The effects of elimination diet on nutritional status in subjects with atopic dermatitis

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Abstract

A food allergy is an adverse health effect arising from a specific immune response that occurs reproducibly upon exposure to a given food. In those with food allergies that are thought to cause aggravation of eczema, food avoidance is important. The objective of this study was to research the nutritional status of patients with food allergies. A total of 225 subjects diagnosed with atopic dermatitis underwent a skin prick test as well as measurement of serum immunoglobulin E. Food challenge tests were conducted using seven food items: milk, eggs, wheat, soybeans, beef, pork, and chicken. At post-food challenge visits to the test clinic, participants completed a three-day dietary record, which included two week days and one weekend day, in order to evaluate energy intake and diet quality during the challenge. We analyzed nutrient intake based on differential food allergens. Subjects with a food allergy to milk showed lower intake of Ca, Zn, and vitamin B2, and subjects with a food allergy to egg showed lower intake of vitamin A, B1, B6, niacin, and cholesterol. Subjects with a food allergy to wheat and soybean showed lower intake of Ca, P, Fe, K, Zn, vitamin B2, vitamin B6, and niacin; and subjects with a food allergy to beef, pork, and chicken showed lower intake of Fe and higher intake of K, vitamin A, B2. Subjects with atopic dermatitis were lacking in several nutrients, including vitamin A and vitamin C. A greater number of food allergies showed an association with a greater number of nutrient intake deficiencies. Allergen avoidance is the basic treatment for atopic dermatitis. However, when the allergen is food, excessive restriction can lead to nutrition deficiency. Findings of this study suggest the necessity for enhanced nutritional education in order to provide substitute foods for patients with food allergies who practice food restriction.

Key Words: Food allergy, atopic dermatitis, food restriction

Introduction

Atopic dermatitis (AD), a chronic relapsing inflammatory skin disease commonly associated with allergy, affects 15% to 30% of children [1]. Many children who develop AD go on to develop other allergic diseases, such as food allergy (FA), asthma, or allergic rhinitis (the ‘atopic march’) [2]. FA is an adverse health effect arising from a specific immune response that occurs reproducibly upon exposure to a given food [3]. The clinical spectrum of FA ranges from mild skin irritation to severe life-threatening anaphylaxis [4].

The majority of FAs are mediated by immunoglobulin E (IgE), however, sensitization to a specific food as confirmed by skin prick test (SPT) or serum-specific IgE does not always imply clinical reactivity. Accurate diagnosis of FAs should be based on an oral food challenge [5-7]. The delayed onset of symptoms in FA (6 hours to as long as 48 hours after the food challenge) makes observation difficult, although as many as 25% of cases will present within 1-2 hours of ingestion [8]. There are no specific laboratory tests for FAs; therefore, diagnoses are made using elimination diets and oral challenges [9,10] as well as food challenge tests [11,12]. Several food challenge methods have been proposed, including double-blind placebo-controlled food challenges (DBPCFC) and open food challenges (OFC) [3]. In an OFC, the patient is not blinded and therefore recognizes the test food. Test results are then interpreted according to objective symptoms. If the patient shows anxiety regarding the challenge and complains of abdominal pain or pruritus, the food challenge test is considered invalid [3]. DBPCFC is regarded as a more accurate method for diagnosis of FAs, because both the patient and the observer are blinded to the test food. While this is the most rigorous method for diagnosis of adverse food reactions [13], conduct of DBPCFCs in the clinical setting is difficult.

The current standard of care for patients with food allergies is based on avoidance of trigger foods with the goal of eventually gaining tolerance [14]. In those with food allergies that are thought to cause aggravation of eczema, food avoidance is important. However, due to the risk of nutritional deficiencies, careful supervision of such diets is also vital, particularly if calcium-rich foods are avoided [15]. In many cases, multiple foods are the

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Received: March 6, 2013, Revised: September 2, 2013, Accepted: September 23, 2013
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cause of AD, and food allergies are not the only cause of AD. More than 50% of FA patients also have food allergies to more than one food, with the average FA patient experiencing symptoms from 2.8 food items [16].

Care should be taken to ensure that diet is nutritionally adequate, especially for those with multiple allergies. Without elimination of allergy provocation, AD cannot be controlled effectively. As a first step toward the diagnosis of food allergy, dietary restrictions should be initiated. If a patient shows improvement in symptoms on an elimination diet, a food allergy to the restricted foods may be strongly suspected. An optimal elimination diet can then be achieved.

