〈Original Article〉

# Estimate of the dietary intake of biotin in infants prescribed special therapeutic infant formulas in Japan

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**Summary** This study aimed to analyze the biotin content of special therapeutic infant formulas produced in Japan and estimate the dietary intake of biotin in infants. The average content of biotin for low birth weight was 1.49 μg/100 kcal in 3 products. However, the average biotin content for protein and amino acids metabolism disorders was under 0.08 μg/100 kcal in 7 products. For milk allergies and intractable epilepsy, the biotin content was an average of 0.23 μg/100 kcal in 5 products, and the biotin content was below the measurable limit for milk allergies in 2 products. The estimates of the daily biotin intakes in infants with carbohydrate metabolism, protein and amino acids metabolism and electrolyte metabolism disorders averaged at 2.27, 0.38 and 1.94 μg/day, respectively. These were lower than 4 μg/day, which are Adequate Intake (AI) ages 0-5 months in Dietary Reference Intakes (DRIs) in Japan. Therefore, the biotin intake is not enough to maintain the health and growth in infants fed with special infant formulas. There are still reports every year of dermatitis, erythema and hair loss causes by biotin deficiency in infants fed with special infant formulas, such as amino acid formulas, produced in Japan. To prevent biotin deficiency in these infants, it is strongly recommended to add biotin to the infant formulas as soon as possible by revising Food for Special Dietary uses and the related act.

**Key words:** Special infant formulas, Biotin content, Deficiency, Dietary intake, Carnitine, Infants

## 1. Introduction

Biotin is one of the water-soluble vitamins which plays an important role as a covalently bound coenzyme for carboxylases in fatty acid synthesis, gluconeogenesis and branched-chain amino acids (BCAA) metabolism in mammals[1]. This vitamin is widely distributed in various foods and produced in intestinal microflora. Biotin deficiency is rare in general dietary habits. However, biotin deficiency

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causes scaly dermatitis, hair loss and neurologic signs when decreasing the dietary intake of biotin and inhibiting biotin absorption by avidin being contained in egg whites for long periods of time.

The human life stage can be generally categorized as 4 stages, the infant stage, youth stage, adult stage and elderly stage. The growth and development are remarkable in the infant stage; the change in physical function, especially, is markedly large in the early infant stage. Therefore, the ingestion intake of the balanced nutrient is necessary for neonates and infants. Many kinds of standard infant formulas (standard formulas) such as starting infant formulas (starting formulas) and follow-up infant formulas (follow-up formulas) are developed and distributed in Japan, as well as many other countries.

In Japan, bottle-feeding neonates and infants is 18.1% at 4-5 months old compared with that of breast-feeding, 55.8%, according to the National Growth Survey on Preschool Children, the Ministry of Health, Labor and Welfare in 2010. Neonatal mass screenings for inherited metabolic diseases started in Japan in 1977. Six inborn errors of metabolism are surveyed within 1 week after birth. The incidence of the neonates with inborn metabolism errors is found to be 1 in 9,330 between 1977 and 2007[2]. A recent report shows that neonates with the phenylketouria commonly appear with the incidence in 1:55,700 in 2011. Special therapeutic infant formulas (special formulas) for these infants are necessary to control the catabolites of phenylalanine and keep a balance in nutrient metabolism. Amino acid formulas that are low in phenylalanine (Low Phenylalanine Formulas® or Non Phenylalanine Formulas®) are prescribed depending on the degree of the clinical signs of the infant patients. Many kinds of infant formulas corresponding to metabolism disorders are developed in Japan, most of which are distributed to infant patients for free. However, it is demonstrated that possible signs of biotin deficiency, such as dermatitis and loss of hair, are induced in the neonates that are fed special formulas.

In Japan, the recommended intake of biotin was set in "Recommended Dietary Allowances -Dietary Reference Intakes (DRIs)-" in 2000. Afterwards, the Adequate Intake (AI) of biotin for infants aged 0-5 months and 6-12 months was set at 4 μg and 10 μg/day in "Dietary Reference Intakes for Japanese, 2010 version," respectively[3, 4]. However, as biotin is not listed in the "Standard Tables of Food Composition in Japan, 5th Revised and Enlarged edition" (Japanese Food Composition Table) (1,878 foods) published in 2005 from the Ministry of Education, Culture, Sports, Science and Technology, the biotin intake from meals and the requirement of biotin could not be estimated by diet surveys using the food composition table. The biotin contents of restricted foods (only 498 foods) were finally added to the Japanese Food Composition Table in 2010[5].

