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Title: A randomized controlled trial of an emollient with ceramide and filaggrin-associated amino acids for the primary prevention of atopic dermatitis in high-risk infants

Running head: An emollient for primary prevention of atopic dermatitis

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Abstract

Background: Skin barrier dysfunction may precede infantile development of clinical atopic dermatitis (AD). Early life emollient therapy to enhance barrier function may prevent or modify AD development in high-risk infants.

Objectives:

- a) To determine whether daily full-body application of an emollient with ceramide and amino acids (study emollient) can reduce the cumulative AD incidence compared to standard skin care at one year of age.
- b) To evaluate the study emollient's effect on skin barrier function, natural moisturizing factor and the microbiome using non-invasive biophysical and biochemical techniques.

Methods: We performed a single center, investigator-blinded, randomized controlled trial enrolling infants at high risk for AD development determined by family history. The intervention was full-body once daily application of the study emollient. The control arm was asked to not apply full body emollient regularly and only use an emollient of their choice for dry skin. The primary outcome was the cumulative incidence of AD diagnosed at 12 months by a blinded investigator.

Results: Less than half the target sample size was enrolled (n=100, goal sample was 208) with 28% lost to follow-up. Across all clinical endpoints, a numerical trend was observed in favor of the intervention, although not statistically significant likely due to lack of power from under-enrollment. AD was diagnosed in 13.2% vs. 25.0% at 12 months ($P=0.204$) and 19.4% vs. 31.0% at two years ($P=0.296$) in intervention vs. control groups, respectively.

There were no significant differences between groups in skin barrier or microbiome assessments. While there were no serious adverse events, there were more cases of reported contact dermatitis in the intervention vs. control arms, 9.3% vs. 4.3%, respectively, however these events were not related to the study emollient and most mild in severity.

Conclusion: The observed trends suggest a protective effect of daily study emollient therapy compared to control.

Trial registration and funding: ClinicalTrials.gov Identifier: NCT01375205

Introduction

Atopic dermatitis (AD) is a highly prevalent chronic inflammatory skin condition affecting up to 10-20 percent of children in developing countries¹. The geographic variability, urban/rural gradient, and increasing global prevalence suggests environmental factors play a role in AD pathogenesis. Given the rising prevalence and significant impact the disease may have on quality of life of patients and families, several disease prevention strategies have been pursued. Allergen avoidance strategies are largely disappointing with no consistent approach emerging.² Recently the skin barrier has been a focus of intense investigation for the treatment and prevention of AD.

Skin barrier function plays an important role in the development and chronicity of AD. Defects in the gene encoding the skin barrier protein, filaggrin (FLG), predispose individuals to developing AD – a finding replicated in multiple populations around the world.³ Kelleher and colleagues found increased transepidermal water loss (TEWL) in the first days and

months of life to be the strongest predictive factor for AD development, independent of *FLG* genotype or a family history of atopic disease.⁴ These studies suggest improving skin barrier function, or reversing skin barrier dysfunction early in life may prevent the development of AD.

A pilot study by Simpson and colleagues suggested a benefit of emollient therapy when compared to the known rate of AD development in the literature.⁵ Two randomized controlled studies confirmed these initial findings and found daily emollient therapy reduced the cumulative incidence of AD up to 50%.^{6,7} Lowe and colleagues found a similar effect size using a ceramide-dominant cream, but results were not significant likely due to the small sample size (n=80).⁸ Additional studies evaluating how emollients affect infant skin physiology and AD development are needed.

The objective of this study was to evaluate the efficacy of daily emollient therapy containing FLG breakdown amino acids and a synthetic ceramide on AD development. Deficiencies in FLG and its breakdown products and ceramides are thought to underlie the barrier dysfunction in AD. We further sought to evaluate how this emollient affected skin barrier physiology using non-invasive testing of the skin barrier, the microbiome, and natural moisturizing factor (NMF) components.

Methods and Materials

Study Design

This single center, investigator blinded, randomized controlled trial recruited newborns at high-risk for the development of AD. The study was approved by the Oregon Health &

Science University (OHSU) Institutional Review Board (#00007484). The parents or guardians of participants were recruited between June 2011 to January 2014 primarily through direct face-to-face recruitment in maternal hospital wards, recruitment letters and advertisement. Inclusion criteria included: parent or guardian over age 18 with a newborn within 21 days considered at high-risk for AD development (1st degree relative with a history of AD, asthma, or allergic rhinitis) and parents/guardians of participants willing to comply with the study procedures. Participants were excluded if they were premature (born prior to 37 weeks gestational age), diagnosed with major congenital anomalies, had significant dermatitis at birth (excluding seborrheic dermatitis), had immunodeficiency disorders, or had a serious medical problem making emollient use inadvisable by increasing the risk of adverse events or inhibiting outcomes assessment. Study visits occurred at 2, 6 and 12 months. Phone calls were performed at 18 and 24 months to discuss development of AD, provide education on emollient use and supply additional product.

Randomization and Intervention

Randomization was completed via a computer-generated randomization list issued by a statistician with block sizes of 10 and concealed from the recruitment coordinator until randomization. The intervention arm was supplied with Cetaphil® Restoraderm® moisturizer and cleanser within 21 days of birth and instructed to apply the moisturizer daily to all body surfaces excluding the scalp and diaper area and use the cleanser only as needed during bathing. The key ingredients in Cetaphil® Restoraderm® moisturizer include shea butter as a lipid source, pseudoceramide-5, and two FLG breakdown products- arginine and sodium pyrrolidone carboxylic acid. No bathing frequency instructions were provided. The control arm was given no specific instructions regarding use of emollients except to use emollients

of their choice on an as needed basis. Participants in the control arm were supplied Johnson's and Johnson's Head-to-Toe® baby wash and shampoo and lotion to use as needed for dryness but were not required to use this product. Our previous studies of a similar population found these products to be commonly used on an as needed basis and represented a standard of care in this population.⁹ The ingredients and properties of the intervention and control can be found in Supplementary Table 1.

