CLINICAL INVESTIGATION OF SKIN-BEAUTIFYING EFFECT OF A BEAUTY SUPPLEMENT CONTAINING RICE-DERIVED CERAMIDE

Objective Evaluation By Microscopic Three-dimensional Imaging Analysis In Dry Skin

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[Summary]

A placebo-controlled double-blind ingestion study of a beauty supplement containing rice-derived ceramide was performed in 33 subjects who always tended to have rough skin due to dryness, and the following results were obtained: (1) Dermatological diagnosis by physicians showed that the supplement significantly improves dryness and itching of the skin. (2) On measurement of water content in the skin, the supplement was shown to significantly increase the water content in the skin. (3) On microscopic three-dimensional analysis of the epidermis, the supplement was shown to improve smoothness, exfoliation, and texture of the skin. The above findings showed that a long-term ingestion of a supplement rice-derived ceramide is effective in moisture-retention and maintaining smoothness of the skin, and thus, is an effective skin-beautifying food.

[Introduction]

Ceramide is one of polar lipids called sphingolipid. Since ceramide was shown to be present in the brain in humans by the German

physician, L.T.W. Thudichen in 1884, it has been extracted from bovine brains as a cosmetic. Synthetic ceramide is now widely used as a substance that holds moisture in the skin corneum and beautifying the skin.

Human epidermis consists of the stratum basale, stratum spinosum, granulosa, and stratum corneum from the inner layer toward outside, and the lipid component differs among the layers. In the stratum basale, phospholipids and cholesterol are the major component. Glycosylceramide gradually increases from the stratum basale toward the granulosa, and glycosylceramide is the major component in the granulosa. In contrast, in the stratum corneum, ceramide is produced using glycosylceramide as a precursor, and accumulates. Therefore, glycosylceramide disappears and ceramide is present as the major component of interstitial lipids between corneocytes.

Previous studies have shown that ceramide in the stratum corneum has superior barrier functions, such as formation and stability of skin structure, moisture-retention, and prevention of invasion of foreign bodies. It has been shown that the amount of ceramide in the stratum corneum is decreased in patients with senile xeroderma and atopic dermatitis, suggesting that lack of ceramide may be a cause of skin disease.

As described above, animal ceramide extracted from bovine brain had been used previously as a cosmetic, but synthetic ceramide is now used mostly. However, in recent years, ceramide is being regarded as a supplement for health and beauty, and natural ceramide has been

attracting attention again, focusing on its safety. Plant ceramide, in particular, is being regarded as a functional food for daily ingestion because of its high safety without risk of adverse events, unlike animal ceramide derived from cattle, which may develop mad cow disease. Oriza Yuka Co., Ltd.(オリザ油化株式会社) has succeeded in extraction and purification of sphingoglycolipids that exist in rice bran and germ in minute amounts and has developed dietary supplement containing 100% rice-derived ceramide. In this study, we investigated the effectiveness of ceramide as a skin-beautifying supplement.

The amount of water perspiration and skin water content varied depending on the environmental factors in evaluation of the skin-beautifying effect, posing a question as to the reliability of the evaluation. Therefore, in this study, in addition to diagnosis by dermatologists and measurements of skin water content, acidity, and oil content, we attempt to evaluate the condition of the epidermis objectively using a newly developed microscopic three-dimensional skin surface analysis system (VISIOSCAN, Courage + Khazaka Electronic Gmbh Co., Germany).

[Subjects]

Individuals who were troubled by rough skin due to chronic dry skin and had not been treated with drugs such as oral or topical drugs, were recruited from the students and faculty of National Osaka Foreign Language University with compensation. Forty-six applicants

underwent measurement of skin water content in the region below the left eye on February 21, 2000 (weather: cloudy, temperature: 18℃, humidity: 48%). Those who showed 50% or higher water content were excluded, and 37 subjects entered into the study. After the study started, four subjects withdrew from the study due to personal reasons (those who forgot to take the supplement and did not return for testing), excluding suspension due to the test supplement. Therefore, 33 subjects were finally evaluated (male: 6, female: 27, age: 25.1 ±7.8 years). This study was performed in conformity to the Helsinki declaration. Before execution of the study, physicians fully explained the purpose of this study to the subjects, and written consents were obtained.