On the other hand, the use of biotin for Foods with Nutrient Function Claims (FNFC) was authorized in 2003, and the health claim and the upper and lower limits (500 and 10 μg/day, respectively) were recognized[6]. The health claim for biotin is "a nutrient which helps to maintain healthy skin and mucosa". However, this vitamin is not yet permitted as a food additive in any food. Therefore, infant formulas including standard and special formulas which are classified in "Food for Special Dietary Uses" may not add biotin either.

Maternal breast milk is the best food for infants. Infant formulas have been improved to contain the same nutritional component as maternal breast milk. As for protein, the amino acid composition of infant formulas has been developed to be similar to maternal breast milk by reducing casein and by increasing whey protein. The biotin content of human breast milk is 5 μg/L[7-10]. The authors have reported on the biotin content of infant formulas made in Japan in 1996 and 1998 and pointed out that the Japanese infant formulas do not sufficiently contain biotin compared with the standard of WHO[11, 12]. However, biotin has not been added to foods, including special foods such as infant formulas, except FNFC as of yet. As for 17 cases with biotin deficiency induced by feeding infants formula made in Japan, their clinical characteristics were reviewed in our previous paper in Japanese[13].

Therefore, this study aimed to analyze the biotin content of special therapeutic infant formulas newly

produced in Japan and estimate the dietary intake of biotin in infants. Also, cases with biotin deficiency that occurred by feeding special infant formulas in Japan during 1991-2013 were reviewed and its nutritional and clinical characteristics were discussed.

## 2. Materials and Methods

2.1. Sampling of infant formulas

Infant formulas determined in this study were 56 products manufactured in Japan (Japanese infant formulas) and 10 products distributed in Thailand (foreign infant formulas). These products are manufactured and imported from the US, Singapore and New Zealand. The infant formulas were roughly classified into 2 categories: standard formulas and special formulas. Standard infant formulas are for healthy infants, which include starting formulas and follow-up formulas. The special infant formulas are for infants and children with low birth weight and inborn metabolism errors[14]. These include 3 products for low birth weight infant, 7 carbohydrate metabolism disorders, 7 protein and amino acid metabolism disorders, 4 organic acid metabolism disorders, 14 electrolyte metabolism disturbances, 8 absorption disorders, 4 milk allergies and 1 intractable epilepsy, as shown in Table 1.

2.2. Biotin determination of infant formulas

The biotin content in infant formulas was determined by the microbiological assay using *Lactobacillus plantarum* ATCC 8014. Biotins contained in foods are in parts in protein-bound form. Therefore, protein-bound biotin was hydrolyzed before biotin was determined. 100 μL of each sample were transferred into microtubes and 100 μL of 2.25 mol/L $H_2SO_4$ were added. After the samples were hydrolyzed by autoclaving for 60 min at 121℃ (2 atoms), they were neutralized by adding approximately 100 μL of 4.5 mol/L NaOH. The supernatant was used as a sample for biotin determination after centrifuging at 10,000 rpm for 5 min. The sample was mixed with the culture medium that was added with *L. plantarum* and cultured in the microtiter plate for 18 hours at 37℃. After culturing, the growth of *L. plantarum* was measured at OD 610 nm. The biotin content of the infant formulas was expressed as μg/100g and μg/100 kcal.

The biotin intake in infants ages 0 through 5 months was calculated based on the average volume of breast milk in Japan (780 mL/day) and the biotin content of infant formulas (μg/100 mL). The infant formulas were made according to the standard direction and the amount of biotin intake was

