Application of moisturizer to neonates prevents development of atopic dermatitis

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Background: Recent studies have suggested that epidermal barrier dysfunction contributes to the development of atopic dermatitis (AD) and other allergic diseases.
Objective: We performed a prospective, randomized controlled trial to investigate whether protecting the skin barrier with a moisturizer during the neonatal period prevents development of AD and allergic sensitization.
Methods: An emulsion-type moisturizer was applied daily during the first 32 weeks of life to 59 of 118 neonates at high risk for AD (based on having a parent or sibling with AD) who were enrolled in this study. The onset of AD (eczematous symptoms lasting >4 weeks) and eczema (lasting >2 weeks) was assessed by a dermatology specialist on the basis of the modified Hanifin and Rajka criteria. The primary outcome was the cumulative incidence of AD plus eczema (AD/eczema) at week 32 of life. A secondary outcome, allergic sensitization, was evaluated based on serum levels of allergen-specific IgE determined by using a high-sensitivity allergen microarray of diamond-like carbon–coated chips.
Results: Approximately 32% fewer neonates who received the moisturizer had AD/eczema by week 32 than control subjects ($P = .012$, log-rank test). We did not show a statistically significant effect of emollient on allergic sensitization based on the level of IgE antibody against egg white at 0.34 kU_A/L CAP-FEIA equivalents. However, the sensitization rate was significantly higher in infants who had AD/eczema than in those who did not (odds ratio, 2.86; 95% CI, 1.22-6.73).
Conclusion: Daily application of moisturizer during the first 32 weeks of life reduces the risk of AD/eczema in infants. Allergic sensitization during this time period is associated with the presence of eczematous skin but not with moisturizer use. (J Allergy Clin Immunol 2014;134:824-30.)

Key words: *Atopic dermatitis, atopy, allergic sensitization, food allergy, IgE, randomized controlled trial*

The prevalence of atopic dermatitis (AD) among children continues to increase, reaching 20% in some parts of the world; almost half of all children experience eczema within the first 2 years of life. AD reduces quality of life, such as by disturbing sleep, and should be considered a significant global burden of disease.[1-4]

Skin barrier dysfunction contributes to the development of AD, and dry skin often causes inflammation of eczematous skin. Filaggrin, a key component of the epidermal differentiation complex, is required for barrier function. Disruption of the gene encoding filaggrin *(FLG)* is associated with development of AD, as well as ichthyosis. Children with mutations in *FLG* have increased transepidermal water loss, even before AD develops.[2,5,6] The skin stratum corneum of infants is intact shortly after birth, but the water-sustaining barrier function of skin becomes adult like only after the first year of life.[7] Therefore it has been proposed that intensive emollient use in early life could prevent AD, especially in infants at high risk for AD (based on having a parent or sibling with AD). This hypothesis was investigated in a pilot study,[8] and a large-scale randomized controlled trial (RCT) is underway (Barrier Enhancement for Eczema Prevention trial; http://www.beepstudy.org/).[9] We initiated an RCT in 2010 to test the effects of an emulsion-type moisturizer (2e [Douhet] emulsion; Shiseido, Tokyo, Japan) in neonates at high risk for AD.

Several cohort studies have provided evidence that infants with eczema tend to have other allergic diseases, such as asthma, rhinitis, and food allergy.[10,11] Moreover, topical application of peanut oil to neonatal skin increased the infant's risk of peanut allergy, indicating epicutaneous sensitization to allergens.[12] Loss-of-function mutations in *FLG* are associated with a wide range of allergic diseases and sensitization to airborne and food antigens, even though filaggrin expression is limited to the skin and oral mucosa and has not been detected in the respiratory or intestinal mucosa.[6,13-15]

Primary prevention of allergic disease has been studied for many years. However, studies of avoidance of food allergens, aeroallergens, or both have generally produced disappointing results.[16] In this study we investigate whether daily application of moisturizer to neonates at high risk for AD prevents allergic sensitization, as well as development of AD. In addition to the outcomes of this RCT, we report that the presence of skin lesions (including AD) is a risk factor for allergic sensitization.

Abbreviations used
AD: Atopic dermatitis
DLC: Diamond-like carbon
FLG: Filaggrin gene
IRB: Institutional review board
NCCHD: National Center for Child Health and Development
OR: Odds ratio
RCT: Randomized controlled trial
UMIN-CTR: University Hospital Medical Information Network Clinical Trials Registry

METHODS

Trial design and participants

We performed an investigator-blinded, randomized, controlled, parallel-group study at the National Center for Child Health and Development (NCCHD) in Tokyo, Japan, from November 2010 through November 2013 (Fig 1). The NCCHD is the only national hospital for mothers and children in Japan, performing more than 1600 deliveries per year. After receiving approval from the institutional review board (IRB) of the NCCHD in August 2010, we invited expectant mothers with family histories of AD who visited the prenatal clinic of the NCCHD to participate in this trial. A high familial risk of AD was defined as a history of physician-diagnosed AD for at least 1 of the unborn baby's parents or siblings. Informed consent was obtained from the parents before delivery. After birth, the study doctors and a dermatology specialist confirmed the eligibility of each neonate on the basis of the inclusion criteria (eg, absence of treatment with corticosteroids) and exclusion criteria (eg, abnormal skin disorders, such as ichthyosis), which had been registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; UMIN000004544). The enrolled neonates were then randomly assigned to the intervention (n = 59) or control (n = 59) group (Fig 2).

The intervention group began receiving daily application of an emulsion-type emollient (2e [Douhet] emulsion) from the first week of life; petroleum jelly was prescribed to each infant in both groups on request by the IRB. Emollient was applied each day for 32 weeks. All infants were examined by the same blinded dermatologist from the NCCHD at scheduled visits and at weeks 4, 12, 24, and 32 of life. At each visit, the dermatologist examined the skin condition of the infant and recorded a diagnosis of AD, eczema, skin rash without pruritus, or healthy skin without any lesions. The worldwide and most validated criteria for diagnosis did not specify a time frame for AD development, describing a chronic or relapsing course,[17-19] and therefore it was not possible to diagnose an infant's AD immediately after his or her pruritic skin lesion emerged.