Study Visits

The study consisted of a screening visit before or after birth. If deemed eligible for enrollment, participants were enrolled within 21 days of birth and randomized. Contact was made via telephone at two weeks to address any concerns, assess usage of the moisturizer, and provide support for families. Three clinic visits were completed in at 2, 6 and 12 months, and two follow up phone calls were completed at 18 and 24 months. During these study timepoints, history of skin rashes, skin care and bathing, topical medications, antibiotic exposure, topical medications, adverse events, and moisturizer adherence were assessed. At 6 months, every infant was tested for *FLG* gene mutations via buccal saliva samples. At every study contact, skin care products were resupplied when needed according to their group allocation. The parents and guardians were encouraged to contact the study coordinators if there was a skin rash or itchy dry skin that persisted over seven days at which point, participants were asked to return to clinic for an AD evaluation during an unscheduled visit by a blinded investigator.

Outcome measures

The primary outcome was the cumulative incidence of AD at 12 months diagnosed by a blinded investigator (“Investigator-confirmed AD”). The criteria for the development of AD were the U.K. Working Party Criteria adapted to identify incident cases of AD rather than a 12-month period prevalence.¹⁰ These criteria include a continuous or intermittent itchy skin condition present for a month or more in addition to three of the following: a history of rash in the skin creases, generally dry skin, flexural dermatitis, first degree relative with hay fever or asthma, or onset younger than 2 years old. Symptoms may have been present over a shorter duration if topical anti-inflammatory therapies were deemed clinically needed by the investigator to treat symptomatic disease. Because of the large drop-out rate, a *post hoc* secondary analysis of the primary outcome was also performed using the cumulative incidence of AD diagnosed by any of the following – a blinded investigator, non-investigator physician, or provider-diagnosed AD recorded in the medical record in the first 12 and 24 months (“Any AD”). Other secondary outcomes included reported adherence with intervention and incidence of treatment-related adverse events. Every adverse event was evaluated for its relationship to the emollient as “none,” “unlikely,” “possible,” “probable,” and “highly probable.” Participants were withdrawn if they withdrew their consent or were unwilling to continue with the study visits or telephone contacts.

FLG analysis

A buccal mucosa sample was collected at 6 months by saliva sponge. Samples were collected, de-identified, isolated at the Oregon Clinical and Translational Research Institute Core Lab and then evaluated in the lab of Irwin MacLean at the University of Dundee. Following previously published methods, genetic analysis was limited to the four most

common mutations linked to AD in European populations, which include 2282del4, R2447X, R501X, and S3247X.⁸

Skin Barrier Substudy

A substudy (n=56) enrolled participants to evaluate differences in skin barrier function including TEWL, skin hydration, skin pH, skin microbiome assessment, skin lipidomic profile, stratum corneum cohesion, and assessment of NMF content using tape stripping. TEWL, skin electrical capacitance and skin pH were measured according to their respective manufacturer's instructions at 2, 6, and 12 months visits. TEWL was measured once with the Aquaflux TEWL unit (Biox, London) for approximately thirty seconds on the dorsal forearm. Skin electrical capacitance, a measure of skin hydration, was evaluated in duplicates from the dorsal forearm by a corneometer CM 820 (Courage and Kazaka, Cologne, Germany). Skin pH was measured in duplicates using a pH probe in one second measurements (Courage and Kazka, Cologne, Germany).

Samples for microbiome analysis were collected with a cotton tipped applicator at 2, 6 and 12 months visits at six anatomical sites, including samples from the extensor forearm, medial cheek, volar forearm, antecubital fossa, nares, and stool sample. A control swab was also collected. These samples were analyzed through 16S rRNA gene phylotyping through Diversigen (Houston, Texas). Briefly, the Illumina MiSeq platform sequenced the 16S rRNA gene amplicons in the target V4 variable region. Within 180 samples, 753 operational taxonomic units (OTUs) were identified using read data with UCLUST binning and the Silva database with a median of 6,844 per sample and a project total of 1,007,776 mapped reads. The data was rarefied to 1,089 reads per sample for diversity analysis which excluded

45 samples, leaving 135 samples for analysis. Observed species, Chao1 and Shannon metrics were used for alpha diversity analysis using PhyloSeq and Kruskal-Wallis significance testing.

To determine stratum corneum integrity, ten D-Squame tape 20mm strips (CuDerm Corporation, Dallas, Texas) were collected from the dorsal forearm with 225 gf/cm² of pressure applied to each strip utilizing the D-Squame D500 instrument (CuDerm Corporation, Dallas, Texas) to ensure equal pressure for each participant. These were used to determine stratum corneum cohesion by CuDerm SquameScan 850A (CuDerm Corporation, Dallas, Texas) by extrapolating the absorbance of the strips to the amount protein density. At 2 and 6 months, three consecutive tapes from the volar forearm skin site were collected for NMF quantification performed by ammonia extraction and subsequent analysis by high performance liquid chromatography using established methods within the laboratory of Sanja Kezic.¹¹ For the analysis, second and third tapes have been analyzed; for data analysis the mean values from both tapes have been taken.

Sample Size

A total of 90 participants in each group would provide the 80% power needed to detect differences between proportions of eczema development at 20% for intervention and 40% for control at the 95% confidence level. A drop-out rate of 15% was predicted, resulting in 208 total required participants.

Data analysis

The main clinical end point was the 12 month cumulative incidence of investigator-confirmed AD. Infants were classified as having AD if either the principal investigator or another medically qualified study-trained investigator diagnosed AD development on examination during the 12-month intervention period. The primary clinical end point analysis was conducted on an intent-to-treat basis using a complete case approach and statistical analysis completed through Chi-square test of two proportions. The substudy was analyzed using independent t-tests between study arms at each time point with the assumption of equal variance and an alpha of 0.05.

Results

The study enrolled less than half of the target sample size due to slow enrollment and eventual termination of funding. Of the 100 participants enrolled in the study, 54 were in the intervention vs. 46 in the control. Six participants were incorrectly randomized to the intervention and two to the control explaining the imbalance. These mistakes in randomization displayed no pattern and were the results of acknowledged study coordinator error. Each participant was analyzed according to the group they were ultimately randomized to by the research coordinator. Lost to follow-up was 28.0% for the primary endpoint. The study flow diagram can be found in **Fig. 1**. The baseline characteristics of the participants were balanced between groups and can be found in **Table**

1.