Table 1 Biotin concentration of infant formulas and daily biotin intake in infants

| Category | No. of samples | Total biotin[1] mean | Total biotin[1] range[4] | Free biotin[1] mean | Free biotin[1] range | Percent of free biotin | Total biotin[2] mean | Total biotin[2] range | Biotin intake[3] mean | Biotin intake[3] range |
|---|---|---|---|---|---|---|---|---|---|---|
| Standard formulas made in Japan | | | | | | | | | | |
| Starting and follow-up formula | 8 | 7.91 | (0.94-14.3) | 7.04 | (0.34-13.8) | 81.2 | 1.61 | (0.18-2.94) | 8.29 | (0.93-15.1) |
| Special formulas for | | | | | | | | | | |
| Low birth weight infant | 3 | 7.56 | (4.70-12.3) | 9.18 | (3.47-14.2) | 121.4 | 1.49 | (1.01-2.37) | 9.10 | (5.50-15.2) |
| Carbohydrate metabolism disorders | 7 | 2.09 | (0.62-5.43) | 0.32 | (0.18-0.44) | 28.5 | 0.49 | (0.13-1.29) | 2.27 | (0.68-5.93) |
| Protein and amino acid metabolism disorders | 7 | 0.43 | (0.00-1.33) | 0.39 | (0.00-1.07) | 71.6 | 0.08 | (0.03-0.23) | 0.38 | (0.00-1.56) |
| Organic acid metabolism disorders | 4 | 0.06 | (0.00-0.15) | 0.07 | (0.00-0.14) | 83.2 | 0.01 | (0.00-0.03) | 0.07 | (0.00-0.17) |
| Electrolyte metabolism disturbances | 14 | 1.74 | (0.45-5.59) | 1.17 | (0.15-4.41) | 63.3 | 0.35 | (0.09-1.17) | 1.94 | (0.46-6.54) |
| Absorption disorders | 8 | 2.90 | (0.53-6.09) | 2.30 | (0.17-5.70) | 63.8 | 0.53 | (0.11-1.27)[5] | 3.02 | (0.31-6.65) |
| Milk allergies and Intractable epilepsy | 5 | 1.09 | (0.00-4.57) | 0.31 | (0.11-0.55) | 26.9 | 0.23 | (0.00-0.99) | 1.24 | (0.00-5.17) |
| Standard formulas purchased in Thailand | | | | | | | | | | |
| Starting and follow-up formula | 10 | 24.8 | (1.47-43.6) | 28.5 | (18.5-49.5) | 119.0 | 5.61 | (3.13-9.08)[6] | 29.5 | (16.4-48.2)[6] |
| FAO/WHO, Codex (1994) | | | | | | | 1.5 | | | |
| Infant Formula Act (1980) | | | | | | | 1.5 | | | |
| American Academy of Pediatrics (1976) | | | | | | | 1.5 | | | |
| AI for infants ages 0-5 months in Japan (2010) | | | | | | | | | 4 | |
| AI for infants ages 0-6 months in US (1998) | | | | | | | | | 5 | |

[1] μg/100g. [2] μg/100kcal. [3] μg/day. [4] min-max. [5] 7 formulas. [6] 9 formulas.

expressed as μg/day.

2.3. Statistical analysis

Values are mean ± SD. Statistical analysis of these data was performed using Statcel 2 (OMS Publishing, Saitama, Japan). To detect the statistical significance of differences between products, the Student's t-test and the nonparametric Mann-Whitney U test were used when appropriate[15]. Differences were considered statistically significant as a probability level of $p < 0.05$ in all analysis.

## 3. Results

Table 1 shows the amounts of biotin in 56 infant formulas manufactured in Japan and 10 foreign infant formulas obtained in Thailand. In the standard formulas for healthy infants, the average amount of biotin in 8 Japanese products including starting formulas and follow-up formulas was 1.61 μg/100 kcal, which range was from 0.18 to 2.94 μg/100 kcal. This was significantly lower than 5.61 μg/100 kcal on average in foreign products, all of which exceeded the 1.5 μg/100 kcal, which is the standard value in Codex Stan 72, FAO/WHO[16] and the lower limit in ESPGHAN (European Society of Pediatric Gastroenterology, Hepatology and Nutrition). However, there was only one product with a biotin content higher than this standard in Japanese infant formulas.

In special formulas, the average content of biotin in 3 products for low birth weight was 1.49 μg/100 kcal, which was equal to the FAO/WHO standard. However, in all other 45 products, the average biotin content was under 1.5 μg/100 kcal. In particular, 4 special formulas for organic acid metabolism disorders showed an average of 0.01 μg/100 kcal, 2 products of which were under the lower limit of the detection by microbiological assay.