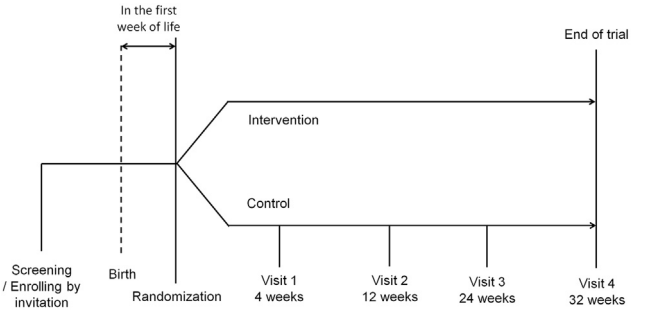

FIG 1. Study design.

Simpson et al[8] have modified the Hanifin-Rajka criteria for an incident case, setting the time for AD development to at least 2 weeks. The same authors proposed setting the time frame as at least 4 weeks.[20] We incorporated these criteria for an incident case of AD according to our definition of infantile eczema and AD. In our trial AD was defined as "itchy eczema at typical locations that lasted for at least 4 weeks," and infantile eczema was defined as the same eczema that lasted at least 2 weeks. Then these criteria were registered. Because AD and infantile eczema, as defined above, were essentially synonymous, we combined them as AD/eczema for this study. If an infant with skin rash or eczematous skin did not show any sign of pruritus, the dermatologist made a diagnosis of skin rash. When given a diagnosis of AD/eczema, infants were immediately removed from the study and treated appropriately. We instructed the parents to visit our outpatient clinic if their infants had any skin problems (Fig 1).

Outcomes

We registered this trial design, including the hypothesis and outcome measures, at UMIN-CTR (UMIN000004544). We proposed that protection of the skin barrier with a moisturizer beginning in the neonatal period would be a safe and effective strategy for prevention of AD and allergic sensitization. The primary outcome measure was the cumulative rate of incidence of AD, eczema, or both by temporal observation. The diagnostic criteria for infantile eczema, AD, or both (AD/eczema) were developed based on a modification of the United Kingdom Working Party's criteria and were applied by a dermatology specialist, as described above. Briefly, those criteria were a pruritic skin condition of at least 2 weeks' duration, visible flexural dermatitis (and/or on the cheeks and extensor surfaces), a history of dry skin, and a family history in a first-degree relative of the enrolled neonate.

Secondary outcome measures were the presence of allergen-specific IgE, transepidermal water loss (to measure stratum corneum hydration and pH at birth [baseline] and at weeks 4, 12, 24 and 32 of life; Vapo Meter, SW-4002; Delfin Technologies, Kuopio, Finland), stratum corneum hydration (Moisture Meter, SC-5; Delfin Technologies), stratum corneum pH (epidermal; Skin-pH-Meter, PH905; Courage & Khazaka Electronic GmbH, Koln, Germany), and skin colonization by *Staphylococcus aureus* (measured at the cheek).

Onset of allergic diseases, such as food allergy (registered on November 10, 2010), and onset of asthma were added as outcome measures on April 12, 2011, in response to a recommendation by the evaluation committee of the Ministry of Health, Labour and Welfare. Skin barrier functions were assessed by using the previously described methods.[21]

Statistical analyses

Analyses of the primary and secondary outcomes were conducted according to the intent-to-treat principle and based on the full analysis set, which included all randomized subjects. For an analysis of allergic sensitization, subjects without serum specific IgE (detected by using the diamond-like carbon [DLC] chip with high-density allergen immobilization and high sensitivity[22] at week 32; n = 2 for the intervention group and n = 5 for the control group) were excluded.

The primary outcome (cumulative rate of incidence of AD, eczema, or both by temporal observation) was analyzed by using the log-rank test. The significance level was set at .05. The Kaplan-Meier method was used to estimate the cumulative incidence of AD/eczema for each group, and the Cox regression model was applied to estimate the hazard ratio between groups. The Mann-Whitney *U* test and χ^2 test with the Yates correction were used with continuous and categorical variables, respectively, to analyze secondary outcomes. Demographic and baseline data are presented as means, SDs, and proportions, as appropriate.

Once the data were collected from all subjects, we conducted several *post hoc* analyses. To evaluate the association between sensitization to foods and AD, we constructed a contingency table that dichotomized serum levels of antigen-specific IgE (based on results from the DLC assay) measured at week 32 at several cutoff values. The odds ratio (OR) and 95% CI were used to evaluate the degree of association. Statistical analyses were conducted with SPSS 17.0 software for Windows (SPSS, Chicago, Ill) and R software (version 3.0.1, http://www.R-project.org).

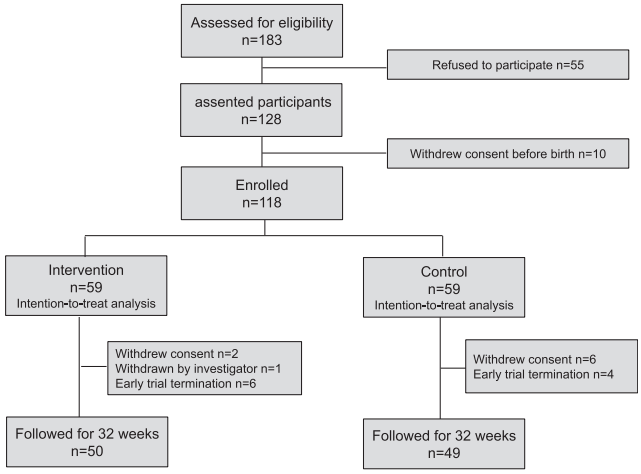

FIG 2. Study flow chart.

Consolidated Standards of Reporting Trials topics

Methods relating to Consolidated Standards of Reporting Trials statement (http://www.consort-statement.org/) and other methods are described in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Characteristics of neonates

We invited 183 expectant mothers from families at high risk for AD to participate in the study; 118 neonates were enrolled and randomly assigned to 2 groups of 59 infants each (Fig 2). Two infants assigned to the control group were found to have accidently received and used the emollient after opening the blinded data; 1 withdrew consent, and another completed the study without skin lesions. All 118 neonates were included as the intent-to-treat population (Table I) and the 2 infants who mistakenly received the intervention were classified into the control group. During the trial, 8 families withdrew informed consent (2 infants in the intervention group and 6 infants in the control group). The dermatologist withdrew an infant in the intervention group from the study because she or he had a hemangioma. After the second scheduled examination, we found that the incidence of AD was significantly lower in the intervention group than in the control group and reported this observation to the IRB of the NCCHD. The trial was discontinued at the recommendation of the NCCHD's IRB on November 30, 2013; by this time, 10 neonates had left the study (6 in the intervention group and 4 in the control group).

