EFFECTS OF SUPPLEMENT CONTAINING GLUCOSYLCERAMIDE EXTRACTED FROM PINEAPPLE WITH HYALURONIC ACID ON SKIN CONDITIONS IN HEALTHY JAPANESE WITH DRY SKIN AND ITCHY SENSATION

Masatomo NAJIMA[1)], Akinobu MIYATA[2)] and Keiichi MAGOSHI[3)]

1) JACTA (Japan Clinical Trial Association)
2) Nihonbashi M's Clinic
3) ATOPY RESCUE CO. LTD.

Abstract

Background: Ceramide is an essential structural component of cell membranes, and has an important role of skin barrier function. We examined to evaluate the efficacy and safety of dietary glucosylceramide extracted from pineapple with hyaluronic acid on skin conditions.
Methods: A randomized, double-blind, placebo-controlled trial was conducted on 40 middle aged healthy subjects with dry skin and itchy sensations. They were assigned to receive either active or placebo for 12 weeks. The efficacy was evaluated using the subjective assessment, stratum corneum hydration and IgE. To evaluate safety, blood biochemical and urine parameters were examined, and adverse events were investigated by questionnaire.
Results: A total of 34 subjects completed the study where efficacy and safety were evaluated. In self-evaluated skin properties, skin conditions including itching, dryness and facial skin conditions significantly improved the active group. SCH (stratum corneum hydration) also significantly increased the active group compared to the placebo group. There was neither an adverse event nor a problematic change in blood biochemical and urine parameters.
Conclusion: These results suggest that the ingestion of glucosylceramide with hyaluronic acid has the potential to improve skin conditions safely.

Key words: pineapple glucosylceramide, hyaluronic acid, atopic skin, skin enhancement

INTRODUCTION

It is known that skin conditions are influenced by various factors including ultraviolet, physiological conditions, aging and dietary habits[1)2)]. To maintain healthy skin is important to keep one's quality of life (QOL) well. Numerous lamella structure films are formed in the horny cell in the outermost layer of skin, and it is an indispensable perspiration barrier for humans[3)]. Fifty percent of the lamella is composed of ceramide (N-acylsphingosine)[4)] which decreases with aging. Therefore it may be said that ceramide is one of the most important factors to maintain youthfulness of the skin. Glucosylceramide is a lipid which exists in many animals and plants and is combined with the glucose and ceramide. Dietary glucosylceramide is absorbed as a sphingolipid from the intestines[5)]. It is suggested that ingestion of sphingolipid enhances ceramide production and improves the skin barrier function through enhancing cornified envelope formulation and tight junction[6)-8)]. The glucosylceramide extracted from pineapple (GP) is used as a functional food which is expected to have a moisturizing effect. Past reports showed GP suppressed water vapor transpiring from the skin surface, keeping the skin's moisture content[9)]. Also, it was observed that the ingestion of hyaluronic acid improved dry skin conditions[10)-12)]. In this study, we examined the effect of GP with hyaluronic acid on skin conditions based on self-evaluation and objective evaluation in Japanese healthy volunteers with dry skin and itchy sensations.

MATERIAL AND METHOD

Subjects

Healthy subjects with dry skin and itchy sensations participated, and only subjects who met all inclusion criteria and did not meet any exclusion criteria were enrolled in this study. The inclusion criteria were as follows; (1) aged between 35 and 59 years, (2) individuals with anxiety about the dryness of their skin, and (3) individuals with mild perennial allergic rhinitis. The

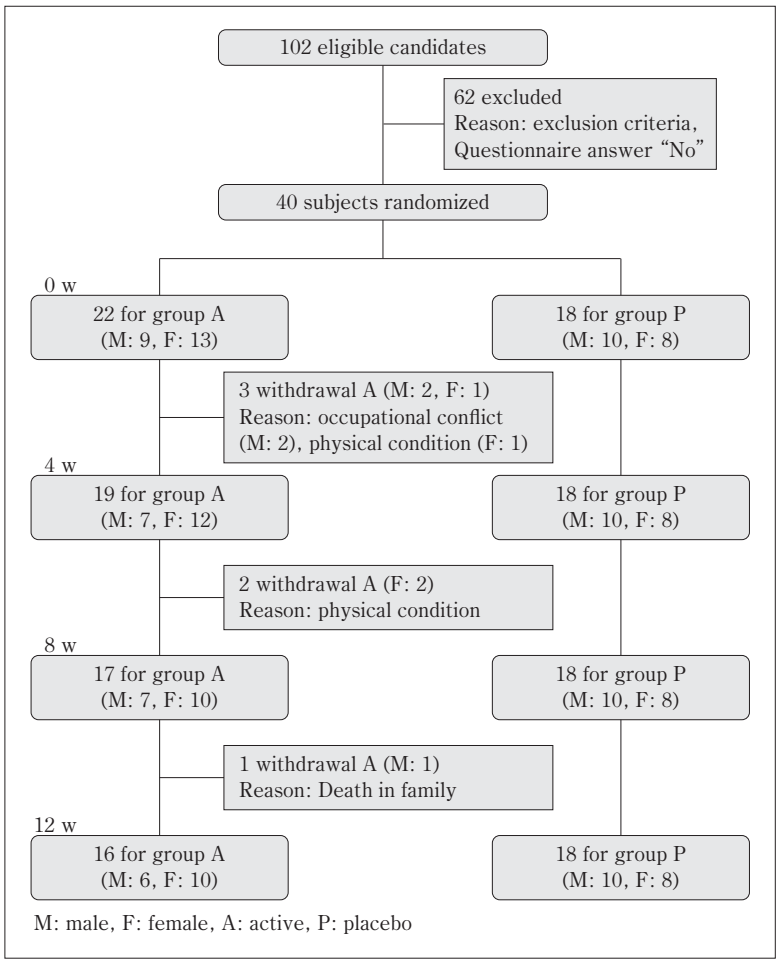

Fig. 1 Subject's flow chart

Table 1 Subject's demographics

Item		Group A	Group P
Number of subjects	Total	16	18
	Male	6	10
	Female	10	8
Age (year, Mean ± SD)	Total	45.2 ± 7.4	47.7 ± 5.4
	Male	46.3 ± 7.6	48.4 ± 4.6
	Female	44.5 ± 7.6	46.8 ± 4.6