The average biotin content in 7 products for protein and amino acids metabolism disorders was also significantly low, which was 0.08 μg/100 kcal. For milk allergies and intractable epilepsy the biotin content in 5 products was 0.23 μg/100 kcal at average, but biotin content was below the measurable limit in 2 products for milk allergies.

The incidence of free biotin in total biotin in infant formulas was 81.2% in Japanese standard formulas but more than 100% in foreign standard formulas. However, the incidence of special formulas was less than 80%, especially special formulas for carbohydrate metabolism disorders was only 28.5%.

As for the estimate of the daily biotin intake in infants using special formulas, the average intake of biotin in infants with low birth weight was 9.10 μg/day. This was the same as 8.29 μg/day in healthy infants fed with starting formulas and follow-up formulas in Japan. However, these are significantly lower than 29.5 μg/day in healthy infants in Thailand.

The estimate of the daily biotin intake in infants with carbohydrate metabolism, protein and amino acid metabolism and electrolyte metabolism disorders averaged at 2.27, 0.38 and 1.94 μg/day, respectively. Also, in other infants with nutrient metabolism disorders, the biotin intake was lower than 4 μg/day which is AI ages of 0-5 months in DRIs in Japan. In infants with organic acid metabolism disorders (2 products) and milk allergies (2 products), the biotin intake was almost 0.

## 4. Discussion

Based on breast milk being one of the best foods for babies, standard infant formulas are improved to get the similar ingredients as close to breast milk as possible. As for protein, whey protein including lactoglobulin, lactalbumin, lactoferrin and essential amino acids is water-soluble and is supplemented in place of milk casein. The composition of the amino acids is as close as possible to that of breast milk. The lipids change parts into vegetable oil to increase essential fatty acid. Carbohydrates are mostly replaced with lactose and partially with oligo saccharides which are easily digestible. As cow's milk is included 3 folds in the mineral of the breast milk, the amount of minerals is decreased and especially the calcium and phosphorous balance is regulated to reduce burden on the kidneys. All vitamins except biotin are increased to be able to make up for respective DRIs. Thus, the infant formulas are prepared and fortified with various

nutrients, the starting formulas commonly contain many proteins and minerals, and less lipids compared to breast milk. However, Japanese infant formulas are regulated as Food for Special Dietary Uses by law, and improvement on these formulas is limited. Biotin has not been added to the standard infant formulas nor the therapeutic special infant formulas as of yet.

Biotin in foods is in free form and protein-bound form. The percentage of free biotin is easy to utilize in the intestine, even in neonates, as the free form of biotin can be absorbed in the intestine without digestive enzymes[1]. On the other hand, the percentage of free biotin in infant formulas for carbohydrate metabolism disorders is markedly lower than that of other special therapeutic infant formulas. This formula contains soybeans as a source of protein, which is different from other special formulas. Therefore, biotin contained in infant formulas for carbohydrate metabolism disorders is difficult to absorb compared with other special infant formulas.

The biotin content in special infant formulas produced in Japan is significantly lower than that in the standard infant formulas in our previous studies[11, 12]. There are many steps in producing special infant formulas. As for infant formulas for milk allergies, protein is hydrolyzed to amino acids and small molecular peptides, and hydrolyzed whey is changed to an amino acids mixture. As a result, the biotin content in these formulas is close to 0. Also, as above mentioned, biotin cannot be added to foods including infant formulas except the FNFC in Japan, resultantly inducing the biotin deficiency in infants.

The biotin content in Japanese infant formulas is low compared with foreign infant formulas[12]. It is demonstrated that standard infant formulas made in the US contain 1.71 $\mu$g/100 mL on average and special infant formulas contain 1.21 $\mu$g/100 mL. On the other hand, Japanese special infant formulas are 0.27 $\mu$g/100 mL on average which is insufficient for 0-5 month old infants. Vitamins including biotin are approved as a GRACE substance in the US, and biotin can be added in most products, such as medicine, cosmetics, foods and animal foods. Also, it can be added to infant formulas in the US. In Europe, biotin is one of the vitamins which are freely added to foods. In Thailand, most infant formulas are imported from New Zealand and Singapore, and biotin is sufficiently contained at a high concentration.