The cumulative incidence of investigator-confirmed AD at 12 months (primary outcome) was 13.2% vs. 25.0% ($P=0.204$) and investigator-confirmed AD at two years was 19.4% vs. 31.0% ($P=0.296$) in intervention vs. control groups, respectively. A sensitivity analysis of AD diagnosed by any provider (any AD) found AD in 14.6% vs. 27.8% at one year ($P=0.156$) and 24.2% vs. 39.4% at 24 months ($P=0.186$) in intervention vs. control groups, respectively. The cumulative incidence of investigator-confirmed and any AD can be seen in **Fig. 2**. Because only 5 participants had an *FLG* mutation, we were unable to determine whether the emollient effect was modified by *FLG* status.

Adherence

High emollient use was defined as applying the intervention or control emollient five or more days per week. At 2, 6 and 12 months, the intervention group had 87%, 72.4% and 66.7% high adherence, respectively, vs. 45.2%, 45.8% and 45.5% in the control group, respectively. At 18 and 24 months, this high adherence decreased in the intervention group to 52.2% and 40.0%, respectively, and remained about the same in the control group at 53.3% and 33.3%, respectively. The adherence in the intervention vs. control group is shown in **Fig. 3**.

Substudy

(a) TEWL, Capacitance, and pH

A total of 56 participants were enrolled into the substudy, 32 from the intervention and 24 from the control. Results for TEWL, average capacitance and skin pH are shown in **Fig. 4(a)**, **Fig. 4(b)**, and **Fig. 4(c)**, respectively. There were no significant differences between intervention vs. control at 2, 6, and 12 months for TEWL ($P=0.427$, $P=0.623$, and $P=0.736$,

respectively) or average capacitance ($P=0.722$, $P=0.906$, and $P=0.406$, respectively). There was a significant increase of pH at 2 months ($P=0.0437$) in the intervention arm (mean 5.46, SD 0.425) vs. the control (mean 4.98, SD 0.393), while there was no significant difference at 6 and 12 months ($P=0.767$ and $P=0.411$, respectively).

(b) Stratum corneum cohesion and NMF content

The stratum corneum cohesion, assessed by average protein density on 10 tape-strips, is shown in **Fig. 4(d)**. There was no significant difference between intervention vs. control at 2 and 6 months ($P=0.955$ and $P=0.348$, respectively). The intervention vs. control groups had similar NMF at 2 months (0.43 vs. 0.44 nmol/ μ g protein, respectively) ($P=0.768$ between groups) and 6 months (0.40 vs. 0.43 nmol/ μ g protein, respectively) ($P=0.595$ between groups). These data can be found in **Supplementary Table 2 and Supplementary Figure 1**.

(c) Microbiome alpha diversity

Microbiome alpha diversity analysis at 6 months is found in **Fig. 5**. We restricted the first analysis to only infants who did not develop AD by 6 months of age to determine the effect of study emollient therapy on the microbiome. Between intervention and control groups, in **Fig. 5** there were no significant differences in the alpha diversity analyses (Operational Taxonomic Units (OTUs) Chao1, and Shannon) of the 6-month samples in participants without AD, indicating similar richness, diversity and evenness. Additionally, there were no significant differences in the alpha analysis of high and low adherence to the study moisturizer (data not shown).

Adverse Events

Skin related adverse events are found in **Table 2**. The incidence over the course of the study in intervention vs. control groups for bacterial skin infections is 7.4% vs. 6.5%, respectively and hypersensitivity reactions is 14.8% vs, 8.7%, respectively. Hypersensitivity reactions included irritant contact dermatitis (ICD) and urticaria. For ICD, identifiable causes included sunscreen or clothing tags, and for urticaria identifiable causes included pollen, food allergies, and antibiotics. However, some reactions did not have an identifiable cause. No participant needed to stop applying the intervention or control due to hypersensitivity reactions. All hypersensitivity reactions were evaluated as having either “no” relationship or “unlikely” relationships to the emollient. There was a group of unspecified transient rashes that did not meet criteria for AD or another diagnosis. They were either parent-reported or observed from the chart diagnosed by another provider, however, they were not diagnosed as AD and did not require stopping the study arm emollient. There were no serious adverse events in either group.

Discussion

While this study found no statistically significant effect of the ceramide and amino acid-containing emollient on AD prevention, the numerical trend suggests regular full-body Cetaphil Restoraderm® use in high-risk infants protects against AD development. The effect size for this study was similar to effect sizes previously reported by Simpson and colleagues and Horimukai and colleagues in studies of emollients for AD prevention.^{1-4,6,7} Although we observed the anticipated effect size, the under-enrollment and higher than expected drop-out rate led to an underpowered study that lacked statistical significance. We hope these

data can be included in future meta-analyses to further characterize the effects of emollients on AD prevention.

One objective for this study was to better understand the role of emollient therapy on infantile skin. Surprisingly, we could not detect significant differences in the emollient group compared to the control group with regards to microbiome diversity, stratum corneum integrity, NMF content, basal TEWL, or stratum corneum hydration. The lack of differences in skin barrier biophysical measurements may be due to the heavy use of emollient in the control group (45% reported regular use) which may have blunted differential effects between the two groups. Previous studies have shown high emollient use in this part of the U.S., thus we planned on testing Restoraderm against the local standard of care.⁹ We hypothesized that the formulation of Restoraderm that includes FLG breakdown products would be superior to more intermittent use of the control moisturizer with fewer lipids. While there were consistent trends favoring Restoraderm, it is possible that the use of the control emollient provided some protective effect to the control group as well. We previously found AD rates in a high-risk population to be closer to 40% within the first year of life⁶ without emollient use, whereas the control group AD prevalence in this study was 25%.

Another explanation for the lack of differences in the skin barrier sub-studies may be that the biological effects of emollients are not completely explained by improvements in biophysical skin properties. Perhaps emollients exert their protective effects through not only skin barrier properties, but act via yet to be defined mechanisms, such as anti-inflammatory effects. While all of the adverse events were thought to have an “unlikely” or

“no” relationship to the treatment, numerically more babies developed mild transient rashes in the intervention group that we are not able to explain but could be due to mild and transient irritancy from the Cetaphil Restoraderm product. However, no participant needed to discontinue the intervention due to this adverse event. Therefore, as seen in other studies, emollient use from early in life appears to be a safe intervention. More studies are needed and would help determine the effects of the active ingredients and breakdown products on skin barrier function.