In Korea, the prevalence of food allergies by OFC testing among patients with AD were as follows: milk, 67.3%; chicken, 64.2%; pork, 62.8%; eggs, 61.0%; beef, 55.4%; wheat, 52.0%; and soybeans, 45.2%. Little research on these seven specific foods has been reported [17]. Unfortunately, these seven foods are also rich in important nutrients. During treatment of FA, prevention of excessive food elimination and proper use of alternatives are required. Various studies have highlighted cases of reported growth delay and nutrient deficiency in patients experiencing excessive food limitation [2,8,15]. However, few studies on food limitation in FA patients in countries including Korea have been reported [10,16].

Therefore, the aim of this study is to identify practically deficient nutrients by researching nutrient status of FA patients. We intend to provide basic information that can be used for establishment of appropriate health and dietary guidelines.

Subjects and Methods

Subjects

A total of 225 AD patients (M:F = 114:111) aged 1-65 years who visited the Department of Allergy and Clinical Immunology at the Seoul Allergy Clinic, Seoul, Korea, and Chungnam National University Hospital Pediatric Allergic Clinic, Daejeon, Korea between June 2000 and August 2010 were enrolled in this study. Age groups were classified according to physiological characteristics and developmental stage. Patients were grouped into age groups of 1-2 years, 3-5 years, 6-11 years, 12-19 years, and over 20 years, as referenced by the age classification of the Korean Nutrition Society [18]. Subjects underwent blood tests and skin prick tests (SPTs), as described below, and fulfilled the criteria of Haniifin and Rajka [19]. Each group was divided into two groups: negative and positive responders to food allergens. Subjects in the positive group showed an allergic response to a minimum of one of the seven food items studied according to the OFC and DBPCFC; while those in the negative group did not. Informed consent was obtained from adult patients or the parents of children participating in the study. This study was approved by the Institutional Review Board of Chungnam University, Daejeon, Korea.

Diagnosis of food allergy

Skin prick tests

All subjects underwent blood tests and SPTs. SPTs were performed simultaneously on the patients’ left forearms using crude and commercial allergy extracts (Bencard, Brentford, England). Histamine hydrochloride (1 mg/ml, Bencard) was used as a positive control. SPTs using vehicles (physiologic saline, distilled water, and glycerol) were used as controls. Reactions were read 15 min after the skin prick. Food allergens (1:10 or 1:20 glycerinated food extract) that elicited a wheal at least 3 mm larger than that of the negative control (not including erythema) were considered positive, while anything less than 3 mm was considered negative.

Blood test

Blood tests included a complete blood cell count (CBC) with differential for determination of the eosinophilic fraction, total serum IgE levels, and food-specific serum IgE levels (which were measured using UniCAP; Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Serum food-specific IgE levels less than 0.35kU A/L were classified as undetectable.

Food challenge test

For all subjects, diagnoses of allergies to milk, eggs, wheat, soybeans, beef, pork, and chicken were made using food challenge tests. Among the seven foods, milk, eggs, wheat, and soybeans were tested using DBPCFCs; and beef, pork, and chicken were tested using OFCs.

Elimination diet

A trial of an elimination diet preceded the food challenge test for all seven foods. Patients were asked to eliminate the suspected allergen according to past history of allergic responses, skin prick tests, and specific IgE level (maximum elimination phase). A replacement diet was designed in order to provide substitutes for the eliminated foods for maintenance of balanced nutrition [17]. To confirm the complete elimination of all foods identified as allergens, dietary diaries were kept by all patients. Analysis of dietary diaries was performed by dietitians as well as by a physician.

Open food challenge tests

Patients consumed one portion of the test food each morning [18]. Three days after the first challenge, the clinical results and the severity score were evaluated. If patients showed increased severity scores or obvious worsening of clinical signs or symptoms, the tests were discontinued. Otherwise, the food challenge tests continued with ingestion of increased quantities of the test food for another four days. If patients showed worsening symptoms, subsequent challenges were delayed until patients recovered to the pre-challenge state. If patients consumed the food that was
to be eliminated during the study, the challenge tests were stopped, and the patients were observed for one week.