In our previous findings, the biotin level in the serum was 2.3 ng/mL and 2.4 ng/mL in infants that were fed maternal breast milk and standard infant formula, respectively[17]. Also, the serum biotin level averaged 2.5 ng/mL in infants with PKU that were fed Low Phenylalanine Formulas® or Non Phenylalanine Formulas®. There were no significant differences compared with 2.3 ng/mL in infants that were fed breast milk. However, the urinary excretion of biotin in PKU infants was 16.8 $\mu$g/g creatinine, a sensitive indicator for dietary biotin intake, and decreased to half compared with in infants fed with standard infant formulas (39.3 $\mu$g/g creatinine). The urinary biotin excretion was 70.2 $\mu$g/g creatinine in infants fed with maternal breast milk. Therefore, the biotin intake was not high enough to maintain health and growth in infants fed with special infant formulas. Biotin deficiency in these infants has only been reported in Japan.

Table 2 shows 25 cases of infants with biotin deficiency that were prescribed special infant formulas after 1990[18-35]. Data from these cases were analyzed in detail. These infants ranged in age from 3 months to 5 years old, 9 cases of whom were under 6 months old and 15 cases under 1 year old. Seventeen cases are diagnosed as having allergy to cow's milk, 8 cases of whom were prescribed Elemental Formula® (Meiji Dairies Co., Tokyo) and 4 cases Milfy-HP® (Meiji Dairies Co., Tokyo). The period when the special infant formulas were given is from 1.5 months to 3 years and 5 months, 12 cases of whom were within 4 months. The characteristic symptoms in infants fed these special infant formulas are the erythematous dermatitis and scaly lesion around the eyes, mouth and neck, and loss of hair. Also, the low biotin level in serum and urine as well as abnormal excretion of 3-HIA (hydroxyisovaleric acid) and 3-MCG (methyl-crotonylglycine) in urine are observed in most cases. However, the appearance of biotin deficiency improved dramatically after oral biotin treatment of 0.2-10 mg/day (1 mg/day in 10 cases). The biotin

Table 2　Reviews of biotin deficiency developed by special infant formulas in Japan

(Continued on page 66)

| References | | Age | Sex | Diagnosis | Special formulas | Serving period | Clinical and biochemical features | Biotin oral doses (mg/day) | Changes by biotin and other treatments |
|---|---|---|---|---|---|---|---|---|---|
| Abe et al.[18] | 1991 | 3M | ? | Milk allergy | Elemental Formula®1) | 2m | · Atopic dermatitis, hair loss, conjunctival injection, fissuring of the lip, and pubic erosive<br>· Low biotin level in serum and urine<br>· Abnormal increase in lactic acid and pyruvic acid in serum<br>· Contain no free carnitine or acylcarnitine in serum<br>· Abnormal excretions of 3-HIA and 3-MCG in urine | 10 | · 10 mg/day of biotin and 500 mg/day of carnitine administration<br>· Improvement of hair loss, conjunctival injection and fissuring of the lip<br>· Increase biotin in serum to normal range after 2 weeks<br>· Decrease lactic acid in serum after 4 weeks |
| Higuchi et al.[19] | 1996 | 11M | M | Milk allergy | Elemental Formula® 1) | 4m | · Dermatitis of diaper area and erythematous dermatitis of eyelids and lip<br>· Hair loss of head and eyebrows<br>· Abnormal increase in lactic acid in serum<br>· Abnormal excretion of 3-HIA in urine | 1 | · Disappearance of dermatitis and erythema after 2 weeks<br>· Improvement of hair loss after 2 months<br>· Increase biotin in serum to normal range after 1 week<br>· Decrease in urinary excretion of 3-HIA to normal level after 1 week |
| | | 5M | M | Milk allergy | Elemental Formula ®1) | 1.5m | · Dermatitis of diaper area<br>· Low biotin level in serum<br>· Abnormal increase in lactic acid in serum<br>· Abnormal excretions of 3-HIA, 3-MCG and methylcitric acid in urine | 1 | · Disappearance of dermatitis of diaper area<br>· Increase in biotin in serum to normal range after 3 weeks<br>· Decrease in lactic acid in serum to normal level after 3 weeks<br>· Decrease in urinary excretion of organic acid to normal level after 1 week |
| Nishihara[20] | 2001 | 4Y | F | Milk allergy | Epitoless®1) | 3y5m | · Erosive and erythema of occipital scalp, mucosa of the body orifices, both of axilla, pubic and buttock<br>· Hair loss of occipital scalp | 1 | · Improvement of erosive and erythema after 1 week<br>· Complete recovery of erosive and erythema after 3 weeks |
| | | 11M | M | Milk allergy | Nobiyaka®1) | 5m | · Infiltrating erythema with eschar and desquamation of anterior ears to cheeks<br>· A walnut-sized erythematous dermatitis includes desquamation of forearm<br>· Soy or walnut-sized dermatitis in the back<br>· Low biotin level in serum | 2 | · Improvement of infiltrating erythema after 1 week |
| Hosoya et al.[21] | 2002 | 4Y | M | Soy and milk allergy | Elemental Formula®1) | 9m | · Bleached hair and hair loss<br>· Low biotin and free carnitine levels in serum<br>· Abnormal excretions of 3-HIA and 3-HPA in urine | 1 | · Improvement of bleached hair and hair loss after 2 weeks<br>· Increase biotin in serum to normal range<br>· Disappearance of urinary excretion of organic acid |
| | | 5Y | F | ? | Elemental Formula®1) | 6m | · Hair loss<br>· Low biotin level in serum | 1 | · Improvement of hair loss<br>· Increase biotin in serum to normal range |
| Fujimoto et al.[22] | 2005 | 5M | M | Milk allergy | Elemental Formula®1) | 4m | · Scaly lesions and erythema in and around the anus and scalp<br>· Hair loss of occipital scalp<br>· Low biotin levels in serum and urine<br>· Abnormal excretions of 3-HIA, 3-MCG and methylcitric acid in urine | 1 | · Improvement of dermatitis and hair loss<br>· Disappearance of urinary excretion of organic acid |
| Kawaba et al.[23] | 2005 | 4M | F | Milk allergy | Amino acid formula4) | 4m | · Abnormal increase in lactic acid and pyruvic acid in serum and metabolic acidosis<br>· Abnormal excretions of 3-HIA, 3-MCG and methylcitric acid in urine<br>· Increase in OH-C5 acylcarnitine in serum<br>· Hypoglycemia<br>· Increase in ketone body concentration and free fatty acid in serum | 1 | · Decrease in lactic acid and pyruvic acid in serum<br>· Decrease in urinary excretion of organic acid<br>· Increase in physical activity of patient after 4 days<br>· Improvement of growth by continuous biotin administration |