The lack of an effect on the microbiome is in contrast to recent findings that emollient therapy appears to diversify the microbiome in infants.^{12,13} It is possible that different emollients have varying effects on the microbiome. Myles and colleagues found certain preservatives and emollients may affect pathogenic and protective strains of bacteria to varying degrees.¹³ Thus, emollients may all affect the microbiome in heterogeneous ways depending on ingredients and formulation.

Future studies are needed with larger sample sizes and in various settings to better understand a skin barrier approach to AD prevention. A more definitive study, the Barrier Enhancement for Eczema Prevention study, is near completion in a high risk population in the U.K.(ISRCTN21528841) with a planned enrollment of 1400 babies. The Community-based Assessment of Skin Care, Allergies and Eczema trial recently opened to enrollment designed to assess barrier protection in a general population of babies with a planned enrollment of 1200 (NCT03409367). Additional information is needed whether bland petrolatum-based emollients are as effective in AD prevention as emollients with certain ceramide ratios or other additives. This study was not designed to evaluate whether adding

the filaggrin breakdown products to the formulation improves the barrier compared to a similar formulation without these additives. Further comparative data regarding clinical endpoints in addition to skin barrier assessments are required to fully understand the added value of more costly emollients especially when developing a cost-effective global approach to AD prevention.

Limitations of this study include under-enrollment – a well-known problem plaguing clinic research.¹⁴ AD prevention trials may be particularly difficult to enroll and maintain given the difficulty in recruiting and maintaining young, busy parents in a cohort evaluating long-term study endpoints. Previous studies found parents are already using emollients on infants,¹⁵ and thus may not be interested in participating in a study where they could be randomized to more restricted emollient use. Lowe enrolled only 5% of the target population in one pilot trial of emollients for AD.⁸ Some possible strategies to improve the efficiency of prevention trials in the future could include recruiting patients after their hospital stay (rather than during) and using a pragmatic trial design that mimics the real-world and reduces the study procedure burden on families. This study also did not score the severity of AD or type of steroids initiated upon diagnosis, which would be beneficial to know if there were a difference in severity upon diagnosis.

In summary, although this trial failed to meet enrollment goals by 50%, results suggest emollient therapy likely reduces the risk of AD development. The mechanisms are currently unclear and there were no observable changes in microbial or biophysical barrier measurements with emollient therapy compared to standard of care to explain the

protective effects of emollients. Larger studies with various populations using various emollients are needed.

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Tables, Legends and Figures

Table 1. Baseline Characteristics

Table 2. Skin Related Adverse Events

Figure 1. Flow diagram.

The primary outcome was the cumulative incidence of atopic dermatitis defined as atopic dermatitis (AD) diagnosed by an investigator in the first 12 or 24 months of life (Invest-confirmed AD). A secondary outcome was the cumulative incidence of atopic dermatitis defined as AD diagnosed by an investigator and/or an outside pediatrician or chart review within 12 or 24 months (Any AD). Participants diagnosed with AD by sources not including the investigator were excluded from Invest-confirmed AD analysis. Participants were withdrawn from the study if they withdrew their consent or unwilling to continue with the study visits or contacts.

*Enrollment goal was 208. Enrollment rate was lower than expected and the study was halted after 100 participants were enrolled.

Figure 2. Cumulative atopic dermatitis diagnosis

The incidence of the primary outcome, defined as investigator-confirmed AD, and the secondary outcome, defined as any AD diagnosed by parental report, chart review, pediatrician diagnosed, and/or a study investigator (Any AD) at 12 and 24 months. Both outcomes did not reach statistical significance, but both had trends of decreased AD incidence in the intervention vs. the control.

Figure 3. Percent of participants in intervention vs. control at each time point with no, low, moderate and high adherence.

The percent of participants with low, moderate or high adherence, defined as having the study group applied 1-2, 3-4, or at least 5 times a week, respectively, are demonstrated in the control vs.

intervention arms. The adherence is high during the being of the study and matches the control at the 18 months and 24 month time point.

Figure 4. Barrier function. This was assessed by (a) the transepidermal water loss (TEWL) [g/m²h] and (b) the average of two capacitance values [arbitrary units] in the intervention vs. control at 2 months (2M), 6 months (6M) and 12 months (12M). There was no significant difference between groups. The skin (c) pH and (d) average protein density [mg/cm²] over 10 tape strips (an indicator of stratum corneum cohesion) are also shown. There was no significant difference between study arms for pH and average protein density, except for pH at 2 months which showed a statistically significant increase in mean pH in the intervention vs. control of 5.5 vs. 5.2 (P=0.0437, independent t-test).

Figure 5. Microbiome alpha diversity.

Between the intervention and control groups, there were no significant differences in the alpha analysis (Operational Taxonomic Units (OTUs) Chao1, and Shannon) in the 6-month samples in participants without atopic dermatitis (AD), indicating similar richness, diversity and evenness of the microbiota. Additionally, there were no significant differences in the alpha analysis of high and low adherence to the study moisturizer at 6 months (not shown).

Supplementary Table 1. Ingredients

Supplementary Table 2. Substudy Results

Supplementary Figure 1. Average Total NMF at 2 and 6 months between intervention and control. There was no significant difference between the average NMF (nmol/ug protein) for the intervention and control at 2 and 6 months. The error bars are the 95% Confidence Intervals.