[Methods]

The study was a placebo-controlled double blind study of six-week ingestion of the supplement containing rice-derived ceramide (ceramide).

a) Test supplement

The test supplement was a soft capsule (Orizaceramide, containing 1.2 mg/day rice-derived sphingoglycolipids) provided by Oriza Yuka Co., Ltd (オリザ油化株式会社) at a daily intake of 40 mg/day of ceramide.

The placebo was a capsule with the same appearance and taste (odorless) as the test supplement.

b) Ingestion and evaluation periods

The ingestion period was six weeks in both groups.

The evaluation was principally performed immediately before the ingestion period, after three weeks of ingestion, and immediately after the end of the ingestion period (after six weeks of ingestion).

c) Evaluation methods

(1) Dermatological diagnosis and inquiry

As general findings, itching, dryness, flush, erosion, exfoliation, papules, blebs, and swelling, and as facial findings, cosmetic rash, dryness, flush, and holding of cosmetic were classified into four grades. The total of facial and general findings were evaluated as overall symptoms. In each evaluation, anchor points (ｱﾝｶｰﾎﾟｲﾝﾄ) were prepared and evaluated by a several physicians including those authorized by the Japanese Society of Dermatology.

(2) Water content, oil content, and acidity (pH)

The water content was measured using a Corneometer CM825 (Courage + Khazaka Electronic Gmbh Co.). This apparatus measures the water content in the epidermis by measuring the electrostatic capacity via the stratum corneum, and is reported to show less errors compared with the current impedance method and infrared spectroscopy.

The oil content was measured using a Sebumeter SM810 (Courage + Khazaka Electronic Gmbh Co.). In this measurement system, a specific tape absorbing only oil is attached to the measured site for 30 seconds, and the oil content is measured from changes in the optic permeability. It has been shown experimentally that this system is not affected by humidity.

The acidity was measured using a PH900 (Courage + Khazaka Electronic Gmbh Co.). In this system, the electrode is connected to the skin surface via an ion-permeable membrane placed adjacent to the glass membrane, and the acidity is electrochemically measured noninvasively.

The measurements were made at a site 1 cm below the left eye, inner side of the left forearm (3 cm upper the elbow), and dorsal neck (3 cm below the spinous process of the neck).

To maintain the same environmental conditions, measurements were performed in a meeting room controlled to the specified conditions (temperature: 18-22℃, humidity: 45-55%) prior to measurement, and the subjects waited in the room for 30 minutes or longer in a resting state (sitting on a chair and watching TV). Each subject was asked to visit at almost the same time in all three measurements and wear a blouse with long sleeves or a T-shirt, and sweaters were prohibited. Makeup on the measurement sites was prohibited from 60 minutes before the test in all subjects. Subjects who visited with makeup removed the makeup during the resting/waiting period and underwent the testing after 60 minutes.

(3) Analysis using a microscopic three-dimensional skin surface analysis system (VISIOSCAN)

The microscopic three-dimensional skin surface analysis system is a digital system for analysis of skin surface (VISIOSCAN) developed by Courage + Khazaka Electronic Gmbh Co. (Germany) in cooperation with Prof. Tronnier (Witten Univ.). The skin surface is irradiated

with a specific ultraviolet light source, and the images are captured in a high performance CCD camera and digitized, then evaluated. Multivariate analysis has been performed on many clinical findings, and the following factors were used as parameters.

1) SE sm (Skin Smoothness)

An index of skin smoothness. It is calculated from the mean width and depth of furrows.

The skin becomes more smooth as the value decreases.

2) SE r (Skin roughness)

An index of roughness of the skin.

The ratio of darker points than the established point to the whole image is calculated.

The skin becomes more smooth as the value decreases, and becomes coarse as the value increases.

3) SE sc (Skin Scaliness)

An index of scale (degree of dryness of the stratum corneum).

Desquamated sites of the skin are counted brighter than the established value in the imaging, and the ratio of areas that is brighter than the established value to the all area is calculated.

The skin becomes more moist as the value decreases, showing less desquamation (scale).

4) SE w (Skin Wrinkles)

An index of the number and width of wrinkles of the skin.

This index presents texture of the skin in vertical and horizontal directions or the number and width of wrinkles.

A greater number means the presence of many wide wrinkles.