**Double-blind placebo-controlled food challenges**

Recipes for DBPCFCs using eggs, milk, wheat and soybeans were developed by dieticians at the Seoul Allergy Clinic. The vehicle used was 15 g of mixed cereal flour, consisting of 15 g of mixed cereal flour by dieticians at the Seoul Allergy Clinic. The vehicle used was 15 g of mixed cereal flour, consisting of brown rice flour (5 g), quinoa flour (5 g), and barley flour (5 g). For the DBPCFC, a 21 g mixture, consisting of 15 g of the above mixed cereal flour and 6 g of freeze-dried egg yachts, seeds, wheat or skim milk powder was used. Foods containing eggs, milk, wheat and soybeans were eliminated from daily diets for at least two weeks prior to testing. Before starting the DBPCFC, it was confirmed that patients tolerated mixed cereal flour by challenging them with the placebo for seven days. If no symptoms were reported, patients proceeded with the DBPCFC. Two challenges separated by seven days were performed, including one with placebo only and one with the suspected food antigen. Challenges separated by seven days were performed, including with placebo only and one with the suspected food antigen within the vehicle. A summary of the diagnostic flow of the food challenge tests in patients with atopic dermatitis is shown in Fig. 1.

**Table 1. Intake of nutrients by age in AD subjects with and without food allergy**

| Age Group | Negative (n = 23) | Positive (n = 38) | Negative (n = 16) | Positive (n = 17) | Negative (n = 12) | Positive (n = 19) | Negative (n = 11) | Positive (n = 15) | Negative (n = 19) | Positive (n = 11) | Negative (n = 41) | Positive (n = 34) |
|-----------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Energy (kcal) | 8056.2 ± 173.09 | 804.18 ± 217.17 | 1147.11 ± 225.51 | 1198.31 ± 229.03 | 1440.65 ± 228.56 | 1418.63 ± 236.64 | 828.24 ± 513.71 | 1341.83 ± 391.66 | 1137.12 ± 328.87 | 1302.85 ± 406.78 | 90.52 ± 19.00 |
| Protein (g) | 32.29 ± 9.29 | 32.20 ± 9.29 | 43.47 ± 8.87 | 41.00 ± 6.54 | 54.17 ± 16.33 | 55.20 ± 16.87 | 51.78 ± 23.06 | 53.57 ± 23.22 | 44.15 ± 17.11 | 52.39 ± 21.19 | 37.90 ± 17.14 |
| Lipid (g) | 23.70 ± 7.32 | 23.60 ± 7.32 | 27.69 ± 8.92 | 24.75 ± 5.93 | 36.49 ± 13.92 | 39.07 ± 18.26 | 36.07 ± 2.12 | 31.33 ± 15.76 | 27.13 ± 18.79 | 31.49 ± 18.64 | 31.49 ± 18.64 |
| Carbohydrate (g) | 127.27 ± 24.76 | 127.22 ± 24.76 | 185.74 ± 40.22 | 204.22 ± 44.61 | 228.11 ± 30.89 | 213.48 ± 39.32 | 191.43 ± 71.45 | 212.19 ± 51.69 | 178.46 ± 50.79 | 201.89 ± 63.95 | 194.5 ± 18.73 |
| Caffeine (mg) | 490 | 490 | 608.62 ± 214.29 | 608.62 ± 214.29 | 608.62 ± 214.29 | 608.62 ± 214.29 | 608.62 ± 214.29 | 608.62 ± 214.29 | 608.62 ± 214.29 | 608.62 ± 214.29 | 608.62 ± 214.29 |

**Dietary intake analysis**

All foods except those that elicited an allergic response were eliminated during the study, the challenge tests were stopped, and the patients were observed for one week.

**Fig. 1.** Diagnostic flow for the food challenge test in patients with atopic dermatitis.
test (OFC or DBPCFC). At each post food challenge test visit to the clinic, participants completed a three-day dietary record, including two weekdays and one weekend day in order to evaluate the energy intake and diet quality. Results of the dietary intake survey were analyzed in terms of the quantity of nutrient intake, age, and sex using Can-Pro 3.0 (The Korean Nutrition Society, Seoul, Republic of Korea).