Table 1. Baseline Characteristics

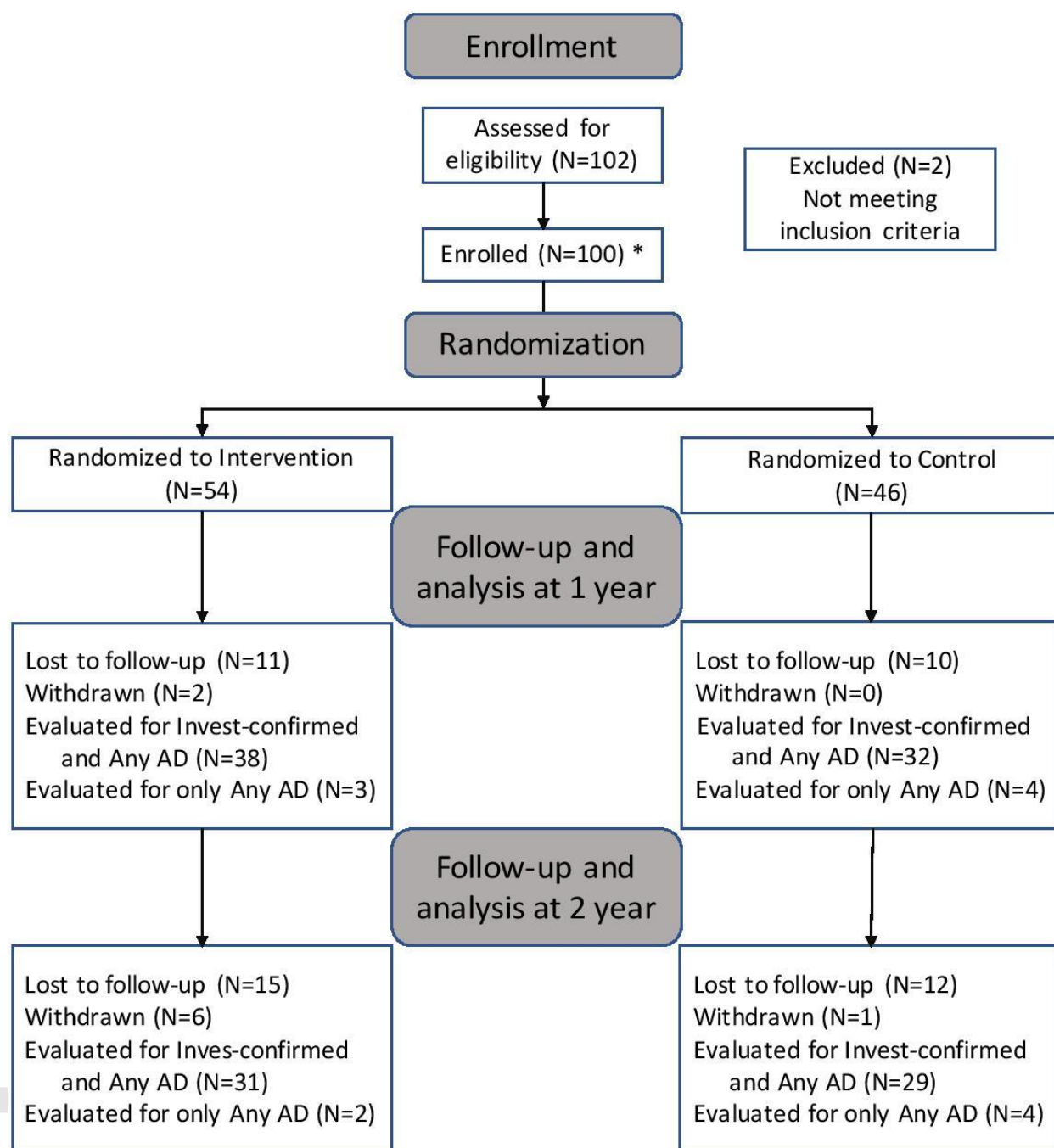
Characteristic		Intervention N=54	Control N=46	Total N=100
Female Gender (%)		24/54 (44.4)	25/46 (54.3)	49/100 (49.0)
Hispanic Ethnicity (%)		12/54 (22.2)	10/46 (21.7)	22/100 (22.0)
English Language (%)		48/54 (88.9)	44/46 (95.7)	92/100 (92.0)
Filaggrin mutation (%)		3/256 (12.015.4)	2/31 (6.5)	56/567 (8.910.5)
Dry skin (%)		10/53 (18.9)	9/46 (19.6)	19/99 (19.2)
Owens a pet in first six months (%)		23/35 (65.7)	21/35 (60.0)	44/70 (62.9)
Cesarean Section Delivery (%)		7/54 (13.0)	13/46 (28.2)	20/100 (20.0)
Previous bathing (%)	None	14/54 (25.9)	8/46 (17.4)	22/100 (22.0)
	Water Only	7/54 (13.0)	5/46 (10.9)	12/100 (12.0)
	Cleanser/Unspecified	33/54 (61.1)	33/46 (71.7)	66/100 (66.0)
Previous shampoo use (%)	Yes	9/54 (16.7)	12/46 (26.1)	21/100 (21.0)
	No	45/54 (83.3)	34/46 (73.9)	79/100 (79.0)
Median DOL at first application of study arm		11 (N=36)	11 (N=33)	11 (N=69)
<i>DOL</i> , Day of life				