5) Kurtosis (correction K)

This parameter indicates the smoothness of whole skin.

It presents the quality of the histogram of skin color points. As the value comes close to 0, the histogram of color points shows a smoother curve, indicating ideal skin.

d) Grouping of subjects

The subjects were randomly divided by the controller into the ceramide group and placebo group. The final numbers of the subjects evaluated were 17 and 16 in the ceramide and placebo groups, respectively. As shown in Table 1, there were no significant differences between the groups in the following items obtained from the inquiry before initiation of the study: Age, height, body weight, sleep time, time of exposure to direct sunlight, the number of smokers, irregular menstruation, occupation, and alcohol drinking habit. Regarding the past dermatological history, three and two in the ceramide and placebo groups, respectively, had atopic dermatitis. However, none of them had been taking a drug at the initiation of the study, and it was confirmed there were no subjects being treated or outpatients in the study.

[Results]

(1) Dermatological diagnosis

The dermatological diagnoses before and after ingestion are shown in Table 2. Each value arbitrarily represents the mean of

scores evaluated by the four grades from 0 (no symptoms) to 3 (severe). As the score decreases, the symptom improves during the course. For statistical analysis, non-parametric analysis was employed. In facial symptoms, a significant improvement was observed in the observation items of "dryness of facial skin", "flush", and "holding of cosmetic" after three and six weeks of ingestion compared to those before ingestion in the ceramide group. In contrast, in the placebo group, "dryness" and "flush" were significantly improved compared to those before ingestion, but "holding of cosmetic" did not show significant improvement. Table 3 shows the improvement rate of each symptom score. The outcome was judged "markedly improved" when the symptom improved by two grades or disappeared after six weeks of ingestion compared to that before ingestion, "improved" when the symptom improved by one grade, "markedly aggravated" when the symptom aggravated by two grades or became severe, and "aggravated" when the symptom aggravated by one grade. Although both test foods were effective on "facial dryness", "flush", and "holding of cosmetic", improvements of all these symptoms were greater in the ceramide group than the placebo group.

Regarding the general findings, as shown in Table 2, significant improvement was observed in "itching", "dryness", and "flush" in both groups. However, in the overall finding, improvement was not significant in the placebo group, while significant improvement was obtained in the ceramide group. In the results shown in Table 3, although improvement was also observed in both groups, the improvement

rate was greater in all symptoms in the ceramide group. In the overall evaluation, the improvement rate was 43.8% in the placebo group, while it was high, 64.7%, in the ceramide group.

(2) Water content, oil content, and acidity

Changes in the water content, oil content, and acidity after ingestion in each group are shown in Table 4. Fig. 1 shows changes in the water content in the region below the left eye. As shown in Fig. 1, there were no marked changes in the water content in the placebo group, while the water content was significantly increased at the dorsal neck after four weeks and at all three measured sites after eight weeks.

Regarding the acidity, the optimal skin pH is considered to be about 5.5 in women. There were no significant changes in either group.

Because the oil content was 0 in most subjects except the region below the left eye, the measurement at the sites in the left forearm and dorsal neck was excluded after the 2nd test. There were no significant changes at the site below left eye in either group.

(3) Results of analyses by microscopic three-dimensional skin surface analysis system (VSIOSCAN)

Changes in each parameter in imaging analysis using VISIOSCAN are shown in Table 5.

In kurtosis, that is smoothness of total skin, a significant improvement was observed in the dorsal neck in the ceramide group. No significant changes were observed in the placebo group.

The SEsm value, which is an index of skin smoothness calculated from the depth, width, and notch of furrows, significantly improved in the dorsal neck in the ceramide group, showing a recovery of skin smoothness. In contrast, no significant changes were observed in the placebo group. Next, in SEr (index of skin roughness), a significant improvement was observed at the sites below the left eye and dorsal neck after six weeks of ingestion compared to that before ingestion. Similarly, SEsc (index of corneous dryness) significantly improved in all sites in the ceramide group, showing that corneous dryness was reduced and scaling was decreased in all regions (Fig. 2). In contrast, no significant improvements were observed in the placebo group. SEw (number and width of skin wrinkles) was significantly improved at the site below the left eye after three weeks of ingestion in the ceramide group, but no other marked changes were observed.