Statistical analysis

All analyses were performed using SPSS version 13.0 (SPSS, Chicago, IL, USA). Results are expressed as mean ± SD. The significance of differences was assessed using the Student’s t-test and one-way analysis of variance (ANOVA) coupled with Duncan multiple range tests. Significance was defined as a p-value less than 0.05.

Results

Intake of nutrients by age in AD subjects

Intake of nutrients reported by age group in AD subjects is shown in Table 1; Dietary Reference Intakes for each age level including two weekdays and one weekend day in order to evaluate the energy intake and diet quality. Results are expressed as mean ± SD. The significance of differences was assessed using the Student’s t-test and one-way analysis of variance (ANOVA) coupled with Duncan multiple range tests. Significance was defined as a p-value less than 0.05.

Table 2. Intake of nutrients by the number of food allergens in subjects with food allergy

| Food Allergen | 1 (n = 45) | 2 (n = 35) | Over 3 (n = 40) |
|---------------|------------|------------|----------------|
| Energy, kcal  | 1240.05 ± 364.59 | 1156.49 ± 386.928 | 1021.30 ± 428.619 |
| Protein, g     | 49.37 ± 19.03 | 44.88 ± 18.788 | 38.08 ± 18.2598 |
| Lipid, g       | 32.06 ± 14.68 | 28.98 ± 15.03 | 21.42 ± 12.1482 |
| Carbohydrate, g | 192.65 ± 62.22 | 178.59 ± 66.00 | 168.41 ± 68.23 |
| CPF ratio      | 62.23:15 | 63.22:15 | 67:18:15 |
| Dietary fiber, g | 12.40 ± 5.25 | 12.23 ± 7.02 | 11.39 ± 6.50 |
| Ca, mg         | 353.47 ± 177.97 | 328.70 ± 195.73 | 307.84 ± 190.55 |
| P, mg          | 684.06 ± 276.59 | 609.04 ± 233.888 | 535.58 ± 247.178 |
| Fe, mg         | 8.78 ± 3.04 | 7.99 ± 3.74 | 7.36 ± 4.138 |
| Na, mg         | 2334.20 ± 1194.9 | 1988.18 ± 947.63 | 1837.39 ± 1281.30 |
| K, mg          | 1781.37 ± 687.18 | 1636.39 ± 622.19 | 1490.09 ± 747.24 |
| Zn, mg         | 6.49 ± 2.55 | 5.82 ± 2.32 | 4.91 ± 2.248 |
| Vit A, µgRE    | 468.95 ± 285.04 | 506.16 ± 446.10 | 425.12 ± 322.45 |
| Vit B6, mg     | 0.71 ± 0.23 | 0.67 ± 0.388 | 0.56 ± 0.268 |
| Vit B12, µg    | 1.35 ± 0.48 | 1.49 ± 0.48 | 1.15 ± 0.55 |
| Niacin, mg     | 10.56 ± 5.00 | 9.18 ± 4.41 | 8.10 ± 3.908 |
| Vit C, mg      | 70.09 ± 43.49 | 74.16 ± 45.38 | 60.03 ± 40.70 |
| Vit E, mg      | 9.27 ± 4.13 | 9.00 ± 5.32 | 8.71 ± 6.67 |
| Cholesterol, mg| 236.44 ± 123.40 | 201.75 ± 121.538 | 137.48 ± 107.058 |

Intake of nutrients by the number of food allergens

Intake of nutrients according to the number of food allergens

Intake of nutrients according to the number of food allergens was analyzed for FA patients who had positive allergic response of OFC and DBPCFC to the seven food items (Table 2). Intake of energy, protein, lipid, phosphorus, zinc, vitamin B6, B12, niacin, and cholesterol decreased as the number of food allergens increased.

Intake of nutrients between positive and negative responses to seven foods each

Intake of nutrients between positive and negative responses to milk and egg by DBPCFC in subjects is shown in Table 3. Subjects who were positive for a milk allergy had significantly lower intake of energy recommendations and nutrients such as lipid, calcium, sodium, zinc, and vitamin B6 than those who were...
Discussion