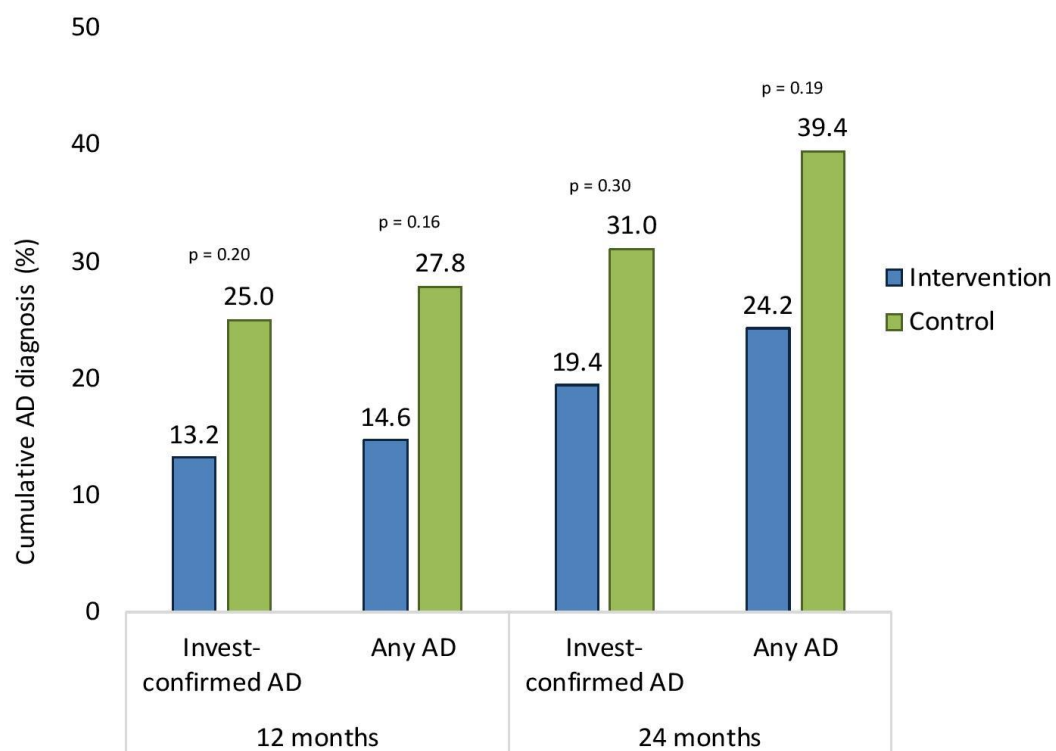
Table 2. Skin Related Adverse Events

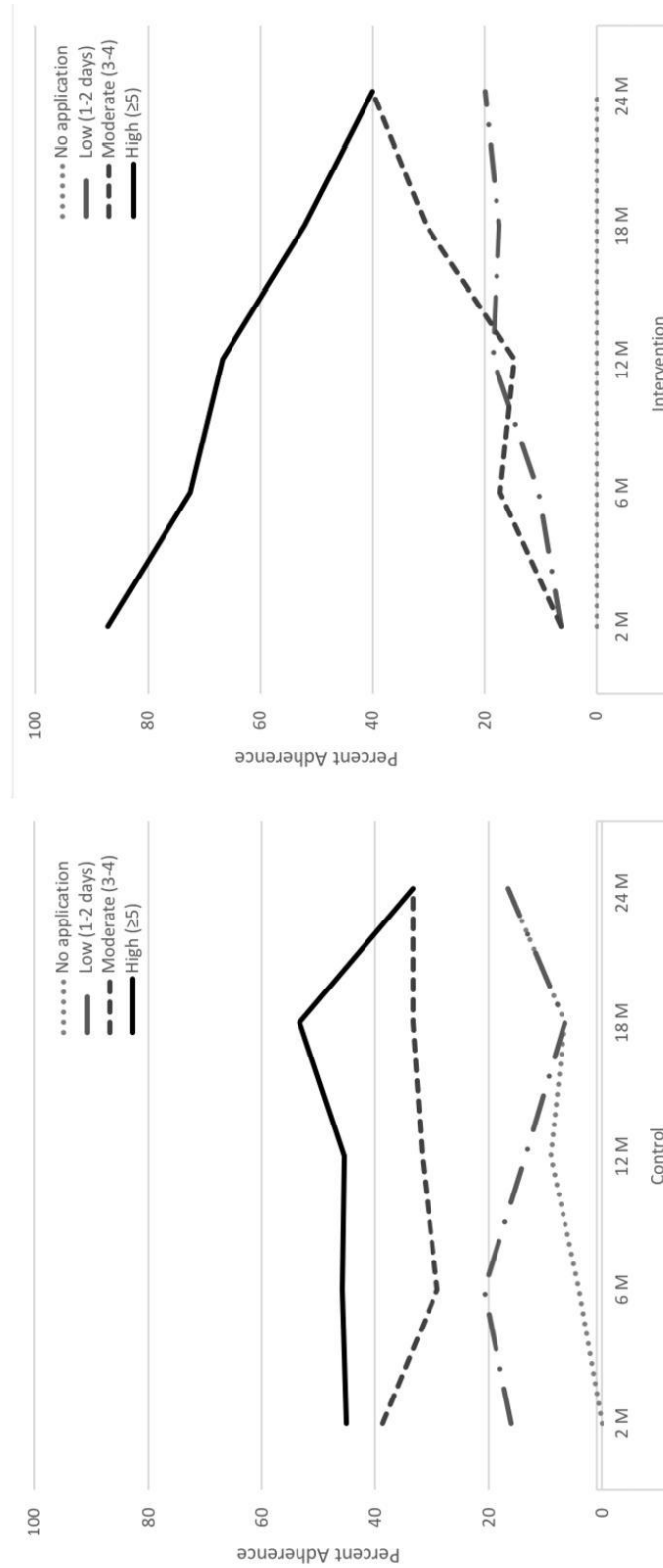
	Intervention N=54	Control N=46	Total N=100
Insect Bite	1/54 (1.8%)	0/46 (0.0%)	1/100 (1.0%)
Birthmark vs. vitiligo	0/54 (0.0%)	1/46 (2.2%)	1/100 (1.0%)
Milia	0/54 (0.0%)	1/46 (2.2%)	1/100 (1.0%)
Baby acne	3/54 (5.6%)	1/46 (2.2%)	4/100 (4.0%)
Erythema toxicum	1/54 (1.8%)	1/46 (2.2%)	2/100 (2.0%)
Viral exanthems	5/54 (9.3%)	2/46 (4.3%)	7/100 (7.0%)
Heat rash/miliaria	4/54 (7.4%)	3/46 (6.5%)	7/100 (7.0%)
Candidiasis	6/54 (11.1%)	8/46 (17.4%)	14/100 (14.0%)
Diaper dermatitis	7/54 (13.0%)	5/46 (10.9%)	12/100 (12.0%)
Seborrheic dermatitis	9/54 (16.7%)	8/46 (17.4%)	17/100 (17.0%)
Bacterial skin infection	4/54 (7.4%)	3/46 (6.5%)	7/100 (7.0%)
Tinea Corporis	0/54 (0%)	1/46 (2.2%)	1/100 (1.0%)
Unspecified transient rashes*	9/54 (16.7%)	3/46 (6.5%)	12/100 (12.0%)
Rash to vaccination/drug rash	2/54 (3.7%)	0/46 (0.0%)	2/100 (2.0%)
Hypersensitivity	8/54 (14.8%)	4/46 (8.7%)	12/100 (12.0%)
ICD	5/54 (9.3%)	2/46 (4.3%)	7/100 (7.0%)
Urticarial rash	3/54 (5.6%)	2/46 (4.3%)	5/100 (5.0%)