exclusion criteria were as follows; (1) individuals undergoing treatment of skin or allergy, (2) individuals with taking medication including herbal medicines, (3) individuals with pollen allergy, (4) individuals who are pregnant, nursing or likely to become pregnant during the study, and (5) individuals considered unsuitable to participate by the investigator. All subjects were enrolled via monitor bank of CROee Inc.(Tokyo). This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the institutional review board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all subjects. This study was funded by ATOPY RESCUE CO. LTD. (Kyoto).

Study design

A randomized, placebo-controlled, double-blind study was conducted to evaluate efficacy and safety of GP with hyaluronic acid on skin conditions at two centers (OZ clinic, Tokyo and JACTA, Tokyo). The study period was 12 weeks, from September 26th to December 19th, 2015. All eligible subjects were assigned based on a random number table to one of the masked products and randomized to active group (group A: 22) and the placebo group (group P: 18). The tablets were prepared by

Table 2　Chronological change in scores of questionnaire ①

Questionnaire		Time points	Scores	
			Group A (n = 16)	Group P (n = 18)
Current skin symptoms	Itching	Baseline	0.6 ± 0.7	0.8 ± 0.8
		Week 4	0.4 ± 0.5	0.8 ± 0.7
		Week 8	0.4 ± 0.6	0.8 ± 0.7
		Week 12	0.2 ± 0.4 *	0.9 ± 0.8
		⊿ 0-4	−0.3 ± 0.6	0.0 ± 0.6
		⊿ 0-8	−0.2 ± 0.7	0.1 ± 0.5
		⊿ 0-12	−0.4 ± 0.6	0.1 ± 0.8 #
	Soreness	Baseline	0.8 ± 0.9	0.5 ± 0.8
		Week 4	0.3 ± 0.5 †	0.4 ± 0.8
		Week 8	0.4 ± 0.8	0.5 ± 0.8
		Week 12	0.4 ± 0.8	0.6 ± 0.9
		⊿ 0-4	−0.4 ± 0.8	−0.1 ± 0.3
		⊿ 0-8	−0.4 ± 0.9	0.0 ± 0.3 ‡
		⊿ 0-12	−0.3 ± 0.9	0.1 ± 0.2 ‡
	Prickling	Baseline	0.4 ± 0.5	0.6 ± 1.1
		Week 4	0.2 ± 0.5	0.6 ± 1.1
		Week 8	0.3 ± 0.8	0.6 ± 1.1
		Week 12	0.4 ± 0.7	0.7 ± 1.1
		⊿ 0-4	−0.2 ± 0.5	0.0 ± 0.0
		⊿ 0-8	−0.1 ± 0.8	0.0 ± 0.3
		⊿ 0-12	0.0 ± 0.7	0.1 ± 0.2
	Dryness	Baseline	1.4 ± 0.5	1.6 ± 1.1
		Week 4	1.1 ± 0.6 *	1.6 ± 1.0
		Week 8	1.1 ± 0.7	1.6 ± 1.0
		Week 12	0.5 ± 0.6 **	1.8 ± 1.1
		⊿ 0-4	−0.4 ± 0.6	−0.1 ± 0.2
		⊿ 0-8	−0.3 ± 0.7	0.0 ± 0.5
		⊿ 0-12	−0.9 ± 0.8	0.2 ± 0.4 ##
QOL due to skin conditions	Interfering with study/work	Baseline	0.3 ± 0.6	0.4 ± 0.6
		Week 4	0.1 ± 0.3	0.3 ± 0.5
		Week 8	0.3 ± 0.4	0.3 ± 0.6
		Week 12	0.2 ± 0.4	0.6 ± 0.7
		⊿ 0-4	−0.2 ± 0.5	−0.1 ± 0.5
		⊿ 0-8	−0.1 ± 0.7	−0.1 ± 0.5
		⊿ 0-12	−0.1 ± 0.6	0.1 ± 0.5
	Poor concentration	Baseline	0.6 ± 0.5	0.7 ± 0.8
		Week 4	0.3 ± 0.4 *	0.5 ± 0.7
		Week 8	0.2 ± 0.5 †	0.2 ± 0.4 *
		Week 12	0.2 ± 0.4 *	0.4 ± 0.5 †
		⊿ 0-4	−0.3 ± 0.5	−0.2 ± 0.8
		⊿ 0-8	−0.4 ± 0.6	−0.5 ± 0.7