TABLE I. Baseline characteristics of the study population

Characteristic	Intervention group (n = 59)	Control group (n = 59)
Infant girl, no. (%)	26/59 (44.1)	24/59 (40.7)
Birth		
Mean ages of mothers at delivery (y)	35.8 ± 4.80	35.0 ± 4.85
Cesarean section, no. (%)	16/59 (27.1)	13/59 (22.0)
Mean gestational age (wk)	39.1 ± 0.97	39.0 ± 1.07
Mean birth weight (g)	3074 ± 363	3034 ± 366
Breast-feeding at 1 mo (%)	29/58 (50.0)	28/58 (48.3)
Family history		
Food allergy (%)	24/59 (40.7)	21/59 (36.8)
Bronchial asthma (%)	24/59 (40.7)	21/59 (36.8)
Allergic rhinitis (%)	46/59 (78.0)	48/59 (84.2)
Mean no. of siblings	0.34 ± 0.58	0.38 ± 0.62
Environmental exposures		
Smoking in the family, no. (%)	10/59 (16.9)	7/57 (12.3)
Any pet, no. (%)	12/58 (21.4)	13/57 (23.2)
Dog, no. (%)	8/58 (13.8)	6/57 (10.5)
Cat, no. (%)	2/58 (3.4)	4/57 (7.0)
Skin barrier function		
TEWL		
Mean lower leg	8.31 ± 2.67	8.40 ± 2.92
Mean forehead	8.29 ± 4.77	7.62 ± 3.15
Stratum corneum hydration		
Mean lower leg	13.7 ± 5.93	13.5 ± 5.94
Mean forehead	20.6 ± 10.7	19.2 ± 11.6
Mean pH	5.65 ± 0.59	5.61 ± 0.39

TEWL, Transepidermal water loss.

Among 118 infants evaluated, 47 had AD/eczema (19/59 in the intervention group and 28/59 in the control group), 13 had skin rash without pruritus (6 in the intervention group and 7 in the control group), and 31 did not have any skin lesions (20 in the intervention group and 11 in the control group). There were 5 infants (2 in the intervention group and 3 in the control group) who used moisturizers for skin disorders other than AD/eczema. The dermatology specialist stopped giving the emollient to 3 infants whose skin lesions seemed to be the result of urticaria or contact dermatitis caused by emulsion-type emollients (related adverse events). After several days, however, the doctor judged that these skin lesions were not adverse events because they disappeared rapidly and similar lesions were not seen when the same emollients were used again. These 3 infants did not have AD/eczema or skin rash when they were followed for 32 weeks. Among 8 families who withdrew consent, 2 families in the intervention group said that it was difficult for them to visit the NCCHD. There were no infants from families that withdrew consent who had skin lesions. In summary, adverse events caused by this emulsion-type emollient were not observed during this RCT.

Because the IRB recommended permitting application of petroleum jelly when the parents thought it necessary, we calculated the amount of these 2 types of moisturizers used by each group based on their diaries. The mean daily amount of emulsion-type moisturizer used by the intervention group was 7.86 ± 4.34 g (0 g for the control group, excluding the 2 infants placed in the wrong group). The mean daily amount of petroleum jelly applied to the control group was 0.101 ± 0.286 g (mean frequency of use, 0.235 d/wk). Petroleum jelly (20 g per bottle) was prescribed to all neonates born at the NCCHD, but we had no information about how much was used by the intervention group. Nevertheless, only a few of the parents occasionally used a small, almost ignorable, amount of the jelly on their infants.

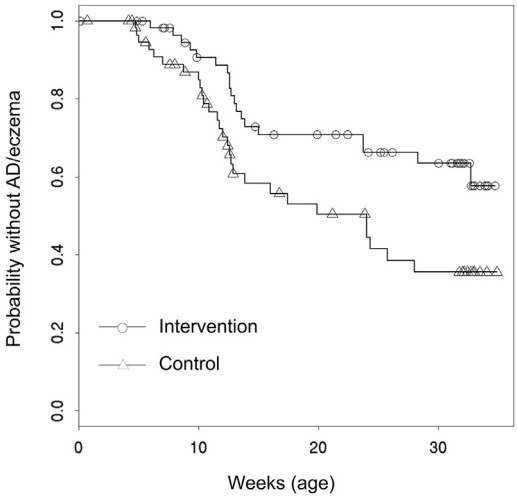

FIG 3. Proportions of infants who did not have AD/eczema. Kaplan-Meier plots show the proportions of infants in the intervention (*circle*) and control (*triangle*) groups with AD/eczema during the first 32 weeks of life. The log-rank test indicated statistically significant differences between groups ($P = .012$).

Primary and secondary outcomes

During their first 32 weeks of life, 19 infants in the intervention group had AD/eczema compared with 28 infants in the control group. Calculation of cumulative incidence values for AD/eczema by using the Kaplan-Meier method showed that the intervention group maintained intact skin for a significantly longer period than the control group ($P = .012$, log-rank test; Fig 3). Cox regression analysis showed the risk of AD/eczema to be significantly lower in the intervention group (hazard ratio, 0.48; 95% CI, 0.27-0.86).

In analyses of secondary outcomes (levels of allergen-specific IgE), we evaluated the serum levels of anti–egg white and anti-ovomucoid IgE in infants at 32 weeks,[22] as described in the Methods section of this article's Online Repository. IgE antibody data were converted to CAP-FEIA data after confirming the correlation between the data sets (see Fig E1 in this article's Online Repository at www.jacionline.org). However, we were not able to demonstrate a statistically significant effect of emollient on the rate of allergic sensitization based on level of IgE antibody against egg white (0.34 kU_A/L CAP-FEIA equivalents); the proportions of infants who were sensitized by allergen were similar in the intervention and control groups (Table II[18] and see Fig E2 in this article's Online Repository at www.jacionline.org).