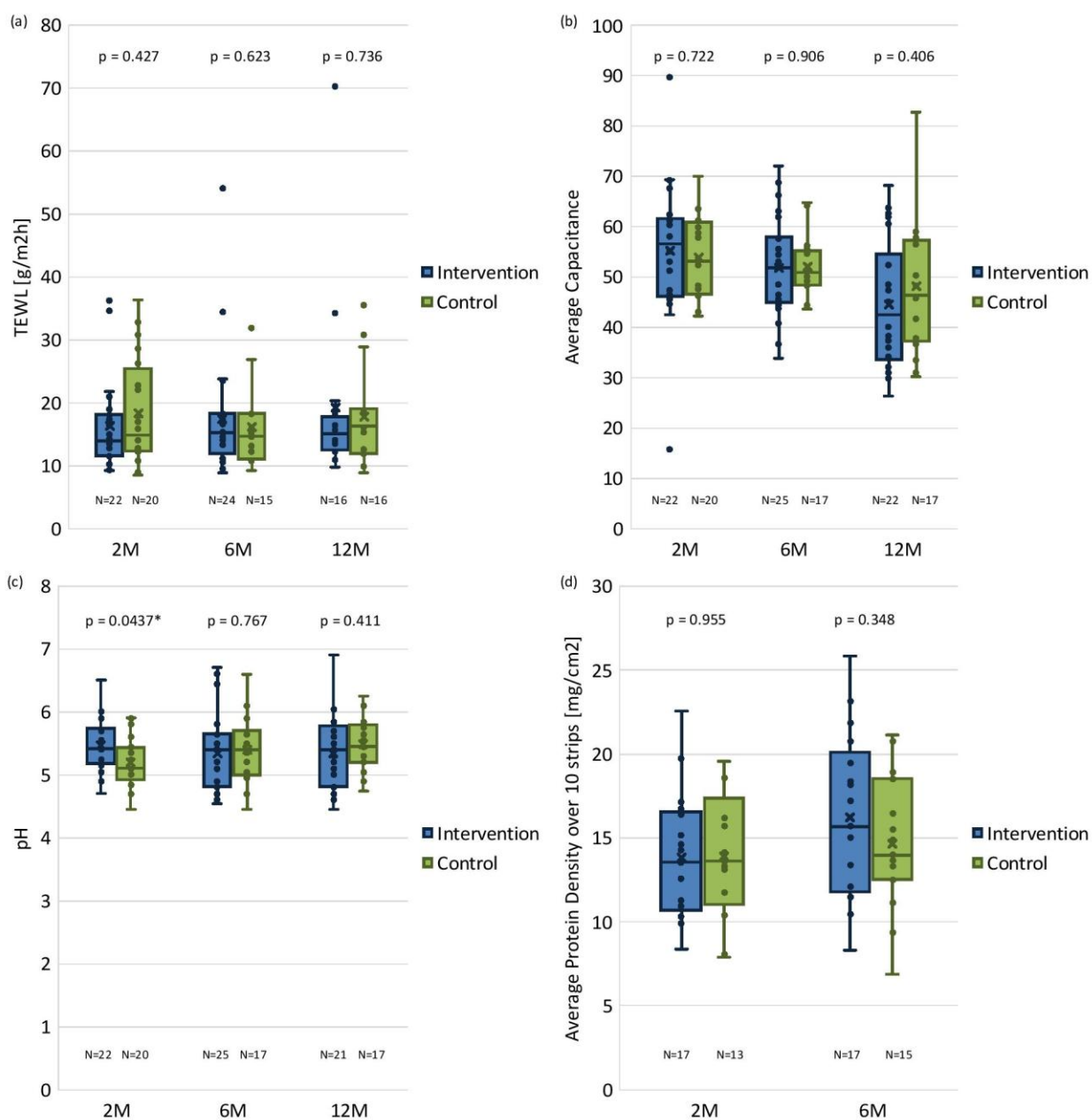
* Includes rashes not meeting criteria for atopic dermatitis that were not otherwise characterized