Fig. 3 shows the VISIOSCAN images (23 years old, below the left eye) before ingestion and after six weeks of ingestion in the ceramide group. It is clear that the water content increased after ingestion, reducing the skin dryness, and the wrinkles became shallow.

The above findings showed that a dietary supplement containing rice-derived ceramide reduces skin dryness and improves the skin conditions in dry skin.

[Discussion]

In previous clinical studies of test products that are expected to have a skin-beautifying effect including external preparations

and cosmetics, the evaluation had to depend on classical dermatological diagnosis by physicians. Therefore, the evaluation greatly depended on the skill of physicians, and it had been difficult to completely eliminate the difference among physicians in a large scale study system. Development of a technique of quantifying the skin conditions by imaging analysis in this study allowed us to performed objective evaluation.

Because this study was performed by the double blind method, the subjects and dermatologists did not know whether the ingested capsule contained the rice-derived ceramide or the placebo. Therefore, subjective findings or diagnoses by the dermatologists showed a clear placebo effect. However, on analysis using the microscopic three-dimensional skin surface analysis system (VISIOSCAN) introduced in this study, no marked changes were observed in the placebo group, and the effects significantly differed between the ceramide and placebo groups. Therefore, ceramide was demonstrated to be effective in prevention of skin dryness and roughness by objective measurement in addition to observation by physicians.

Sphingolipids have been shown to be involved in homeostasis of cell functions of the biological membranes, and play an important role in apoptosis of cancer cells and inhibition of cell growth. The absorption process of orally ingested plant ceramide has also been experimentally examined. Plant ceramide is absorbed from the small intestine as the unchanged form or the metabolized forms in

sphingosine and fatty acids, then reconstructed into ceramide, and enters the stratum corneum and the interstitium between corneocytes via capillary blood vessels. Moreover, ceramide is distributed in tissues such as the brain. Therefore, the dietary supplement containing rice-derived ceramide evaluated in this study may be a safe and superior beautifying agent, showing the effect by oral ingestion.

[Acknowledgment]

We deeply appreciate students and faculty of Osaka Foreign Language University and clinical investigators at the Institute of General Medical Science for cooperation. We also thank Oriza Yuka Co., Ltd.(オリザ油化株式会社) for providing product samples of ceramide.

Table 1 Subject Backgrounds

	Ceramide group	Placebo group
Number of subjects	17	16
Age (years old)	24.9 ± 5.8	25.3 ± 9.8
Height (cm)	162.5 ± 7.4	162.2 ± 8.4
Body weight (kg)	53.9 ± 7.6	56.3 ±10.0
BMI	20.3 ± 1.6	21.3 ±2.3
Number of smokers	3	3

* No significant differences were observed in any of the background items including other factors of skin trouble, time and regularity of sleep, time of exposure to direct sunlight (hour/day), irregular menstruation, occupation, or alcohol drinking habit.

Table 2 Results of dermatological diagnosis before and after ingestion of the test food

		Ceramide group				Placebo group			
		Number of subjects with symptoms	Before ingestion	After three weeks	After six weeks	Number of subjects with symptoms	Before ingestion	After three weeks	After six weeks
face	Cosmetic rash	4	1.25	1.00	1.00	3	1.00	1.00	1.00
	Dryness	17	2.00	1.29**	1.18**	16	2.13	0.69*	1.63**
	Flush	14	1.86	1.29**	1.21*	15	1.93	1.53*	1.47*
	Holding of cosmetic	8	1.88	1.13*	1.00*	5	1.40	1.20	1.20
General	Itching	17	1.65	1.24*	0.94**	12	1.92	1.50	1.17**
	Dryness	17	2.18	1.47**	1.18**	16	2.00	1.44	1.38*
	Flush	7	1.71	1.29	0.86*	10	1.80	1.30*	1.20*
	Erosion	2	2.00	1.00	0.00	3	2.33	2.00	2.00
	Squamation	4	1.50	1.25	0.75	5	1.80	1.60	1.60
	Papules	3	1.33	1.33	1.33	4	1.75	1.50	1.75
	blebs	2	1.50	1.50	1.50	2	1.50	1.50	2.00
	Swelling	0				3	2	1.67	1.67
	Overall	17	1.71	1.24*	1.00**	16	1.69	1.25	1.31

* The value represents the mean in each group. (non-parametric analysis was employed for statistical analysis)