The intervention group had significantly higher levels of stratum corneum hydration in the lower leg at weeks 12 and 24 compared with those seen in the control group (see Fig E3 in this article's Online Repository at www.jacionline.org). In both groups 6.1% of infants (7/115 cases measured) had positive test results for *S aureus* in cheek samples at birth, and 22.4% had positive test results (19/85 cases measured) at week 12. There was no significant difference between percentages of infants with positive test results for S aureus in the intervention (26.0%

TABLE II. Allergic sensitization at week 32

Level of specific IgE	Intervention group (n = 48)	Control group (n = 44)	*P* value†
Egg white (kU_A/L*)			
≥0.35	42% (20/48)	45% (20/44)	.88
≥0.70	38% (18/48)	45% (20/44)	.57
Ovomucoid (kU_A/L*)			
≥0.35	19% (9/48)	6.8% (3/44)	.17
≥0.70	13% (6/48)	4.5% (2/44)	.33

*We converted the levels of specific IgE (binding unit of IgE *[BUe]*/mL) measured with a DLC chip into CAP-FEIA equivalents (kU_A/L) based on a previously described method.[22] Cutoff values for allergic sensitization were set at 0.35 or greater or 0.7 or greater.
†The χ^2 test was used to calculate the difference between the 2 study groups.

TABLE III. Numbers of Infants with AD/eczema and allergic sensitization at week 32

Level of specific IgE	With AD/eczema (n = 43)	Without AD/eczema (n = 49)	*P* value†
Egg white (kU_A/L*)			
≥0.35	56% (24/43)	33% (16/49)	.043
≥0.70	56% (24/43)	29% (14/49)	.015
Ovomucoid (kU_A/L*)			
≥0.35	19% (8/43)	8.2% (4/49)	.24
≥0.70	12% (5/43)	6.1% (3/49)	.57

*We converted the levels of specific IgE (binding unit of IgE *[BUe]*/mL) measured with a DLC chip into CAP-FEIA equivalents (kU_A/L) based on a previously described method.[22] Cutoff values for allergic sensitization were set at 0.35 or greater or 0.7 or greater.
†The χ^2 test was used to calculate the difference between the 2 study groups.

[13/50 cases]) and control (17.1% [6/35 cases]) groups at week 12 (χ^2 analysis).

Post hoc analysis

Recent epidemiologic studies raised the possibility of epicutaneous sensitization to food allergens,[23] whereas others reported that some allergic diseases can be treated by repeated epicutaneous exposure to allergens.[24] Therefore we proposed the hypothesis that allergic sensitization can occur through eczematous but not healthy skin.[23] In a *post hoc* analysis of our data, we compared allergic sensitization in infants with and without AD/eczema at 32 weeks. We found that a greater proportion of infants with AD/eczema had allergic sensitization based on the serum levels of anti–egg white IgE (cutoff level of 0.34 kU_A/L CAP-FEIA equivalents) than infants without AD/eczema (P = .043, Table III).[18] The OR for allergic sensitization in infants with AD/eczema was 2.86 (95% CI, 22-6.73; Table IV[22] and see Fig E4 in this article's Online Repository at www.jacionline.org).

Thirteen infants of a total population had skin rash without pruritus. Six of these 13 infants also had allergic sensitization, and therefore we investigated whether there was an association between allergic sensitization and the presence or absence of skin lesions. The OR for allergic sensitivity (cutoff level of 0.34 kU_A/L CAP-FEIA equivalents) in infants with skin lesions compared with that in infants without skin lesions was 3.73 (95% CI, 1.49-9.36; Table IV and see Fig E4, *B* in this article's Online Repository at www.jacionline.org).

We have shown in this and previous studies that measurements of IgE by using a DLC chip correlate with those determined by using CAP-FEIA (see Fig E1). To prove the accuracy of CAP-FEIA equivalents measured by using a DLC chip in allergic sensitization, we calculated ORs for allergic sensitization using 25 different cutoff levels, ranging from 0.1 to 8.0 kU_A/L (Table IV). We found that ORs for allergic sensitization were greater for infants with AD/eczema than those without AD/eczema and for infants with compared with those without skin lesions when cutoff values were set at 25 different levels.

TABLE IV. Allergic sensitization based on cutoff levels of IgE specific for egg white at week 32

Cutoff values for specific IgE for egg white			
DLC chip (BUe/mL)	CAP-FEIA (kU_A/L)*	Skin lesion (+) vs others, OR (95% CI)	AD/eczema (+) vs others, OR (95% CI)
54.0	0.10	3.01 (1.27-7.16)	2.34 (1.001-5.48)
67.4	0.13	3.38 (1.41-8.07)	2.54 (1.09-5.95)
72.9	0.14	3.79 (1.58-9.14)	2.76 (1.18-6.48)
82.7	0.16	3.49 (1.46-8.39)	3.00 (1.28-7.06)
92.3	0.17	3.23 (1.35-7.71)	3.27 (1.39-7.72)
124.3	0.23	2.99 (1.26-7.11)	2.95 (1.26-6.90)
126.1	0.23	2.77 (1.17-6.57)	2.66 (1.15-6.20)
151.6	0.28	2.57 (1.08-6.08)	2.41 (1.04-5.59)
167.8	0.31	2.90 (1.21-6.93)	2.63 (1.13-6.12)
170.6	0.32	3.29 (1.36-7.97)	2.88 (1.23-6.72)
170.8	0.32	3.05 (1.26-7.39)	2.61 (1.12-6.08)
173.2	0.32	2.84 (1.17-6.85)	2.38 (1.02-5.52)
182.2	0.34	3.24 (1.32-7.96)	2.61 (1.12-6.08)
361.7	0.66	3.73 (1.49-9.36)	2.86 (1.22-6.73)
364.4	0.67	4.35 (1.69-11.2)	3.16 (1.33-7.49)
412.5	1.21	4.04 (1.57-10.4)	2.88 (1.21-6.81)
474.8	2.18	3.76 (1.46-9.67)	2.62 (1.11-6.20)
540.3	3.20	3.50 (1.36-8.99)	2.90 (1.21-6.94)
607.0	3.93	4.13 (1.55-11.0)	3.23 (1.33-7.82)
754.2	5.28	3.84 (1.44-10.2)	2.94 (1.22-7.12)
801.1	5.71	3.57 (1.34-9.51)	2.68 (1.11-6.50)
824.6	5.92	4.31 (1.55-12.0)	3.00 (1.22-7.39)
843.4	6.09	4.00 (1.44-11.1)	3.39 (1.35-8.49)
1004.2	7.56	3.71 (1.33-10.4)	3.09 (1.23-7.74)
1049.7	7.98	3.44 (1.23-9.62)	2.81 (1.12-7.06)