| References | | Age | Sex | Diagnosis | Special formulas | Serving period | Clinical and biochemical features | Biotin oral doses (mg/day) | Changes by biotin and other treatments |
|---|---|---|---|---|---|---|---|---|---|
| Mamada et al.[24] | 2008 | 4M | F | Milk allergy | Elemental Formula®[1)] | 3m | · Erosion around the eyes, lips, neck and buttocks<br>· Hair loss<br>· Low biotin level in serum<br>· Abnormal excretions of 3-HIA and 3-MCG in urine | 1 | · Dramatic improvement of erosives and erythema after 1 week<br>· Increase in biotin in serum to normal range after 1 week<br>· Decrease in urinary excretion of organic acid after 1 week |
| Goto et al.[25] | 2009 | 5M | F | Milk allergy | New MA-1®[2)] | 4m | · Skin desquamation and erythema around the eyes, mouth and cheeks<br>· Erythema with psoriasis and scaly lesion of body trunk and limbs<br>· Edematous erythema with scaly lesion from external genitals to anus<br>· Hair loss<br>· Abnormal excretions of 3-HIA, 3-MCG, methylcitric acid and lactic acid in urine | 1 | · Improvement of skin desquamation and erythema after 1 week<br>· Disappearance of dermatitis and erythema after 6 weeks<br>· Decrease in urinary excretion of organic acid to normal level after 6 weeks |
| Kase et al.[26] | 2009 | 5M | M | Milk allergy | Milfy-HP®[1)] | 4m | · Scaly erythema of the eyelids, around the mouth, neck and inguinal area<br>· Erythematous occipital with scaly lesion and pustules of the scalp and body trunk<br>· Erythematous of fingers and brittle pachyonychia<br>· Hair loss | 2 | · Dramatic improvement of all symptoms after 2 weeks |
| Honma et al.[27] | 2010 | 9M | M | Milk allergy | Whey Hydrolyzed forimula[4)] | 7m | · Hair loss and hypotonia<br>· Metabolic acidosis, ketosis and increase lactic acid and pyruvic acid in serum<br>· Hyper-ammonemia<br>· Abnormal excretions of 3-HIA, 3-MCG, 3-HPA and methylcitric acid in urine<br>· Low biotin level in urine<br>· Low biotinidase activity in serum | 10 | · Improvement of clinical sign<br>· Decrease lactic acid and pyruvic acid in serum to normal level |
| Teramura et al.[28] | 2010 | 3M | M and F (twins) | Milk allergy | Amino acid formula[4)] | 3m | · Deveropment of similar skin lesions in twins<br>· Well-circumscribed, erosive and scaly erythematous lesion of anogenital regions and around the body orifices<br>· Psoriasiform erythema on scalp in only female | 0.2 | · 0.2 mg/day of biotin administration without any other treatment<br>· Treatment and non-recurrent of all symptoms by biotin administration |
| Ihara et al.[29] | 2011 | 3Y | F | Glycogen storage disease type 1b | GSD-D and GSD N formulas[5)] | >2y | · Hair loss<br>· Hypoglycemia and status epilepticus and hypoglycemic encephalopathy<br>· Administration of carbamazepine (antiepilepsy drug)<br>· Skin eruption on eyelids and angles of mouth after 1 month administration of carbamazepine<br>· Low free biotin and carnitine levels in serum and abnormal excretion of 3-MCG, 3-HIA and methylcitric acid after 5 months the onset of skin symptoms<br>· Remarkable elevations of C5-OH and C3/C2 ratio | 0.3 | · Treatment of skin lesions and hair loss by 0.3 mg/day of biotin and 300 mg/day of L-carnitine at 3 weeks<br>· Improvement of blood acylcarnitines level to normal range |
| Takemoto et al.[30] | 2011 | 5Y | M | Multiple food allergy | Elemental Formula®[1)] | 1y11m | · Systemic atopic dermatitis and hair loss<br>· Low biotin level in urine<br>· Abnormal excretion of 3-HIA in urine | 0.3 | · Improvement of hair loss after 4 weeks<br>· Decrease urinary excretion of organic acid to normal level |