0 (no symptoms) - 1 (mild) -2 (moderate) -3 (severe)

Table 3 Improvement rate of each symptom

Symptoms	Test food	Total number of subjects with symptoms	Improvement rating					Improvement rate (Improved or better)
			Markedly improved	Improved	Unchanged	Aggravated	Markedly aggravated	
Facial symptoms								
Cosmetic rash	ceramide	4	0	1	3	0	0	25.0%
	placebo	3	0	0	3	0	0	0.0%
Facial dryness	ceramide	17	3	8	6	0	0	64.7%
	placebo	16	0	8	8	0	0	50.0%
Facial flush	ceramide	14	2	8	2	2	0	71.4%
	placebo	15	2	3	10	0	0	33.3%
Holding of cosmetic	ceramide	8	2	3	3	0	0	62.5%
	placebo	11	0	1	4	0	0	9.1%
Somatic symptoms								
Itching	ceramide	17	5	4	8	0	0	52.9%
	placebo	12	4	1	7	0	0	41.7%
Dryness	ceramide	17	6	5	6	0	0	64.7%
	placebo	16	1	8	7	0	0	56.3%
Flush	ceramide	6	2	2	2	0	0	66.7%
	placebo	10	2	3	5	0	0	50.0%
Erosion	ceramide	2	2	0	0	0	0	100.0%
	placebo	3	0	1	2	0	0	33.3%
Squamation	ceramide	4	2	1	0	1	0	75.0%
	placebo	5	0	1	4	0	0	20.0%
Papules	ceramide	3	0	0	3	0	0	0.0%
	placebo	4	0	1	2	1	0	25.0%
blebs	ceramide	2	0	1	0	1	0	50.0%
	placebo	2	0	0	1	1	0	0.0%
Overall	ceramide	17	3	8	5	1	0	64.7%
	placebo	16	0	7	8	1	0	43.8%

Table 4 Measurement results of water content, pH, and oil content before and after ingestion of the test food

		Ceramide group (n=17)		
		Before ingestion	After three weeks	After six weeks
Water content	Below the left eye	43.2 ± 5.5	48.0 ± 14.3	52.2 ± 12.1 **
	Left forearm	37.0 ± 5.6	41.1 ± 11.0	43.2 ± 35.7 **
	Dorsal neck	43.5 ± 10.8	51.2 ± 11.76 **	55.9 ± 11.1 **
Acidity (pH)	Below the left eye	5.8 ± 0.7	5.6 ± 0.6	5.8 ± 0.5
	Left forearm	5.5 ± 0.5	5.5 ± 0.6	5.8 ± 0.5
	Dorsal neck	5.9 ± 1.1	5.5 ± 0.5	5.4 ± 0.4
Oil content	Below the left eye	42.3 ± 34.8	49.9 ± 35.1	38.1 ± 25.9

		Placebo group (n=16)		
		Before ingestion	After three weeks	After six weeks
Water content	Below the left eye	43.4 ± 5.4	43.2 ± 9.2	41.7 ± 9.4
	Left forearm	35.7 ± 5.6	37.7 ± 7.0	35.7 ± 9.0
	Dorsal neck	49.1 ± 8.8	51.0 ± 10.4	56.1 ± 20.5
Acidity (pH)	Below the left eye	5.9 ± 0.8	5.8 ± 0.6	5.9 ± 0.7
	Left forearm	5.5 ± 1.0	5.6 ± 0.8	5.9 ± 0.5
	Dorsal neck	5.9 ± 0.8	5.5 ± 0.5	5.8 ± 0.4
Oil content	Below the left eye	58.4 ± 55.8	29.5 ± 22.0	40.8 ± 33.3

* Each value represents the mean±S.D. (non-parametric analysis was employed for statistical analysis)

Fig. 1 Changes in water content (below the left eye)

Water content (%)

Table 5 Parameter values measured by VISIOSCAN before and after ingestion of the test food