A food allergy can only be diagnosed with certainty by elimination-provocation tests [20]. Probable allergens are removed in order to create the elimination diet. If symptoms disappear during elimination, a food allergy is likely the cause of the symptoms. The cause can be established by reintroducing foods one at a time. Some subjects were positive for SPT and blood IgE tests, despite not showing allergic responses during food challenge tests (OFC and DBPCFC). No specific food was limited for consumption; however, most of the subjects did not consume the recommended amount of food and nutrients. For proteins, lipids, and carbohydrates, subjects under the age of 5 who were positive for FA consumed less than those who were negative for FA. The opposite was true for patients age 6 and older. At all ages, the subjects showed deficient Ca intake, and Ca intake was lower in FA-positive subjects in the group of patients who were 12 years old and younger—the ages of rapid growth. According to Chang et al. [21], parents of AD patients with chronic atopic disease, especially mothers, suffer from acute stress. They often use scientifically unproven therapy as a result of stress. They often use scientifically unproven therapy as a result of stress.

Table 3. Intake of nutrients between positive and negative milk and egg allergies in subjects with food allergy

|                | Negative (n = 77) | Positive (n = 43) | Negative (n = 79) | Positive (n = 41) |
|----------------|------------------|------------------|------------------|------------------|
| Energy, kcal  | 1184.96 ± 357.79 | 1067.18 ± 463.26* | 1190.06 ± 387.88 | 1051.61 ± 414.52* |
| (72.24 ± 22.55) | (71.26 ± 23.31) | (75.35 ± 23.34) | (65.22 ± 20.14) | |
| Protein, g     | 46.20 ± 17.91    | 40.89 ± 20.07    | 46.26 ± 19.26    | 40.52 ± 18.58    |
| (160.97 ± 89.22) | (158.67 ± 74.25) | (170.21 ± 74.68) | (138.85 ± 63.01) | |
| Lipid, g       | 29.49 ± 14.19    | 24.25 ± 15.11*   | 29.74 ± 14.82    | 26.53 ± 13.68    |
| Dietary fiber, g| 12.52 ± 6.19     | 11.10 ± 6.15     | 12.37 ± 5.70     | 11.32 ± 7.06     |
| Ca, mg         | 370.66 ± 189.77  | 260.07 ± 160.49* | 340.98 ± 190.56  | 311.86 ± 199.41  |
| (56.39 ± 29.17) | (51.22 ± 27.26)  | (52.11 ± 27.26)  | (48.86 ± 29.33)  | |
| P, mg          | 684.04 ± 243.83  | 549.38 ± 279.82  | 641.60 ± 267.39  | 556.97 ± 240.09  |
| (98.15 ± 52.48) | (89.98 ± 41.02)  | (98.90 ± 38.76)  | (98.94 ± 33.41)  | |
| Fe, mg         | 8.42 ± 3.48      | 8.47 ± 3.92      | 8.21 ± 3.42      | 7.82 ± 4.10      |
| (88.96 ± 35.58) | (90.71 ± 42.70)  | (91.92 ± 37.35)  | (95.08 ± 38.89)  | |
| Na, mg         | 2198.12 ± 1136.51| 1776.94 ± 1202.85| 2181.92 ± 1206.79| 1770.70 ± 1066.06*|
| K, mg          | 1756.57 ± 664.55 | 1436.62 ± 709.26 | 1715.28 ± 688.79 | 1500.78 ± 694.00 |
| Zn, mg         | 6.04 ± 2.21      | 5.28 ± 2.81*     | 6.00 ± 2.49      | 5.32 ± 2.36      |
| (138.73 ± 43.44) | (124.23 ± 50.14) | (137.25 ± 48.26) | (126.38 ± 41.82) | |
| Vit A, ugRE    | 501.36 ± 357.92  | 400.44 ± 327.41  | 509.86 ± 374.75  | 379.14 ± 278.56* |
| (101.72 ± 67.54) | (93.77 ± 69.58)  | (111.15 ± 72.70) | (75.22 ± 51.14)  | |
| Vit B1, mg     | 0.68 ± 0.30      | 0.59 ± 0.28      | 0.69 ± 0.31      | 0.57 ± 0.26*     |
| (86.36 ± 35.02) | (84.75 ± 41.35)  | (94.68 ± 39.34)  | (70.75 ± 26.65)  | |
| Vit B2, mg     | 0.71 ± 0.29      | 0.53 ± 0.26*     | 0.68 ± 0.30      | 0.57 ± 0.26*     |
| (77.33 ± 41.63) | (63.35 ± 30.81)  | (77.67 ± 41.21)  | (62.01 ± 30.80)  | |
| Vit B6, mg     | 1.27 ± 0.48      | 1.15 ± 0.56      | 1.29 ± 0.50      | 1.12 ± 0.52      |
| (130.89 ± 52.17) | (128.47 ± 52.52) | (139.02 ± 55.23) | (110.69 ± 40.17) | |
| Niacin, mg     | 9.54 ± 4.47      | 9.97 ± 4.77      | 9.82 ± 4.87      | 8.40 ± 3.80*     |
| (92.23 ± 38.64) | (100.97 ± 47.31) | (102.05 ± 44.02) | (82.48 ± 34.68)  | |
| Vit C, mg      | 71.43 ± 45.21    | 61.65 ± 39.01    | 70.58 ± 40.77    | 62.81 ± 47.60    |
| (114.61 ± 87.29) | (102.86 ± 80.33) | (123.12 ± 87.56) | (98.38 ± 76.32)  | |
| Folic acid, µg | 161.90 ± 105.10  | 142.99 ± 101.26  | 160.45 ± 94.54   | 144.86 ± 120.04  |
| (63.79 ± 47.25) | (60.17 ± 47.27)  | (69.47 ± 49.31)  | (55.33 ± 41.41)  | |
| Vit E, mg      | 9.48 ± 4.43      | 8.16 ± 6.76      | 9.59 ± 5.32      | 7.88 ± 5.41      |
| Cholesterol, mg| 206.00 ± 118.34  | 170.64 ± 131.92  | 221.83 ± 119.95  | 138.43 ± 113.99*|