(Continued on page 67)

| References | | Age | Sex | Diagnosis | Special formulas | Serving period | Clinical and biochemical features | Biotin oral doses (mg/day) | Changes by biotin and other treatments |
|---|---|---|---|---|---|---|---|---|---|
| Yamaguchi et al.[31] | 2011 | 8M | M | Milk allergy | Milfy-HP®1) | 3m | · Eczemas of facial, head and around the eyelid, mouth and anus<br>· Hyperlactacidemia<br>· Abnormal excretions of 3-HIA, 3-MCG, 3-HPA and methylcitric acid in urine | 10 | · 10 mg/day of biotin and 300 mg/day of carnitine administration<br>· Improvement of eczemas |
| Suzaki et al.[32] | 2011 | 8M | F | Milk allergy | Milfy-HP®1) | 5m | · Erosive and scaly erythema of the eyelids, body trunk and limbs and around the nasal cavity, mouth and anus<br>· Erythema of skin of around the nail and trachyonychia<br>· Abnormal excretion of 3-HIA in urine | 1～2 | · Improvement of erosive and scaly erythema after 3 days at 2 mg/day biotin administration<br>· Decrease in urinary excretion of 3-HIA<br>· Increase in biotin in serum and urine<br>· Despite 1mg/day of biotin administration from 2 weeks, no problems |
| Sato et al.[33] | 2012 | 9M | M | Non-ketotic hyperglycinemia | S-22®3) | 4m | · Erythema with eschar of around the mouth<br>· Scaly erythema of the auricle, axilla and buttocks<br>· Erythematous of the forearm, dorsal hand, lower legs and ankle<br>· Convulsion similar myoclonus | (0.1～0.4) | · 0.1 mg/day intramuscular administration of biotin during the first 8 days<br>· 0.4 mg/day oral administration of biotin after day 9<br>· Improvement of erythema after 15 days<br>· Disappearance of convulsion similar myoclonus |
| Ito et al.[34] | 2013 | 10M | F | Milk allergy in Down's syndrome | Milfy-HP®1) | 3m | · Erythema with scaly lesion eyelids, scalp and buttocks<br>· Low biotin level in serum and urine | 2 | · Beginning improvement of skin rash after 2 days at biotin administration<br>· Complete improvement of skin rash after 3 weeks at biotin administration<br>· Increase in biotin level in serum and urine at 3 weeks, but it was not normal range<br>· Biotin treatment period was 6 months |
| Nozaki et al.[35] | in pre-para-tion | 1Y6M | F | Milk allergy | Milfy-HP®1) | 1y | · Hair loss of occiput, intractable steroid-resistant erythematous lesion of back and around the eyes<br>· Hepatomegaly<br>· Hyperammonemia and hyperlactacidemia<br>· Low biotin level in serum and urine<br>· Abnormal excretion of 3-HIA in urine<br>· Hypocarnitinemia and hyposelenemia | 1 | · Improvement of all symptoms 4 months after the start of biotin (1 mg/day), carnitine (300 mg/day) and selenium (12.5 μg/day) administration |