		⊿ 0-12	−0.4 ± 0.5	−0.3 ± 0.7
	Disruption of thought	Baseline	0.2 ± 0.4	0.6 ± 0.6
		Week 4	0.0 ± 0.0	0.2 ± 0.4 †
		Week 8	0.1 ± 0.3	0.1 ± 0.3 *
		Week 12	0.1 ± 0.3	0.2 ± 0.4 *
		⊿ 0-4	−0.2 ± 0.4	−0.3 ± 0.6
		⊿ 0-8	−0.1 ± 0.5	−0.4 ± 0.5
		⊿ 0-12	−0.1 ± 0.6	−0.3 ± 0.5
	Memory deterioration	Baseline	0.2 ± 0.4	0.4 ± 0.7
		Week 4	0.1 ± 0.3	0.2 ± 0.4
		Week 8	0.1 ± 0.3	0.1 ± 0.3 †
		Week 12	0.1 ± 0.3	0.3 ± 0.5
		⊿ 0-4	−0.1 ± 0.5	−0.2 ± 0.5
		⊿ 0-8	−0.1 ± 0.5	−0.3 ± 0.6
		⊿ 0-12	−0.1 ± 0.5	−0.1 ± 0.5
QOL due to skin conditions	Interfering with outdoor activity	Baseline	0.4 ± 0.6	0.4 ± 0.7
		Week 4	0.1 ± 0.3	0.2 ± 0.5
		Week 8	0.2 ± 0.5	0.2 ± 0.4
		Week 12	0.1 ± 0.5	0.2 ± 0.5
		⊿ 0-4	−0.3 ± 0.7	−0.2 ± 0.5
		⊿ 0-8	−0.2 ± 0.8	−0.2 ± 0.5
		⊿ 0-12	−0.3 ± 0.7	−0.2 ± 0.4
	Interfering with social activity	Baseline	0.3 ± 0.7	0.3 ± 0.5
		Week 4	0.0 ± 0.0	0.2 ± 0.5
		Week 8	0.1 ± 0.3	0.2 ± 0.5
		Week 12	0.1 ± 0.3	0.2 ± 0.5
		⊿ 0-4	−0.3 ± 0.7	−0.1 ± 0.5
		⊿ 0-8	−0.2 ± 0.8	−0.1 ± 0.5
		⊿ 0-12	−0.1 ± 0.8	−0.1 ± 0.5
	Interfering with social relationship	Baseline	0.3 ± 0.7	0.3 ± 0.5
		Week 4	0.0 ± 0.0	0.2 ± 0.4
		Week 8	0.1 ± 0.3	0.2 ± 0.4
		Week 12	0.1 ± 0.3	0.2 ± 0.4
		⊿ 0-4	−0.3 ± 0.7	−0.1 ± 0.4
		⊿ 0-8	−0.2 ± 0.8	−0.1 ± 0.4
		⊿ 0-12	−0.2 ± 0.8	0.2 ± 0.4
	Interfering with conversation	Baseline	0.2 ± 0.5	0.1 ± 0.3
		Week 4	0.0 ± 0.0	0.2 ± 0.4
		Week 8	0.1 ± 0.3	0.3 ± 0.5
		Week 12	0.1 ± 0.3	0.3 ± 0.5
		⊿ 0-4	−0.2 ± 0.5	0.1 ± 0.2
		⊿ 0-8	−0.1 ± 0.6	0.2 ± 0.4
		⊿ 0-12	−0.1 ± 0.6	0.2 ± 0.4
	Worry about symptoms	Baseline	0.9 ± 0.6	1.5 ± 1.0
		Week 4	0.6 ± 0.5	0.9 ± 0.9 *
		Week 8	0.6 ± 0.7	0.8 ± 0.7 *
		Week 12	0.7 ± 0.8	0.7 ± 0.8 *
		⊿ 0-4	−0.3 ± 0.6	−0.6 ± 0.9
		⊿ 0-8	−0.3 ± 0.8	−0.7 ± 0.9
		⊿ 0-12	−0.2 ± 0.8	−0.8 ± 1.0 ‡
	Worry about other's thoughts	Baseline	0.4 ± 0.6	0.9 ± 1.0
		Week 4	0.3 ± 0.4	0.5 ± 0.6 †
		Week 8	0.2 ± 0.4	0.4 ± 0.5 *
		Week 12	0.4 ± 0.5	0.4 ± 0.5 *
		⊿ 0-4	−0.2 ± 0.4	−0.4 ± 0.9
		⊿ 0-8	−0.3 ± 0.7	−0.5 ± 1.0
		⊿ 0-12	−0.1 ± 0.9	−0.6 ± 0.9
	Sleep disorder	Baseline	0.3 ± 0.5	0.4 ± 0.6
		Week 4	0.1 ± 0.3 †	0.3 ± 0.6
		Week 8	0.2 ± 0.4	0.2 ± 0.4
		Week 12	0.1 ± 0.3	0.4 ± 0.5
		⊿ 0-4	−0.3 ± 0.4	−0.1 ± 0.8
		⊿ 0-8	−0.1 ± 0.5	−0.2 ± 0.4
		⊿ 0-12	−0.2 ± 0.5	0.0 ± 0.5
	Sense of fatigue	Baseline	0.4 ± 0.7	0.5 ± 0.7
		Week 4	0.1 ± 0.3	0.4 ± 0.6
		Week 8	0.4 ± 0.6	0.2 ± 0.4 †
		Week 12	0.2 ± 0.4	0.2 ± 0.4
		⊿ 0-4	−0.3 ± 0.8	−0.1 ± 0.6
		⊿ 0-8	−0.1 ± 0.9	−0.3 ± 0.6
		⊿ 0-12	−0.3 ± 0.9	−0.3 ± 0.7

mean ± SD.

⊿ : amount of change from baseline.

†: $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$ against baseline.

‡: $p < 0.1$, #: $p < 0.05$, ##: $p < 0.01$ between-group difference in change from baseline.