		Ceramide group			Placebo group		
		Before ingestion	After three weeks	After six weeks	Before ingestion	After three weeks	After six weeks
Kurtosis (Ideal value: 0)	Below the left eye	0.38	0.37	0.35	0.39	0.38	0.38
	Left forearm	0.35	0.39	0.40	0.43	0.43	0.40
	Dorsal neck	0.40	0.40	0.30 *	0.40	0.40	0.40
SE sm (Ideal value: low value)	Below the left eye	377.4	364.4	342.1	368.0	354.1	347.7
	Left forearm	339.4	304.8	308.5	326.1	317.3	334.2
	Dorsal neck	386.8	327.8 *	333.1 *	355.2	349.2	354.5
SE r (Ideal value: low value)	Below the left eye	0.29	0.26	0.25 *	0.30	0.31	0.30
	Left forearm	0.26	0.20	0.16	0.31	0.26	0.25
	Dorsal neck	0.18	0.15	0.14 *	0.31	0.30	0.30
SE sc (Ideal value: low value)	Below the left eye	49.6	47.6 *	46.8 *	46.6	46.8	46.6
	Left forearm	48.9	47.9 *	47.6 *	48.3	48.9	48.4
		46.1	44.5 *	42.9 *	46.4	46.9	46.4
SE w (Ideal value: low value)	Below the left eye	36.1	32.3 *	33.9	36.0	33.0	35.5
	Left forearm	26.7	24.7	27.1	27.5	24.5	27.7
	Dorsal neck	28.4	24.7	26.1	28.2	25.9	30.6

* The value represent the mean in each group.

Fig. 2 Changes in SEsc (moisture/smoothness of skin) (dorsal neck)

Skin squamation (+), Roughness (+)

Moisture (+), Smoothness (+)

Fig. 3 A 23-year-old woman, below the left eye

Top: Before ceramide ingestion

Bottom: After six weeks of ceramide ingestion

Fig. 1 Changes in water content (below the left eye)

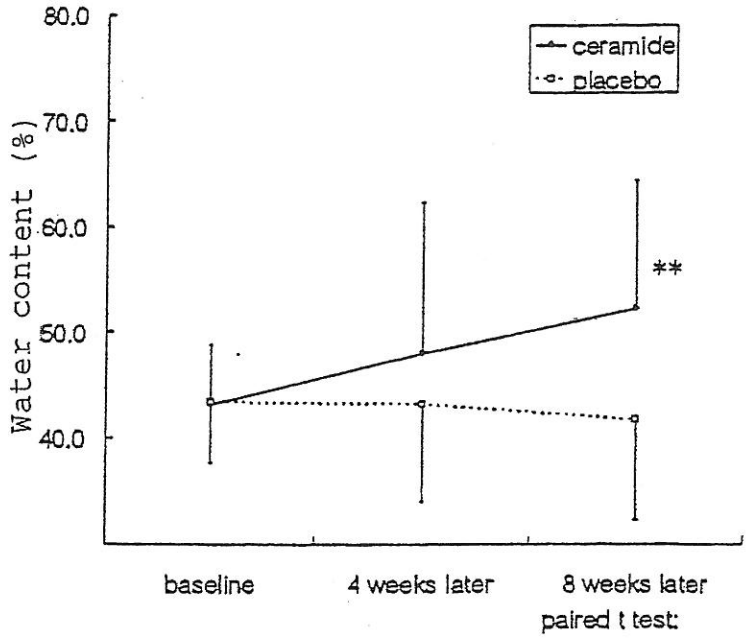


Fig. 2 Changes in SEsc (moisture/smoothness of skin) (dorsal neck)