Energy (C) represents percent of estimated energy requirement. Nutrients (C) represents percent of recommended nutrient intake.
Data are expressed as mean ± SD.
* Significant difference between positive and negative food allergy according to independent t test.
of fears and worries. They also show extreme concern and tension. Such behaviors may cause limited selection of foods. Dietary factors, such as a low intake of antioxidants may affect the risk of AD and asthma [22,23]. Several studies conducted in AD and asthma patients reported that intake of several foods containing high levels of vitamin C and vitamin A was related to a decrease in the risk of these diseases [23-25]. Our results showed that AD subjects were lacking in several nutrients, including vitamin A and vitamin C. A greater number of food allergens showed an association with an increase in number of deficient nutrients.

Allergen avoidance is the treatment for AD. However, when the allergen is food, excessive restriction can lead to nutrition deficiency. Relevant to atopic diseases, the effects of nutrition on the manifestation and severity of atopic disorders were carefully reviewed in a previous study [26]. In this regard, two different approaches have been studied as preventive measures for various atopic diseases: (1) an elimination diet aimed at omission of causative nutrients and (2) a supplementary diet aimed at addition of protective nutrients [27].

We analyzed nutrient intake according to the kinds of food allergen in subjects with FA for seven food items. Subjects with milk allergy had lower intake of Ca, Zn, and vitamin B2, and subjects with egg allergy had lower intake of vitamin A, vitamin B1, vitamin B2, niacin, and cholesterol. Subjects with FA to wheat and soybean had lower intake of Ca, P, Fe, K, Zn, vitamin B2, vitamin B6, and niacin. Subjects with FA to beef, pork, and chicken had lower intake of Fe and higher intake of Ca, K, vitamin A, B2.

Because most causative agents of FAs are foods that provide valuable nutrients, consumption of replacement food containing similar nutrients should be emphasized in order to prevent malnutrition. Avoidance of symptom-causing foods should be in accordance with tolerance: thus, if food-induced symptoms are
mild and occur only after ingestion of higher doses, there is no need for a strict elimination diet [20].

In this study, we investigated seven food items as causative foods for food allergy symptoms, the result of food limitation to AD patients, and associated nutrient intake. Findings of this study suggest the necessity for enhanced nutritional education for replacement foods in order to provide FA patients with information necessary for acquisition of adequate nutrition. Following this study, investigation of the impact of excessive food limitation on growth, malnutrition, and AD management is needed. Further studies should investigate systems and strategies for mitigation of harm from excessive limitation. Regarding the limitations of this study, the number of subjects in each age group is not large enough, even though FA patients were studied for 10 years, from 2000 to 2010. Despite a long period of time for collection or gathering of information in order to obtain accurate results from FA patients, the number of patients is insufficient. Therefore, in the future, this study is expected to continue.

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