Table 2 Chronological change in scores of questionnaire ②

Questionnaire		Time points	Scores	
			Group A (n = 16)	Group P (n = 18)
QOL due to skin conditions	Getting tired easily	Baseline	0.5 ± 0.7	0.6 ± 0.9
		Week 4	0.2 ± 0.5	0.4 ± 0.7
		Week 8	0.4 ± 0.6	0.4 ± 0.6
		Week 12	0.3 ± 0.4	0.4 ± 0.6
		⊿ 0-4	−0.3 ± 0.8	−0.2 ± 0.5
		⊿ 0-8	−0.1 ± 0.9	−0.2 ± 0.9
		⊿ 0-12	−0.3 ± 0.9	−0.2 ± 0.8
	Feeling down	Baseline	0.4 ± 0.6	0.6 ± 0.7
		Week 4	0.3 ± 0.4	0.5 ± 0.7
		Week 8	0.3 ± 0.6	0.4 ± 0.5
		Week 12	0.2 ± 0.4	0.4 ± 0.6
		⊿ 0-4	−0.1 ± 0.5	−0.1 ± 0.5
		⊿ 0-8	−0.1 ± 0.8	−0.1 ± 0.6
		⊿ 0-12	−0.2 ± 0.7	−0.2 ± 0.5
	Irritation	Baseline	0.5 ± 0.9	0.7 ± 0.8
		Week 4	0.2 ± 0.4	0.4 ± 0.8
		Week 8	0.3 ± 0.7	0.4 ± 0.5 *
		Week 12	0.2 ± 0.5	0.4 ± 0.6 †
		⊿ 0-4	−0.3 ± 0.8	−0.3 ± 0.8
		⊿ 0-8	−0.3 ± 0.9	−0.3 ± 0.5
		⊿ 0-12	−0.3 ± 0.7	−0.3 ± 0.7
	Depression	Baseline	0.4 ± 0.7	0.6 ± 0.8
		Week 4	0.1 ± 0.3 *	0.4 ± 0.7
		Week 8	0.2 ± 0.4	0.4 ± 0.5
		Week 12	0.3 ± 0.6	0.4 ± 0.6
		⊿ 0-4	−0.3 ± 0.5	−0.2 ± 0.9
		⊿ 0-8	−0.3 ± 0.7	−0.2 ± 0.7
		⊿ 0-12	−0.2 ± 0.7	−0.2 ± 0.8
	Unsatisfaction of life	Baseline	0.4 ± 0.9	0.4 ± 0.6
		Week 4	0.1 ± 0.3	0.4 ± 0.7
		Week 8	0.1 ± 0.3	0.3 ± 0.5
		Week 12	0.3 ± 0.6	0.3 ± 0.6
		⊿ 0-4	−0.3 ± 0.7	0.0 ± 0.5
		⊿ 0-8	−0.3 ± 0.8	−0.1 ± 0.3
		⊿ 0-12	−0.1 ± 0.8	−0.1 ± 0.5
Psychosomatic states	Bowel movement or diarrhea	Baseline	0.6 ± 0.6	0.5 ± 0.6
		Week 4	0.3 ± 0.4 *	0.3 ± 0.5
		Week 8	0.4 ± 0.5	0.3 ± 0.5
		Week 12	0.3 ± 0.5 †	0.6 ± 0.7
		⊿ 0-4	−0.4 ± 0.6	−0.2 ± 0.6
		⊿ 0-8	−0.3 ± 0.6	−0.2 ± 0.4
		⊿ 0-12	−0.3 ± 0.6	0.1 ± 0.3 ‡
	Falling sleep	Baseline	0.9 ± 0.8	0.3 ± 0.7
		Week 4	0.7 ± 0.7	0.4 ± 0.7
		Week 8	0.4 ± 0.5 *	0.4 ± 0.7
		Week 12	0.6 ± 0.8 *	0.4 ± 0.6
		⊿ 0-4	−0.2 ± 0.5	0.1 ± 0.2
		⊿ 0-8	−0.5 ± 0.6	0.1 ± 0.6 #
		⊿ 0-12	−0.3 ± 0.5	0.1 ± 0.5
Psychosomatic states	Quality of awaking	Baseline	0.9 ± 0.6	0.6 ± 0.8
		Week 4	0.8 ± 0.7	0.6 ± 0.8
		Week 8	0.8 ± 0.7	0.6 ± 0.8
		Week 12	0.6 ± 0.7 †	0.3 ± 0.6 **
		⊿ 0-4	−0.2 ± 0.4	0.0 ± 0.0
		⊿ 0-8	−0.1 ± 0.6	0.0 ± 0.5
		⊿ 0-12	−0.3 ± 0.6	−0.3 ± 0.6
	Fatigue	Baseline	1.4 ± 0.8	0.8 ± 0.9
		Week 4	1.3 ± 0.8	0.7 ± 1.0
		Week 8	0.9 ± 0.7 *	0.9 ± 0.9
		Week 12	0.8 ± 0.8 **	0.6 ± 0.7
		⊿ 0-4	−0.1 ± 0.3	−0.1 ± 0.3
		⊿ 0-8	−0.4 ± 0.5	0.1 ± 0.7 #
		⊿ 0-12	−0.2 ± 0.8	−0.2 ± 0.7
	Skin problems	Baseline	1.4 ± 0.7	0.6 ± 0.5
		Week 4	1.1 ± 0.7 †	0.7 ± 0.7
		Week 8	0.8 ± 0.7 *	1.0 ± 0.8 *
		Week 12	0.5 ± 0.6 **	0.9 ± 0.7 *
		⊿ 0-4	−0.3 ± 0.6	0.1 ± 0.4
		⊿ 0-8	−0.6 ± 0.6	0.4 ± 0.7 ##
		⊿ 0-12	−0.9 ± 0.6	0.3 ± 0.6 ##
	Susceptible to catch a cold or disease	Baseline	0.7 ± 0.8	0.7 ± 0.9
		Week 4	0.5 ± 0.6	0.3 ± 0.6 †
		Week 8	0.2 ± 0.4 †	0.4 ± 0.6
		Week 12	0.3 ± 0.6 †	0.3 ± 0.5 *
		⊿ 0-4	−0.2 ± 0.7	−0.4 ± 0.8
		⊿ 0-8	−0.5 ± 0.9	−0.3 ± 0.8
		⊿ 0-12	−0.4 ± 0.8	−0.4 ± 0.8
	Low body temperature or poor circulation	Baseline	1.2 ± 1.2	1.2 ± 1.3
		Week 4	0.6 ± 0.7 †	0.4 ± 0.7 **
		Week 8	0.4 ± 0.5 *	0.7 ± 1.0
		Week 12	0.6 ± 1.0 †	0.3 ± 0.6 **
		⊿ 0-4	−0.6 ± 1.1	−0.8 ± 1.0
		⊿ 0-8	−0.8 ± 1.1	−0.6 ± 1.3
		⊿ 0-12	−0.6 ± 1.0	−0.9 ± 1.0
	Hard texture of skin	Baseline	1.6 ± 0.6	1.6 ± 0.7
		Week 4	0.9 ± 0.6 **	1.3 ± 0.5
		Week 8	0.8 ± 0.7 *	1.6 ± 0.6
		Week 12	0.8 ± 0.6 **	1.5 ± 0.6
		⊿ 0-4	−0.8 ± 0.8	−0.3 ± 0.7 ‡
		⊿ 0-8	−0.8 ± 1.0	−0.1 ± 0.5 ##
		⊿ 0-12	−0.9 ± 0.9	−0.1 ± 0.6 ##
Dryness of facial skin	Facial skin conditions	Baseline	2.4 ± 0.5	1.9 ± 0.6
		Week 4	1.6 ± 0.7 **	2.2 ± 0.7
		Week 8	1.6 ± 0.8 **	2.3 ± 0.6 *
		Week 12	1.5 ± 0.7 **	2.4 ± 0.6 *
		⊿ 0-4	−0.9 ± 0.6	0.3 ± 0.8 ##
		⊿ 0-8	−0.8 ± 0.7	0.4 ± 0.6 ##
		⊿ 0-12	−0.9 ± 0.6	0.5 ± 0.6 ##