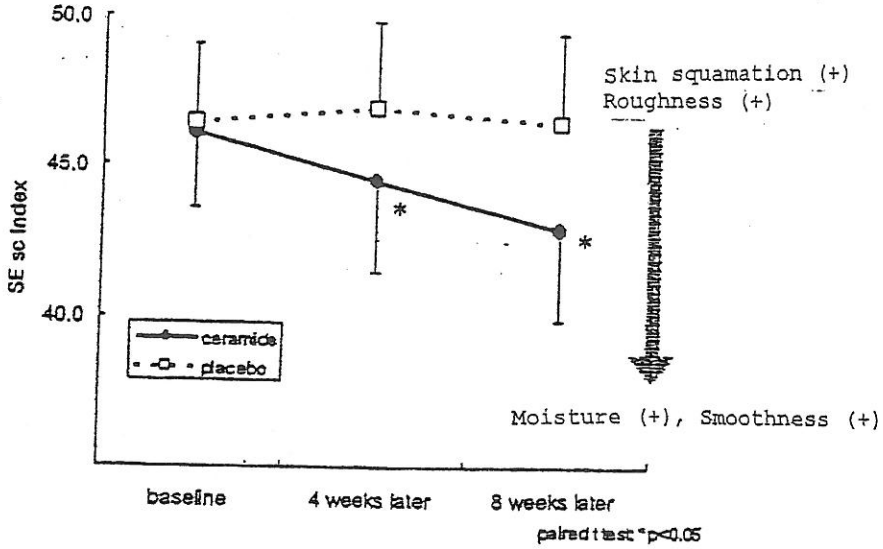
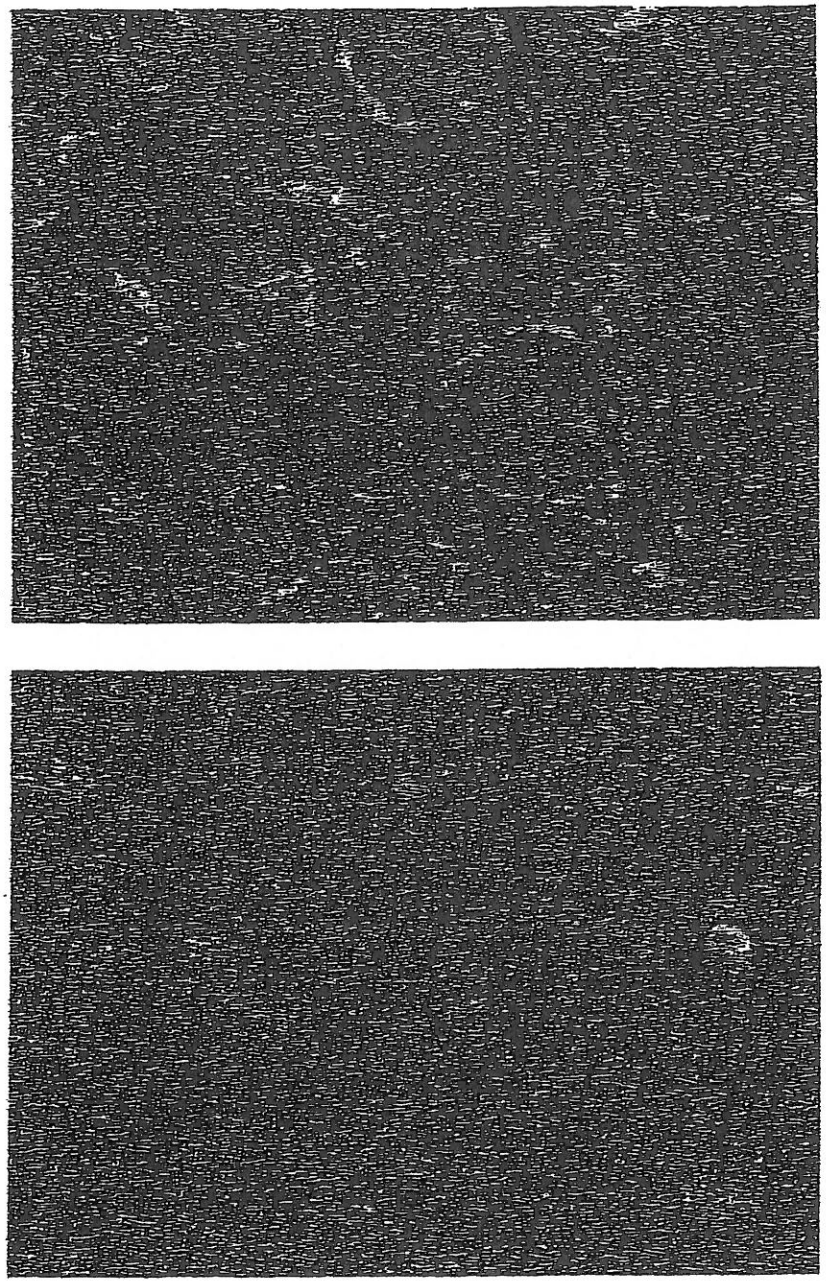


Fig. 3 A 23-year-old woman, below the left eye



Top: Before ceramide ingestion

Bottom: After six weeks of ceramide ingestion