BUe, Binding unit of IgE.
*The levels of specific IgE (BUe/mL) measured with a DLC chip were converted into CAP-FEIA equivalents (kU_A/L) by using a previously described method.[22]

We detected loss-of-function mutations in *FLG* in 6 of the 57 DNA samples from infants. We were not able to demonstrate whether development of AD/eczema correlates with the presence of mutations, probably because of the small sample size (data not shown).

DISCUSSION

In a prospective RCT we investigated whether protection of the skin barrier with an emollient during the first 32 weeks of life prevents AD/eczema development in infants. A previous uncontrolled pilot study investigated whether a moisturizer can prevent AD,[8] but to our knowledge, this is the first RCT to investigate this question.

This trial was performed at only the NCCHD, mainly because of its logistic support. We tested the effects of an emulsion-type moisturizer (2e [Douhet] emulsion) because it is widely used, including for infants, and its composition has been disclosed. Studies to investigate the effects of other moisturizers on other populations are needed to support our findings.

One limitation of our study involves the diagnosis of AD. Worldwide and most validated criteria for the diagnosis of AD did not define the time frame of signs and symptoms,[17-19] resulting in its inability in diagnosis for early onset of AD in infancy. For this trial, we made the diagnosis of AD/eczema based on modified criteria proposed by Simpson et al.[8,20]

Intensive use of a moisturizer was reported to increase hydration of the stratum corneum in neonatal skin[25]; we confirmed this observation in our study. Daily application of an emulsion-type moisturizer during the first weeks of life increased stratum corneum hydration at week 12 compared with that seen in infants who occasionally received the minimum amount of petroleum jelly (control subjects). We found no statistically significant differences between the intervention and control groups in detection of *S aureus* in cheek samples or *FLG* mutations. This lack of association could be a result of insufficient statistical power, and therefore further studies are needed.

Primary prevention of allergic sensitization

Several cohort studies revealed that early-onset eczema increases the risk for allergic diseases, such as asthma, allergic rhinitis, and food allergy.[10,11] The presence of AD was the main skin-related risk factor for food allergen sensitization in young infants.[26] We confirmed that levels of anti–egg white and anti-ovomucoid IgEs measured by using a DLC chip correlate with those from CAP-FEIA. IgE-mediated egg allergy is one of the most common forms of food allergy; IgE against egg white is often used as a marker of atopy in infants.[27,28] In our study we were not able to show the significant effect of emollient on the prevention of allergic sensitization based on the level of IgE antibody against egg white; similar proportions of infants were sensitized in the intervention and control groups. However, we showed that a higher proportion of infants with AD/eczema had allergic sensitization based on serum concentrations of anti–egg white IgE compared with infants without AD/eczema. Furthermore, we found infants with skin lesions to have a more than 3-fold greater risk for allergic sensitization than infants without skin lesions based on 20 of 25 different cutoff points (range, 0.1-8.0 kU_A/L CAP-FEIA equivalents). Collectively, these findings indicate that the presence of eczematous skin, rather than a lack of emollient use, induces or promotes sensitization to allergens, such as egg white, during the first 8 months of life.

The mechanisms of this process are unclear. Levels of tight junction proteins (eg, claudin-1) between epidermal cells are significantly decreased in patients with AD compared with those seen in nonatopic subjects.[29] Also, Langerhans cells were reported to elongate their dendrites, penetrate keratinocyte tight junctions, and take up antigens when the Langerhans cells were activated by means of tape stripping.[30] These results could provide information on how eczematous skin promotes allergen sensitization.

Future directions

Findings from our RCT support our hypothesis that daily application of a moisturizer would prevent development of AD/eczema during the first 32 weeks of life. Contrary to our hypothesis, however, allergic sensitization, as assessed on the basis of acquisition of anti–egg white IgE, was not affected by application of the emollient. Our *post hoc* analysis revealed that the incidence of allergic sensitization was significantly increased among infants with skin lesions, including those caused by AD/eczema, compared with that seen in infants without these lesions. However, studies of a larger number of subjects might find that moisturizer use reduces allergic sensitization by preventing development of AD/eczema. In this *post hoc* analysis skin rash that did not fulfill the present criteria for AD/eczema, such as a lack of pruritus, was proposed to contribute to allergen sensitization. Allergic sensitization sometimes precedes and predicts the development of eczema,[31] and we have described the presence of low-affinity IgE against food antigens in blood and cord blood samples from newborns.[32] Therefore further studies should examine whether sensitization might occur through the placenta or neonatal gastrointestinal tract. It will be interesting to examine the temporal sequence of allergic sensitization, especially of epicutaneous sensitization to food antigens, by separately measuring levels of low-affinity and ordinary IgEs against food antigens.

We thank Professor Emiko Noguchi for providing information regarding the primer design for *FLG* mutations. We also thank Ms Kazuko Hayase and Ms Akiko Maruta of the NCCHD for their excellent assistance.

Clinical implications: Daily application of emollient reduces the risk of AD/eczema by 32 weeks. We might be able to reduce the prevalence of allergic sensitization by preventing the development of AD/eczema.

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METHODS

Interventions, randomization, and blinding

The research pediatricians (K.H. and K.M.) at the Division of Allergy of the NCCHD enrolled participants who met our criteria. Randomization of neonates into 2 groups was performed by means of random permuted blocks of size 4 at the Clinical Research Center of the NCCHD. The effect of intervention was evaluated as the cumulative incidence of AD/eczema, as registered at the UMIN-CTR. Dermatologists in the Division of Dermatology of the NCCHD examined the infants at scheduled visits in an investigator-blinded manner. The list of randomization was kept at the Clinical Research Center of the NCCHD until the end of the study to maintain the blinded state of the investigators.