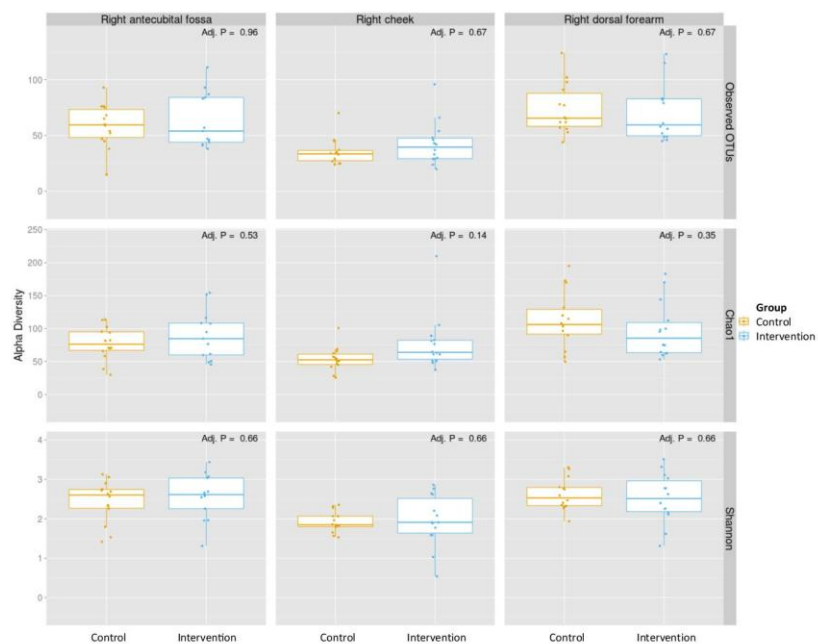
ICD, Irritant contact dermatitis

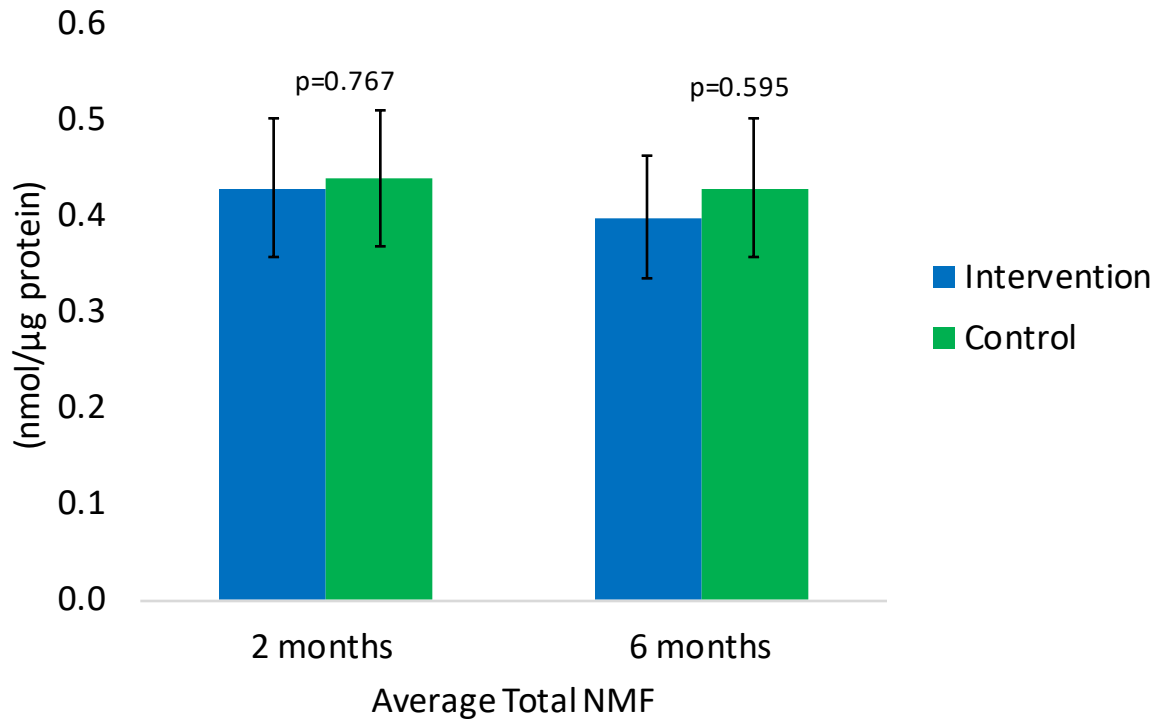












Supplementary Table 1. Ingredients list

		Cetaphil Restoraderm ® Gentle Body Wash	Cetaphil Restoraderm ® Gentle Body Moisturizer	Johnson's Head-to-Toe ® Wash and Shampoo	Johnson's Head-to-Toe ® Lotion
pH		5.5-6.7	5.5-5.9	5.0-5.6	5.1-5.9
Ingredients	Vehicle	Water	Water	Water	Water
	Emollients	Butyrospermum parkii, helianthus annuus (sunflower) seed oil, caprylyl glycol, 1,2-hexanediol	Caprylic/capric triglyceride, helianthus annuus (sunflower) seed oil, pentylene glycol, butyrospermum parkii, cyclopentasiloxane, caprylyl glycol, dimethiconol, cetyl alcohol=		Hydrogenated Palm Glycerides
	Ceramide		Hydroxypalmitoyl sphinganine (ceramide; moisturizer, barrier repair)		
	Surfactant	Sodium trideceth sulfate, sodium lauroamphoacetate, sodium chloride, cocamide MEA		Cocamidopropyl Betaine*, PEG-80 Sorbitan Laurate, , Sodium Methyl Cocoyl Taurate*, Decyl Glucoside	
	Skin conditioner/humectant	Butyrospermum parkii (shea butter; emollient), helianthus annuus (sunflower) seed oil, glycerin, sodium PCA, allantoin, guar hydroxypropyltrimonium chloride	Glycerin, pentylene glycol, butyrospermum parkii, sorbitol, hydroxypalmitoyl sphinganine, niacinamide (Vitamin B3, skin conditioner), allantoin, panthenol (Vitamin B5, moisturizer, skin conditioner), arginine, sodium PCA, caprylyl glycol, sodium hyaluronate	Glycerin, Ethylhexylglycerin	Glycerin, Cocoglycerides, Ethylhexylglycerin, p-Anisic Acid
	pH adjuster	Citric Acid	Citric Acid	Citric Acid	Sodium Hydroxide
	Filaggrin breakdown products	Sodium PCA, arginine	Sodium PCA, arginine		
	Vitamins	Niacinamide (Vitamin B3,smoothing agent), tocopheryl acetate (Vitamin E, antioxidant)	Tocopheryl acetate (Vitamin E, antioxidant), niacinamide (Vitamin B3, skin conditioner), panthenol (Vitamin B5, moisturizer, skin conditioner)		
	Preservatives / stabilizers	Potassium sorbate	Sodium polyacrylate	Phenoxyethanol, Sodium Benzoate	Cetyl Alcohol, Phenoxyethanol
	Chelate	Disodium EDTA, disodium EDTA	Disodium EDTA		Disodium EDTA
	Emulsifier		Cetearyl alcohol, behenyl alcohol, glyceryl stearate, disodium ethylene dicocamide PEG-15 disulfate, glyceryl stearate citrate, cetareth-20		Potassium Cetyl Phosphate
	Thickener			PEG-150 Pentaerythrityl Tetrastearate, PPG-2 Hydroxyethyl Cocamide	

		Cetaphil Restoraderm ® Gentle Body Wash	Cetaphil Restoraderm ® Gentle Body Moisturizer	Johnson's Head-to-Toe ® Wash and Shampoo	Johnson's Head-to-Toe ® Lotion
	Fragrance			Dipropylene Glycol, Hedione, Florosa, Hexyl Salicylate, Ethylene Brassylate	Dipropylene Glycol, Methyl Dihydrojasmonate
	Absorbent				Zea Mays (Corn) Starch

Supplementary Table 2. Substudy Results

Substudy	2 month			6 month			12 month		
	Control	Intervention	P value	Control	Intervention	P value	Control	Intervention	P value
TEWL [g/m2h]									
Mean	18.265	16.316	0.427	16.075	17.449	0.623	17.745	19.166	0.736
SD	8.587	7.123		6.176	9.516		7.817	14.744	
Average Capacitance [arbitrary units]									
Mean	53.935	55.214	0.722	52.103	51.786	0.906	48.144	44.625	0.406
SD	8.154	13.954		6.062	9.782		13.212	12.781	
pH									
Mean	5.200	5.464	0.044*	5.400	5.346	0.766	5.494	5.352	0.411
SD	0.393	0.425		0.526	0.604		0.410	0.597	
Average Protein Density (over 10 strips) [mg/cm2]									
Mean	13.927	13.846	0.955	14.652	16.195	0.348			
SD	3.812	3.795		4.052	4.975				
NMF [nmol/μg protein]									
Mean	0.441	0.425	0.768	0.431	0.405	0.595			
SD	0.206	0.220		0.185	0.211				

- Statistically significant (P<0.05)