Abbreviations:
M, male; F, female; m, month; y, year; M, months old; Y, years old.
3-HIA, 3-Hydroxyisovaleric acid; 3-MCG, 3-methylcrotonylglycine; 3-HPA, 3-hydroxypropionic acid.
?: no information.
1)Meiji Dairies Co., Tokyo.
2)Morinaga Milk Industry Co., Ltd., Tokyo.
3)Megmilk Snow Brand Co., Ltd., Tokyo.
4)Products are unknown.
5)For glycogen storege disease.

content in serum and urine recovered within the normal range after 2-3 weeks. The dermatitis, erythema and erosion improved in most cases 2-4 weeks after injecting biotin.

Carnitine is essential in β-oxidation to transport long-chain fatty acids across the inner mitochondria membrane[36]. Carnitine is especially necessary to obtain energy from fats stored in the adipose tissue in neonatals. As the concentration of total carnitine in human breast milk remains at a constant mean level near 62.9 nmoles/mL (10.1 mg/L) during the first 21 days postpartum and 35.2 nmoles/mL (5.7 mg/L) until the 40-50th day[37], its content of infant formulas is recommended at more than 1.2 mg/100 kcal in the standard of Codex/WHO. However, it is noted that carnitine is not detected in most special infant formulas produced in Japan, as well as biotin. It is suggested that urinary excretion of 3-HIA-carnitine increases in response to biotin deficiency. Kawaba et al.[23] reported that C5-OH acylcarnitine increased in serum in biotin deficient infants fed with special infant formulas. Ihara et al.[29] showed that free biotin and carnitine was low in serum and C5-OH and C3/C2 ratios were remarkably elevated in these infants. Also, it is known that patients with 3-methylcrotonyl-CoA carboxylase have a severe secondary deficiency of free carnitine in their plasma[38]. Shigematsu et al.[39] suggest that carnitine supplementation is possibly beneficial for patients with a holocarboxylase synthetase deficiency who respond incompletely to biotin therapy. From these findings, biotin and carnitine have simultaneously been treated in biotin deficient infants by special infant formulas in previous studies[18, 29, 31]. We also demonstrated that when an infant aged 1.5 years old fed with Milfy-HP$^{R}$ was given carnitine (300 mg/day) together with biotin (1 mg/day), all symptoms ameliorated promptly[35]. Therefore, it is suggested that the co-administration of biotin and carnitine may be more certain and effective in infants with biotin deficiency induced by special infant formulas.

The Human Disorders of Biotin Metabolism urgently recommend the following: The addition biotin to infant formulas is not currently authorized by the Food Sanitation Act in Japan. The physician treating the gastrointestinal allergy should keep biotin deficiency prevention in mind in cases of the prescription of the amino acid formulas till the addition of biotin to infant formulas is permitted[40]. Also, the Japan Pediatric Society and the Imperial Gift Foundation Boshi-Aiiku-Kai suggest that care must be taken when treating patients with specific milk[41, 42].

## 5. Conclusion

It is still reported every year that dermatitis, erythema and hair loss has been caused by biotin deficiency in infants fed with special therapeutic infant formulas such as amino acid formulas produced in Japan. This is one reason why the biotin content of Japanese infant formulas is too low to meet the AI for infants in DRIs. To prevent biotin deficiency in infants, it is strongly recommended to add biotin to the infant formulas as soon as possible by revising Food for Special Dietary uses and the related act.

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