mean ± SD.

⊿ : amount of change from baseline.

† : $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$ against baseline.

‡ : $p < 0.1$, #: $p < 0.05$, ##: $p < 0.01$ between-group difference in change from baseline.

ATOPY RESCUE CO. LTD. The daily intake was a 1 tablet weighing 247.5 mg and composed of ceramide derived from pineapple, hyaluronic acid, lactobacillus and etc., while the placebo consisted mainly of cellulose. Both tablets were indistinguishable in shape, color, odor and taste. Subjects received following instructions; to maintain a usual lifestyle and habits; to avoid excessive intake of food, drink and alcohol; to avoid tanning and

Table 3　Chronological change in stratum corneum hydration (SCH)

Time points	Values (index)	
	Group A (n = 16)	Group P (n = 18)
Baseline	45.7 ± 11.0	59.6 ± 10.3
Week 4	48.7 ± 10.9 †	60.1 ± 11.2
Week 8	52.5 ± 9.8 *	50.9 ± 10.8 **
Week 12	55.5 ± 11.1 **	50.6 ± 12.0 **
⊿ 0-4 w	3.0 ± 6.1	0.5 ± 6.6
⊿ 0-8 w	6.8 ± 10.3	− 8.6 ± 6.8 ##
⊿ 0-12 w	9.8 ± 9.7	− 9.0 ± 7.6 ##

mean ± SD.

⊿ : amount of change from baseline.

† : $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$ against baseline.

: $p < 0.01$ between-group difference in change from baseline.

Table 4　Change in IgE level

Time points	Values (IU/mL)	
	Group A (n = 16)	Group P (n = 18)
Baseline	198.8 ± 528.7	209.8 ± 279.7
Week 12	204.9 ± 539.7 †	198.8 ± 249.2
⊿ 0-12	6.2 ± 17.6	− 11.0 ± 56.7

mean ± SD.

⊿ : amount of change from baseline.

† : $p < 0.1$ against baseline.

excessive exercise; to record a dairy of the daily intake of tablet. The allocation list was sealed and strictly controlled in a safety deposit box of JACTA until the end of the study.

Outcome measurements

The subjective assessment of skin properties using a questionnaire was performed as a primary outcome. The questionnaire which was made based on the Japanese Dermatological Association Criteria for the diagnosis of atopic dermatitis (2009) consisted of 30 questions on their current skin condition, QOL due to skin conditions, psychosomatic state and dryness of facial skin. Each question has 5-choices scored from 0 to 4, and subjects chose the most suitable answer by their own will. Five-choices of questions related to their current skin condition and QOL due to skin conditions were as follows; 0: None/No, 1: Mild, 2: Moderate, 3: Severe, 4: Extremely severe. Similarly, the 5-choices of questions relate to dryness of facial skin were as follows; 0: Moist condition, 1: Slightly moist condition, 2: Slightly dry condition, 3: Dry condition, 4: Itchy/Painful. The score of each choice that subjects selected was recorded as a self-evaluated skin condition. Appendix the stratum corneum hydration (SCH) was measured using a skin surface hydrometer (Corneometer® CM825; Courage + Khazaka electronic GmbH, Germany) as an indicator of the horny layer moisture level. In addition, an allergen-specific immunoglobulin E (IgE) level was examined. To evaluate the safety of the test products as a secondary outcome, blood biochemical and urine parameters were examined at the baseline and 12 weeks, and adverse events were recorded using a questionnaire during the study. Efficacy analysis was performed on all subjects who did not meet any of the following criteria; (1) subjects who consumed less than 80% of the expected dose, (2) subjects without adequate records, (3) subjects who fell under the exclusion criteria after enrolment, (4) subjects who got a suntan during the study, (5) subjects who had justifiable reason for exclusion.