The emollient used was an emulsion-type moisturizer, 2e emulsion, which was purchased from Shiseido. It was selected because it is commercially available and in widespread use in Japan, including for patients with AD and infants, and its composition has been disclosed. It contains glycerin, xylitol, butylene glycol, behenyl alcohol, batyl alcohol, hydrogenated polydecene, dimethicone, squalane, pentaerythrityl tetraethylhexanoate, Simmondsia chinesis (JOJOBA) seed oil, PEG-60 glyceryl isostearate, PEG-5 glyceryl isostearate, carbomer, potassium hydroxide, sodium metaphosphate, phenoxyethanol, tocopherol, and water (see also http://2e.shiseido.co.jp/products/emulsion.html) but not preservatives or mineral oils. The moisturizer was applied at least once daily to the whole body surface of infants in the intervention group. The participating families in both groups were routinely given a 20-g bottle of petroleum jelly at birth. As recommended by the IRB, we permitted all the families to use the petroleum jelly when they believed it necessary. They recorded the amounts of emulsion-type moisturizer and petroleum used each day. The families also kept a daily diary regarding their infants' skin condition (rash, erythema, itch, or scratch) and the areas to which the moisturizers were applied. We instructed the parents/caregivers to use commercially available soap with mild cleansing potency for their baby's bathing. Parents were instructed to bath their babies at least once a day. These instructions were just based on local customs. Blood samples (200 μL) were collected from each infant at weeks 1 (birth), 12, and 32. Swab samples to determine skin colonization were collected at weeks 1, 4, 12, and 32. Physical condition and skin barrier functions, such as the stratum corneum water concentration, were also evaluated at weeks 1, 4, 12, and 32.

Sample size

The sample size was calculated based on the preliminary results of our unpublished cohort study at the NCCHD. In that study infants at 6 to 8 months of age had a 47% cumulative prevalence of eczema, which was based on a modification of the questionnaire described in the International Study of Asthma and Allergies in Childhood report.[E1] Our experience shows that the rate of eczema assessed by using the modified International Study of Asthma and Allergies in Childhood questionnaire is always considerably higher than actual diagnoses by dermatologists; on the other hand, our invited participants (families) had a high risk of AD. Because we have no other verification tools for estimation, we estimated that 47% of infants who received a moisturizer in this study and 20% of infants who did not receive a moisturizer would have eczema, with 80% power at the 5% significance level and assuming a dropout rate of 5%. It was estimated that 37 cases were needed in each group. We noted that the rate of eczema is fairly high among infants born in the NCCHD compared with those born in other regions in Japan, although the reason is unclear. One might speculate that a high socioeconomic status could affect the rate because the average income of expectant parents at the NCCHD was estimated to be twice that of expectant parents in other regions.[E2] In addition, the IRB of the NCCHD did not allow us to use "participants who do not use emollients"; we gave petroleum jelly to all the participating parents so that they could apply it when they thought their baby's skin was very dry. Thus we adopted an adaptive study design; that is, we decided to re-estimate the sample size based on the results of interim analyses. The IRB of the NCCHD approved our study design in August 2010. In November 2012, based on the scheduled plan, we had performed the first interim analysis when half of the estimated participants reached the end point. The sample size of each group was calculated as 108 cases based on the first interim analysis. However, 4 control group participants had withdrawn informed consent, whereas only 1 intervention group participant had done so. As a result, we decided to emphasize the importance of the control group to the RCT when explaining the study to potential participants.

The second interim analysis was performed in November 2013, as had been scheduled. We enrolled a total of 118 neonates (59 in each group) and found that the incidence of AD was significantly lower in the intervention group than in the control group. We reported this to the IRB of the NCCHD according to its due process. We decided to discontinue the study at the recommendation of the IRB of the NCCHD on November 30, 2013, at which time 10 neonates (6 in the intervention group and 4 in the control group) were continuing in the study.

Methods to measure allergen-specific IgE antibodies

As secondary outcome measures registered at the UMIN-CTR, serum levels of several allergen-specific IgE antibodies were measured by using a novel allergen microarray on a DLC-coated chip, a high-sensitivity detection method for allergen-specific antibodies, as previously described.[E3] We used mainly a DLC chip method to measure allergen-specific IgE antibodies because it requires less than 2 to 5 μL of blood, although we sometimes measured the same allergen-specific IgE antibodies using the ImmunoCAP solid-phase IgE assay (CAP-FEIA; Thermo Scientific, Uppsala, Sweden) when the blood sample volume was sufficient. The DLC chip, but not CAP-FEIA, can detect low-affinity IgE antibodies that are present in fetuses and neonates.[E4] IgE antibody levels measured with a DLC chip correlate well with those determined by using CAP-FEIA when adult samples are used, and we confirmed this correlation by using our own neonatal samples when we had a sufficient blood volume to test. We successfully measured 3 allergen-specific IgE antibodies (to egg white, ovomucoid, and milk) using both a DLC chip and CAP-FEIA methods (Fig E1). For anti-milk antibody, correlation between the values obtained by using the 2 methods was not sufficiently high, suggesting the presence of low-affinity IgE antibodies. As a consequence, levels of anti–egg white and anti-ovomucoid IgE antibodies measured with a DLC chip correlated significantly with those determined by using CAP-FEIA and were used in further analyses. We were not able to validate the correlation between IgE antibodies detected with the DLC chip and those detected with CAP-FEIA in our samples at 1 and 12 weeks.

