander-specific IgEs were also significantly correlated with the biotin level, as well as showing significant reciprocal relationships (data not shown). One exception was egg
Cytokines were induced in human CD4+ T cells cultured in biotin-deficient condition (25). In contrast, Th17 cell differentiation was suppressed by a specific inhibitor of ACC in the biotin carboxylase domain in human and mice CD4+ T cells (26). These controversial findings may reflect the complex roles of biotin not only as coenzyme but also in the regulation of gene expression (2). Furthermore, epidemiological and molecular biological studies will be required to clarify the role of biotin supplied by diets and synthesized by gut flora in allergic sensitization and diseases.

Alternatively, biotin metabolism may be affected by allergic sensitization and diseases. Neither the effect of cells related to IgE production on biotin metabolism nor the role of biotin on IgE production has been clarified so far. Therefore, it is necessary to investigate whether various cells related to IgE production and the regulation of gene expression in these cells change biotin metabolism and whether they are affected by biotin deficiency or excess.

Limitations of this study include its cross-sectional design. We therefore do not conclude the causal relationship between serum biotin levels and the allergen-specific IgE levels and the onset of cedar pollinosis at this moment. Another limitation is no adjustment of potential confounding factors such as dietary intakes that may affect serum biotin levels. More detailed investigation on this factor should be included in future studies.

In conclusion, this study demonstrated the correlation of serum biotin levels with the presence of cedar pollinosis and several allergen-specific IgE levels in more than 400 Japanese schoolchildren. Further prospective studies are warranted to evaluate the involvement of biotin in the development of allergic sensitization and diseases.

Disclosure of state of COI

The authors declare no conflicts of interest.

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Supporting information

Supplemental online material is available on J-STAGE.

REFERENCES

1) McMahon RJ. 2002. Biotin in metabolism and molecular biology. *Annu Rev Nutr* **22**: 221–239.
2) Leon-Del-Rio A. 2019. Biotin in metabolism, gene expression, and human disease. *J Inherit Metab Dis* **42**: 647–654.
3) Cowan MJ, Wara DW, Packman S, Ammann AJ, Yoshino M, Sweetman L, Nyhan W. 1979. Multiple biotin-dependent carboxylase deficiencies associated with defects in T-cell and B-cell immunity. *Lancet* 2: 115–118.

4) Mock DM. 1991. Skin manifestations of biotin deficiency. *Semin Dermatol* 10: 296–302.

5) Kimura M, Fukui T, Tagami Y, Fujiwaki T, Yokoyama M, Ishioka C, Kumasaka K, Terada N, Yamaguchi S. 2003. Normalization of low biotinidase activity in a child with biotin deficiency after biotin supplementation. *J Inherit Metab Dis* 26: 715–719.

6) Okabe N, Urate K, Fujita K, Yamamoto T, Yao T, Doi S. 1988. Biotin effects in Crohn’s disease. *Dig Dis Sci* 33: 1495–1496.

7) Kuroishi T. 2015. Regulation of immunological and inflammatory functions by biotin. *Can J Physiol Pharmacol* 93: 1091–1096.

8) Kumar M, Axelrod AE. 1978. Cellular antibody synthesis in thiamin, riboflavin, biotin and folic acid-deficient rats. *Proc Soc Exp Biol Med* 157: 421–423.

9) Baez-Saldana A, Diaz G, Espinoza B, Ortega E. 1998. Biotin deficiency induces changes in subpopulations of spleen lymphocytes in mice. *Am J Clin Nutr* 67: 431–437.

10) Baez-Saldana A, Ortega E. 2004. Biotin deficiency blocks thymocyte maturation, accelerates thymus involution, and decreases nose-rump length in mice. *J Nutr* 134: 1970–1977.

11) Kuroishi T, Kinbara M, Sato N, Tanaka Y, Nagai Y, Iwakura Y, Endo Y, Sugawara S. 2009. Biotin status affects nickel allergy via regulation of interleukin-1beta production in mice. *J Nutr* 139: 1031–1036.

12) Saburi S, Bohl JA, Kapadia R, Cogburn K, Ghosal A, Lambrecht NW, Said HM. 2016. Role of the sodium-dependent multivitamin transporter (SMVT) in the maintenance of intestinal mucosal integrity. *Am J Physiol Gastrointest Liver Physiol* 311: G561–570.