Statistics

All analyses were performed on the on-treatment population in the study. Statistics were expressed as mean ± standard deviation (SD). Chronological changes from the baseline in SCH, IgE, and blood biochemical and urine parameters were assessed using the paired t-test. Student's t-test was performed for intergroup comparisons of the amount of change from the baseline in each variable. To evaluate the change in subjective assessment from the baseline, the Wilcoxon signed-rank test was performed. The Mann-Whitney U test was

Table 5 Biochemical blood and urine parameters

Item	Unit	Time points	Values	
			Group A (n = 16)	Group P (n = 18)
Total Bilirubin	mg/dL	Baseline	0.61 ± 0.18	0.74 ± 0.35
		Week 12	0.51 ± 0.17 †	0.58 ± 0.25 **
		⊿ 0-12	− 0.11 ± 0.24	− 0.16 ± 0.18
Total Protein	g/dL	Baseline	7.5 ± 0.3	7.4 ± 0.3
		Week 12	7.6 ± 0.3	7.3 ± 0.3
		⊿ 0-12	0.1 ± 0.4	− 0.0 ± 0.3
Albumin	g/dL	Baseline	4.4 ± 0.3	4.6 ± 0.2
		Week 12	4.5 ± 0.3	4.5 ± 0.2
		⊿ 0-12	0.0 ± 0.3	− 0.1 ± 0.2
AST	U/L	Baseline	22.9 ± 8.8	20.8 ± 5.2
		Week 12	25.9 ± 9.3	21.4 ± 7.1
		⊿ 0-12	3.1 ± 8.2	0.7 ± 5.4
ALT	U/L	Baseline	22.7 ± 12.8	17.5 ± 6.7
		Week 12	24.6 ± 12.1	16.7 ± 8.1
		⊿ 0-12	1.9 ± 9.8	− 0.8 ± 7.4
ALP	U/L	Baseline	199.1 ± 61.9	196.4 ± 53.4
		Week 12	217.8 ± 63.6 *	191.9 ± 56.4
		⊿ 0-12	18.7 ± 27.9	− 4.5 ± 28.8 #
LDH	U/L	Baseline	185.1 ± 28.3	180.2 ± 28.8
		Week 12	198.5 ± 32.2 †	192.1 ± 30.6 *
		⊿ 0-12	13.4 ± 30.5	11.9 ± 23.5
g-GTP: male	U/L	Baseline	57.7 ± 38.0	26.8 ± 11.7
		Week 12	62.8 ± 42.0	23.8 ± 7.9
		⊿ 0-12	5.2 ± 8.7	− 3.0 ± 7.6 ‡
g-GTP: female	U/L	Baseline	17.7 ± 8.2	22.3 ± 16.1
		Week 12	18.6 ± 7.0	24.1 ± 17.4
		⊿ 0-12	0.9 ± 4.5	1.8 ± 2.9
CK: male	U/L	Baseline	161.7 ± 91.9	246.0 ± 169.1
		Week 12	190.3 ± 107.9	198.4 ± 128.2
		⊿ 0-12	28.7 ± 145.8	− 47.6 ± 171.5
CK: female	U/L	Baseline	110.8 ± 72.7	95.3 ± 42.4
		Week 12	92.0 ± 32.2	72.1 ± 11.3
		⊿ 0-12	− 18.8 ± 52.0	− 23.2 ± 42.1
Total cholesterol	mg/dL	Baseline	190.4 ± 29.1	207.8 ± 36.7
		Week 12	1809.9 ± 37.5	206.3 ± 35.0
		⊿ 0-12	− 0.5 ± 18.5	− 1.5 ± 17.1
TG	mg/dL	Baseline	132.4 ± 63.4	136.2 ± 75.4
		Week 12	122.4 ± 104.4	91.9 ± 46.9 **
		⊿ 0-12	− 10.0 ± 83.1	− 44.3 ± 43.2
Na	mEq/L	Baseline	141.5 ± 1.8	141.9 ± 1.9
		Week 12	141.8 ± 1.8	141.8 ± 2.2
		⊿ 0-12	0.3 ± 2.0	− 0.1 ± 1.3
Cl	mEq/L	Baseline	103.1 ± 2.0	103.2 ± 1.7
		Week 12	103.8 ± 2.5	103.7 ± 2.2
		⊿ 0-12	0.6 ± 2.4	0.6 ± 2.3
K	mEq/L	Baseline	4.0 ± 0.2	4.0 ± 0.2
		Week 12	4.4 ± 0.4 **	4.4 ± 0.4 **
		⊿ 0-12	0.4 ± 0.4	0.4 ± 0.4
Ca	mg/dL	Baseline	9.7 ± 0.2	9.8 ± 0.3
		Week 12	9.5 ± 0.3 *	9.6 ± 0.2 *
		⊿ 0-12	− 0.2 ± 0.3	0.2 ± 0.4
Phosphorus	mg/dL	Baseline	3.5 ± 0.5	3.1 ± 0.6
		Week 12	3.5 ± 0.5	3.3 ± 0.5
		⊿ 0-12	0.1 ± 0.5	0.1 ± 0.6
BUN	mg/dL	Baseline	13.7 ± 4.5	12.4 ± 2.6
		Week 12	15.2 ± 3.7	13.5 ± 2.9 *
		⊿ 0-12	1.5 ± 3.6	1.1 ± 2.0
Creatinin: male	mg/dL	Baseline	0.74 ± 0.10	0.87 ± 0.16
		Week 12	0.74 ± 0.10	0.84 ± 0.12
		⊿ 0-12	− 0.00 ± 0.04	− 0.03 ± 0.11
Creatinin: female	mg/dL	Baseline	0.59 ± 0.07	0.61 ± 0.06
		Week 12	0.57 ± 0.07	0.61 ± 0.04
		⊿ 0-12	− 0.01 ± 0.06	0.00 ± 0.04
Blood glucose	mg/dL	Baseline	66.5 ± 8.7	67.6 ± 14.5
		Week 12	71.6 ± 15.1	68.6 ± 20.1
		⊿ 0-12	5.1 ± 15.0	0.9 ± 21.7
Specific gravity of urined	mg/dL	Baseline	1.018 ± 0.011	1.016 ± 0.007
		Week 12	1.020 ± 0.007	1.017 ± 0.006
		⊿ 0-12	0.003 ± 0.009	0.001 ± 0.006
urine pH	g/dL	Baseline	6.4 ± 0.6	6.9 ± 1.0
		Week 12	6.3 ± 0.9	6.4 ± 0.8
		⊿ 0-12	− 0.1 ± 1.1	− 0.5 ± 1.2