FLG mutation analysis

The representative *FLG* mutation sites found in the Japanese population were detected by using the primer sets described below. The p.R501*, p.S2889*, and p.S3296* mutations were screened by using TaqMan analysis (Life Technologies, Thermo Fisher Scientific, Waltham, Mass), as described previously.[E5,E6] The following mutations were screened for by using TaqMan analysis with newly developed primers and probes. The c.3321delA mutation was screened with 2 primers (5′-TGATAGTGAGGGACATTCAGAGGA-3′ and 5′-TTCATGAGTGCTCACCTGGTAGAT-3′) and 2 probes (5′-VIC-ACCTCCCCCTGACCAG-MGB-3′ and 5′-FAM-ACCTCCCCCGACCAG-MGB-3′). The p.Q1701* mutation was screened with 2 primers (5′-AGCA GACAGCTCCACAGACT-3′ and 5′-CTGTGTGTCTGACTCTTCTGAG-3′) and 2 probes (5′-VIC-CAGACAAGATTCATCTGT-MGB-3′ and 5′-FAM-GCAGATAAGATTCATCTGT-MGB-3′). The p.S2554* mutation was screened with 2 primers (5′-GCAAGCAGACAAACTCGTAACGAT-3′ and 5′-CTGGCTAAAACTGGATCCCCA-3′) and 2 probes (5′-VIC-CCAGGGA CAATCAGA-MGB-3′ and 5′-FAM-CCAGGGACAATGAGA-MGB-3′). The p.K4022* mutation was screened by using TaqMan analysis with 2 newly developed probes (5′-VIC-CGTTTGGTAAAGATCATC-MGB-3′ and 5′-FAM-CGTTTGGTTAAGATCAT-MGB-3′) and 2 primers (5′-TGTT TTCAAGGAAAGATCTGATATCTG-3′ and 5′-ATATATCACTAGAATG GCCACATAAACC-3′).

Bacterial culture of *Staphylococcus aureus*

Bacteria on the swabs obtained from the cheeks of infants were inoculated onto No. 110 *Staphylococcus* species–selective agar plates (Nissui Pharmaceutical, Tokyo, Japan) and cultured. Each bacterial colony was examined regarding the expression of *femA* and *femB* genes to confirm the presence of *S aureus*.

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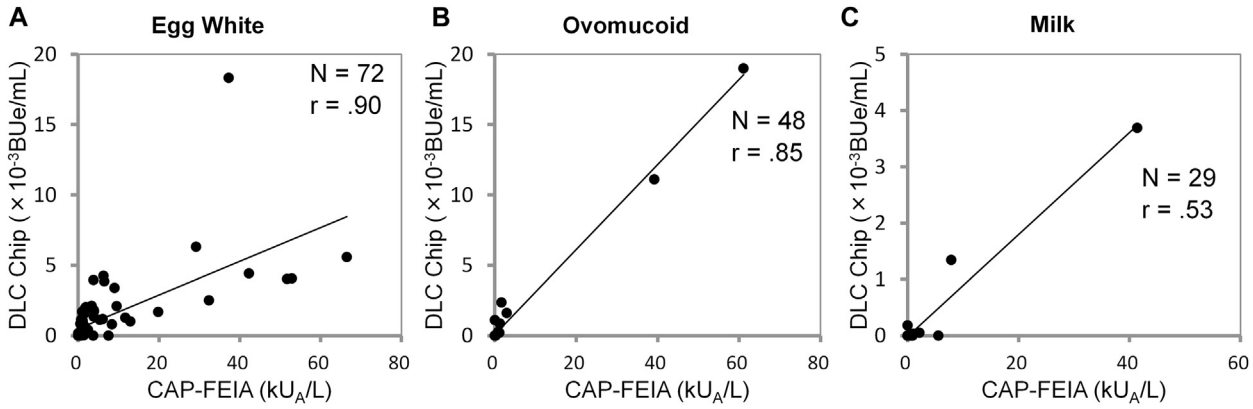

FIG E1. Correlation of allergen-specific IgE values determined by using a DLC chip system and CAP-FEIA. The values of anti-egg white **(A)**, anti-ovomucoid **(B)**, and anti-milk **(C)** IgE antibodies derived from 72, 48, and 29 infants, respectively, could be determined by using both the CAP-FEIA and DLC chip methods, and the correlations between these values obtained from the same samples were tested by means of linear regression analysis. *BUe*, Binding unit of IgE.

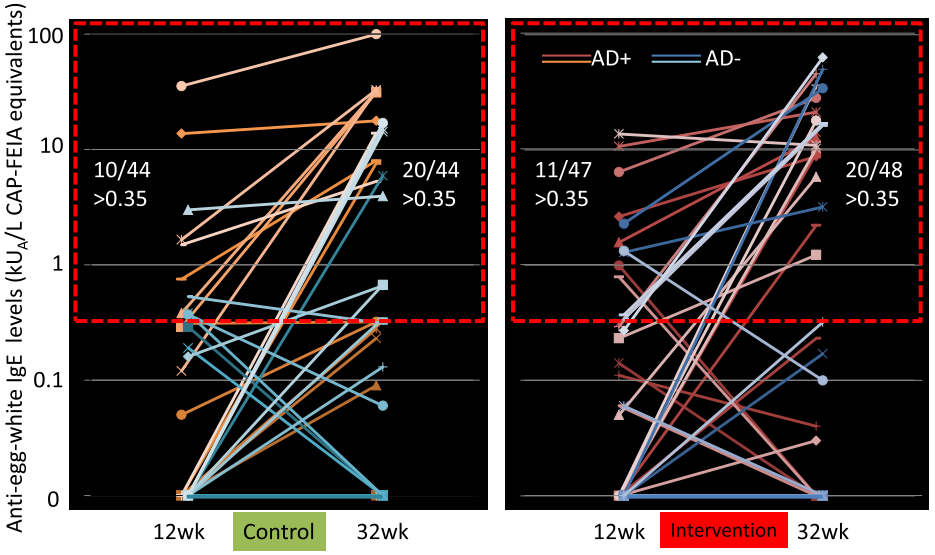

FIG E2. Allergic sensitization at weeks 12 and 32: comparison between the intervention and control groups. The serum levels of egg white–specific IgE (binding unit of IgE *[BUe]*/mL) in infants at weeks 12 and 32 were measured with a DLC chip and converted into CAP-FEIA equivalents (kU$_A$/L) by using a previously described method.[E3] Note that high correlation between these 2 data sets with the present samples was confirmed only at week 32. The values obtained from AD/eczema-positive infants are shown in warm colors, and those from AD/eczema-negative infants are shown in cold colors.