13) Griffin JB, Rodriguez-Melendez R, Zempleni J. 2003. The nuclear abundance of transcription factors Sp1 and Sp3 depends on biotin in Jurkat cells. *J Nutr* 133: 3409–3415.

14) Rodriguez-Melendez R, Schwab LD, Zempleni J. 2004. Jurkat cells respond to biotin deficiency with increased nuclear translocation of NF-kappaB, mediating cell survival. *Int J Vitam Nutr Res* 74: 209–216.

15) Suzuki Y, Hattori S, Mashimo Y, Funamizu M, Kohno Y, Okamoto Y, Hata A, Shimojo N. 2009. CD14 and IL4R gene polymorphisms modify the effect of day care attendance on serum IgE levels. *J Allergy Clin Immunol* 123: 1408–1411 e1401.

16) Asher MI, Keil U, Anderson HR, Beusley R, Crane J, Martinez E, Mitchell EA, Pearce N, Sibbald B, Stewart AW, Strachan D, Weiland SK, Williams HC. 1995. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 8: 483–491.

17) Inoue H, Mashimo Y, Funamizu M, Yonekura S, Horiguchi S, Shimoo N, Kohno Y, Okamoto Y, Hata A, Suzuki Y. 2012. Association of the MMP9 gene with childhood cedar pollen sensitization and pollinosis. *J Hum Genet* 57: 176–183.

18) Sato K, Nakazawa T, Sahashi N, Kochibe N. 1997. Yearly and seasonal changes of specific IgE to Japanese cedar pollen in a young population. *Ann Allergy Asthma Immunol* 79: 57–61.

19) Wakabayushi K, Kodama H, Ogawa E, Sato Y, Motoyama K, Suzuki M. 2016. Serum biotin in Japanese children: Enzyme-linked immunosorbent assay measurement. *Pediatr Int* 58: 872–876.

20) Makino Y, Osada K, Sone H, Sugiyama K, Komai M, Ito M, Tsunoda K, Furukawa Y. 1999. Percutaneous absorption of biotin in healthy subjects and in atopic dermatitis patients. *J Nutr Sci Vitaminol* 45: 347–352.

21) Hayashi A, Mikami Y, Miyamoto K, Kamada N, Sato T, Mizuno S, Naganuma M, Teratani T, Aoki R, Fukuda S, Suda W, Hattori M, Amagai M, Ohyama M, Kanai T. 2017. Intestinal dysbiosis and biotin deprivation induce alopecia through overgrowth of Lactobacillus murinus in mice. *Cell Rep* 20: 1513–1524.

22) Nomiyama R, Okano M, Fujitwara T, Maeda M, Kimura Y, Kino K, Yokoyama M, Hira H, Nagata K, Hara T, Nishizaki K, Nakamura M. 2008. CRTH2 plays an essential role in the pathophysiology of Cry j 1-induced pollinosis in mice. *J Immunol* 180: 5680–5688.

23) Odamaki T, Xiao JZ, Iwabuchi N, Sakamoto M, Takashashi N, Kondo S, Iwatsuki S, Tokugawa T, Soga H, Enomoto T, Benno Y. 2007. Fluctuation of fecal microbiota in individuals with Japanese cedar pollinosis during the pollen season and influence of probiotic intake. *J Invest Allergol Clin Immunol* 17: 92–100.

24) Wakisashin H, Hirose K, Masezawa Y, Kagami S, Sato A, Watanabe N, Saito Y, Hatano M, Tokuhisa T, Iwakura Y, Fuccetti P, Iwamoto I, Nakajima H. 2008. IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 178: 1023–1032.

25) Elahi A, Sabui S, Narasappa NN, Agrawal S, Lambrecht NW, Agrawal A. Said HM. 2018. Biotin deficiency induces Th1- and Th17-mediated proinflammatory responses in human CD4(+) T lymphocytes via activation of the mTOR signaling pathway. *J Immunol* 200: 2563–2570.

26) Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmralls K, Sandouk A, Hesse C, Castro CN, Bahre H, Tschirner SK, Gorinski N, Gohmert M, Mayer CT, Hueln J, Ponimaskin E, Abraham WR, Muller R, Lochner M, Sparwasser T. 2014. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med* 20: 1327–1333.