Mean ± SD.
⊿ : amount of change from baseline
† : $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$ against baseline.
‡ : $p < 0.1$, #: $p < 0.05$ between-group difference in change from baseline.

performed for intergroup comparisons of the amount of change from the baseline in each self-evaluated score. Student's t-test was used to compare subject backgrounds between groups. Statistical analyses were performed using Statcel 3 (Yanai, 2011). The results were considered significant at the ＜5% level in the two-sided test.

RESULTS

Subject's flow and characteristics

102 subjects were recruited and 40 subjects who fulfilled eligibility were enrolled in this study (**Fig. 1**). 22 subjects were allocated to group A and 18 subjects to group P. 6 subjects in group A were withdrawn (occupational conflict: 2, physical condition: 3 and death in family: 1). Thus, 34 subjects completed the study (group A: 16 and group P: 18). No significant differences were observed in the gender or age between groups (**Table 1**).

Efficacy

Table 2 shows the chronological change in self-evaluated scores. In the section of 'current skin symptoms', scores of itching and dryness significantly decreased in group A, and there were significant differences in the amount of change in scores compared to group P after 12 weeks. In the section of 'psychosomatic states', scores on falling asleep, fatigue, skin problems, and hard texture of skin significantly decreased in group A, and there were significant differences in the amount of change in scores compared to group P. The scores of facial skin conditions in the section of 'dryness of facial skin' significantly decreased in group A, and there were significant differences in the amount of change in scores compared to group P. In total, there were significant differences in 7 of 30 questions between groups in the self-evaluated skin condition questionnaire. The chronological change in SCH was shown in **Table 3**. SCH significantly increased in group A at week 8 and 12 respectively, and there were significant differences in the amount of change compared to group P in week 0-8 and week 0-12. **Table 4** shows the results of an allergen-specific IgE test. There was no significant change in amount of IgE in both groups.

Safety

The changes in blood biochemical and urine parameters are shown in **Table 5.** A significant difference was observed in changes of ALP, K and Ca of group A after 12 weeks. However, the investigator judged it as the range of physiological variation (or clinically safe). No adverse event was reported during the study.

DISCUSSION

Ceramide, an essential structural component of cell membranes, has the function of immunomodulation[13], and enhances the skin barrier function[14]. This barrier function plays an important role in protection for ultraviolet, extracellular stimulation and loss of water[15]. Based on above findings, there are many reports that dietary glucosylceramide improves skin conditions[6)16)-21)]. However, there are only a few reports which evaluate SCH along with self-evaluated skin conditions. This study was conducted to evaluate the effect of dietary GP with hyaluronic acid on skin condition based on self-evaluation and objective evaluation using a skin surface hydrometer in Japanese healthy volunteers with dry skin and itchy sensations. In the results of the self-evaluation, the scores of itching, dryness, hard texture of skin, and facial skin conditions significantly improved without any adverse events in subjects talking dietary GP with hyaluronic acid. In addition, the skin surface hydrometer test showed SCH significantly increased compared to the baseline value. Mori et al. reported that the pruritus perception threshold is significantly correlated with skin surface hydration and inversely with trans-epidermal water loss in patients with atopic dermatitis and healthy subjects[22]. These findings correspond with the role of the ceramide with hyaluronic acid and the rationale mentioned above. Specifically, dietary glucosylceramide improves skin conditions based on moisturizing effects resulting from improving the skin barrier function through enhancing ceramide production. Therefore, it can be argued that this study demonstrated the efficacy of dietary GP with hyaluronic acid on skin conditions and it also strongly supports the current theoretical background on the physiological role of ceramide.

Itching and dryness are very common symptoms in adults. Dalgard et al. reported that 18% of men and 9% of women complain about itching and 18% of men and 12% of women complain about dryness of skin in East Asia[23]. GP with hyaluronic acid may contribute to improve skin conditions in many people with itchy sensations and dryness of skin.

CONCLUSION

This study demonstrated dietary GP with hyaluronic acid significantly improved skin conditions safely in healthy Japanese adults with dry skin and itchy sensations.

CONFLICT OF INTEREST

Keiichi Magoshi is a principal of ATOPY RESCUE CO. LTD. All remaining authors have declared no conflicts of interest.

REFERENCES

1) Danno K, Horio T, Takigawa M, et al. Role of oxygen intermediates in UV-induced epidermal cell injury. J Invest Dermatol. 1984; **83**(3): 166-8.
2) Cosgrove MC, Franco OH, Granger SP, et al. Dietary nutrient intakes and skin-aging appearance among middle-aged American women. Am J Clin Nutr. 2007; **86**(4): 1225-31.
3) Uchida Y, Holleran WM. Omega-O-acylceramide, a lipid essential for mammalian survival. J Dermatol Sci. 2008; **51**(2): 77-87.
4) Feingold KR. Thematic review series: skin lipids. The role of epidermal lipids in cutaneous permeability barrier homeostasis. J Lipid Res. 2007; **48**(12): 2531-46.
5) Sugawara T, Kinoshita M, Ohnishi M, et al. Digestion of maize sphingolipids in rats and uptake of sphingadienine by Caco-2 cells. J Nutr. 2003; **133**(9): 2777-82.