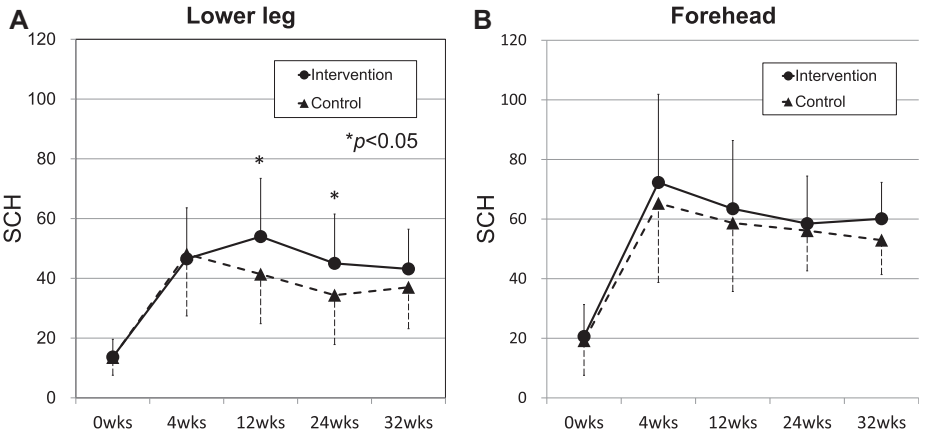

FIG E3. Stratum corneum hydration *(SCH)* change in the lower leg **(A)** and forehead **(B)** in each group. SCH values (relative impedance) on the outside of the lower leg (Fig E3, *A*) and forehead (Fig E3, *B*) were shown at baseline (week 0) and at 4, 12, 24, and 32 weeks of age. *Symbols* (*circles* and *triangles*) and *bars* stand for means and SDs. SCH values were significantly higher for the lower leg in the intervention group at 12 weeks of age compared with those in the control group ($P < .05$, ANOVA).

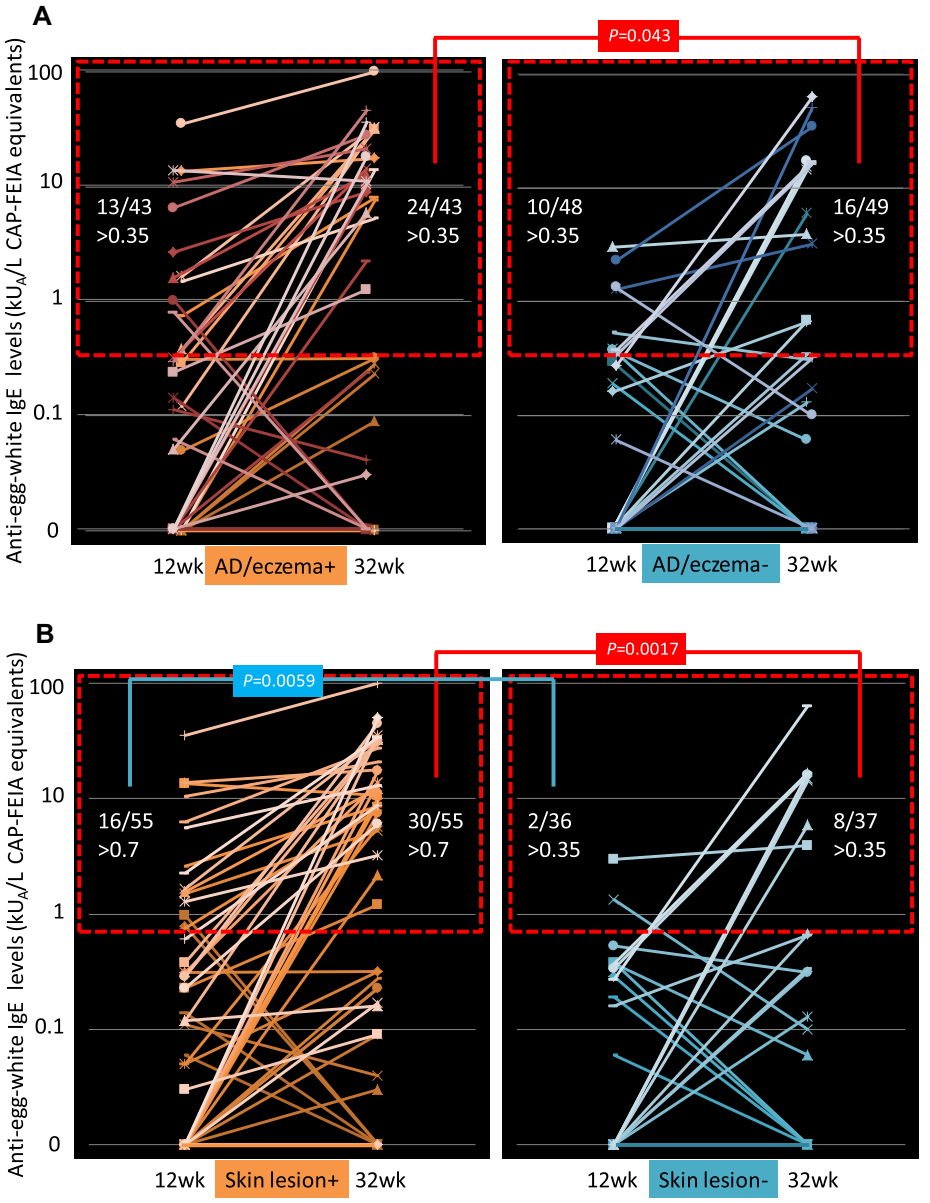

FIG E4. Allergic sensitization at weeks 12 and 32. Serum levels of egg white–specific IgE (binding unit of IgE *[BUe]*/mL) in infants at weeks 12 and 32 were measured with a DLC chip and converted into CAP-FEIA equivalents (kU_A/L). **A,** The AD/eczema-positive group had a higher proportion of infants sensitized with egg white at 0.35 kU_A/L CAP-FEIA equivalents at week 32 compared with the other group (P = .043). **B,** The skin lesion–positive group had a higher proportion of infants sensitized with egg white at 0.70 kU_A/L CAP-FEIA equivalent at week 12 (P = .0059) and week 32 (P = .0017) compared with the other group.