6) Duan J, Sugawara T, Hirose M, et al. Dietary sphingolipids improve skin barrier functions via the upregulation of ceramide synthases in the epidermis. Exp Dermatol. 2012; **21**(6): 448-52.
7) Ideta R, Sakuta T, Nakano Y, et al. Orally administered glucosylceramide improves the skin barrier function by upregulating genes associated with the tight junction and cornified envelope formation. Biosci Biotechnol Biochem. 2011; **75**(8): 1516-23.
8) Kawada C, Hasegawa T, Watanabe M, et al. Dietary glucosylceramide enhances tight junction function in skin epidermis via induction of claudin-1. Biosci Biotechnol Biochem. 2013; **77**(4): 867-9.
9) Uchiyama T, Nakano Y, Ueda O. Oral intake of glucosylceramide improves relatively higher level of transepidermal water loss in mice and healthy human subjects. J Health Sci. 2008; **54**(5): 559-66.
10) Sato T, Sakamoto W, Odanaka W, et al. Clinical effects of dietary hyaluronic acid on dry, rough skin. Aesthetic Dermatology. 2002; **12**: 109-20.
11) Sato T, Yoshida T, Kanemitu T, et al. Clinical effects of hyaluronic acid diet for moisture content of dry skin. Aesthetic Dermatology. 2007; **17**: 33-9.
12) Kawada C, Yoshida T, Yoshida H, et al. Ingestion of hyaluronans (molecular weights 800 k and 300 k) improves dry skin conditions: a randomized, double blind, controlled study. J Clin Biochem Nutr. 2015; **56**(1): 66-73.
13) Kawano T, Cui J, Koezuka Y, et al. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. Science. 1997; **278**(5343): 1626-9.
14) Jennemann R, Sandhoff R, Langbein L, et al. Integrity and barrier function of the epidermis critically depend on glucosylceramide synthesis. J Biol Chem. 2007; **282**(5): 3083-94.
15) Chuong CM, Nicholoff BJ, Elias PM, et al. What is the 'true' function of skin? Exp Dermatol. 2002; **11**(2): 159-87.
16) Hasegawa T, Shimada H, Uchiyama T, et al. Dietary glucosylceramide enhances cornified envelope formation via transglutaminase expression and involucrin production. Lipids. 2011; **46**(6): 529-35.
17) Tokudome Y, Endo M, Hashimoto F. Application of glucosylceramide-based liposomes increased the ceramide content in a three-dimensional cultured skin epidermis. Skin Pharmacol Physiol. 2014; **27**(1): 18-24.
18) Kawada C, Hasegawa T, Watanabe M, et al. Dietary glucosylceramide enhances tight junction function in skin epidermis via induction of claudin-1. Biosci Biotechnol Biochem. 2013; **77**(4): 867-9.
19) Duan J, Sugawara T, Hirose M, et al. Dietary sphingolipids improve skin barrier functions via the upregulation of ceramide synthases in the epidermis. Exp Dermatol. 2012; **21**(6): 448-52.
20) Hasegawa T, Shimada H, Uchiyama T, et al. Dietary glucosylceramide enhances cornified envelope formation via transglutaminase expression and involucrin production. Lipids. 2011; **46**(6): 529-35.
21) Miyanishi K, Shiono N, Shirai H, et al. Reduction of transepidermal water loss by oral intake of glucosylceramides in patients with atopic eczema. Allergy. 2005; **60**(11): 1454-5.
22) Mori T, Ishida K, Mukumoto S, et al. Comparison of skin barrier function and sensory nerve electric current perception threshold between IgE-high extrinsic and IgE-normal intrinsic types of atopic dermatitis. Br J Dermatol. 2010; **162**(1): 83-90.
23) Dalgard F, Holm JO, Svensson A, et al. Self reported skin morbidity and ethnicity: a population-based study in a Western community. BMC Dermatol. 2007; **7**: 4.

Appendix Subjective assessment of skin properties through self-reporting on the questionnaire.

No	Item
Please answer your current status of skin	
1	How itchy has your skin been?
2	How sore has your skin been?
3	How stinging has your skin been?
4	How dry has your skin been?
Please answer the quality of life.	
5	How much has your skin problem interfered with your studying, working or housekeeping?
6	Has your skin affected your concentration?
7	How much has your skin caused a decline in thinking power?
8	How much has your skin caused a decline in memory?
9	How much has your skin interfered with your doing sport or going picnic?
10	How much has your skin interfered with you going out?
11	How much has your skin created problems with your partner or any of your close friends or relatives?
12	How much has your skin interfered with having conversation on the phone?
13	How conscious have you been of your skin?
14	How embarrassed have you feeling because of your skin?
15	How much has your skin caused sleep disorder?
16	How much has your skin caused fatigue?
17	How much has your skin caused getting exhausted easily?
18	How much has your skin caused feeling cheerless?
19	How much has your skin caused irritating you?
20	How much has your skin caused depressed you?
21	How much has your skin prevented you from being satisfied with your life?
Please answer how you feel	
22	Bowel movements or diarrhea
23	Quality of getting off to sleep
24	Quality of awaking
25	Fatigable
26	Skin problems
27	Susceptible to catch a cold or disease
28	Low body temperature or poor circulation
29	Rigid feeling or dryness of your skin
Please answer your facial status of skin dryness	
30	Condition of your skin

Responses to each question were rated on an ordinal scale of 0 to 4, with lower scores indicating a better condition.