

Effects of astaxanthin-containing drinks on skin

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Abstract

Objectives: To investigate the effect of drinks containing astaxanthin (AX) on the skin.

Methods: Subjects were Japanese women aged 30 to 49 years old who were concerned about dull skin or skin deterioration such as sagging and dryness due to aging. A double-blind comparison study was performed to evaluate the results objectively. A total of 20 subjects were randomly divided into 2 groups for intake of AX-containing drink (3 mg per serving, AX group) or placebo drink (control group). Skin conditions were evaluated based on erythema, water content, transepidermal water loss, viscoelasticity, and complexion analysis results (VISIA) Before and 4 and 8 weeks after the commencement of the study.

Results: Intergroup comparison showed that water content, transepidermal water loss, viscoelasticity, erythema, and texture were significantly better in the AX group than in the control group.

Conclusions: AX suppressed damage to the skin barrier mechanism, alleviated skin dryness by preserving the water-retention capacity of the skin, and improved erythema, viscoelasticity, and texture. No adverse events attributable to the test drinks were observed. Taken together, these results suggest that AX-containing drinks are safe and beneficial to the skin.

Key words: Astaxanthin, skin moisture, skin barrier, water content in skin, transepidermal water loss

Introduction

Astaxanthin (AX), a carotenoid widely distributed in nature, is an extremely potent singlet oxygen scavenger with anti-lipid peroxidation activity^{1,2}. In a study investigating the effects of AX on the skin, AX suppressed the ultraviolet B (UVB)-induced secretion of proinflammatory prostaglandin 2 (PGE2) and interleukin (IL)-8 in human epidermal keratinocytes³. In addition, the beneficial effect of AX in atopic dermatitis has been shown in human studies⁵ and in an animal model of atopic dermatitis⁴. Furthermore, AX suppressed UV-induced skin pigmentation in adult women⁶, garnering attention in terms of skin anti-aging strategy. In this double-blind comparison study, we objectively investigated the effect of AX-containing drinks on the skin of adult women by establishing a control group for the intake of placebo drinks.

Materials and Methods

1. Subjects

This study enrolled 20 healthy adult women who provided written consent to participate in the study, fulfilled study inclusion criteria, and were not in conflict with exclusion criteria.

(1) Inclusion criteria:

- i) Japanese women aged 30 to 49 years at the time of signing the consent form.
- ii) Those who had concerns about dull skin.
- iii) Those who had concerns about skin deterioration such as sagging and dryness due to aging.
- iv) Those who agreed not to change or add cosmetics during the study period.
- v) Those who agreed to avoid intentional sun exposure, which might cause sunburn, and to utilize protective measures against UV radiation.

(2) Exclusion criteria:

- i) Those who had chronic diseases and were taking medication.
- ii) Those who had skin disorders such as atopic dermatitis and perioral dermatitis.
- iii) Those who had a history of food allergy or its complications.
- iv) Those who continuously consumed, during the last 3 months, health foods that may affect the study results.
- v) Those who had or were involved in one or more of the following in the last 3 months:
 - Hormone replacement therapy.
 - Cosmetic procedures or medical cosmetic treatments that may influence the evaluation of the study area.

• Regular use of cosmetics (such as a makeup sealer and face patches) that might improve skin moisture and wrinkles.

vi) Excessive alcohol consumption.

vii) Those who might modify their lifestyles (for example, starting to work a night shift or taking a long trip) during the study period.

viii) Those who were pregnant or lactating or may be/become pregnant.

ix) Those who were considered ineligible for the study by the principal investigator and physician.

2. Study method

A double-blind comparison study was conducted at Shirokane Exe Clinic. Subjects were randomly divided into 2 groups for intake of either AX-containing drinks (AX group) or placebo drinks (control group). The scientific and ethical aspects of this study were evaluated and approved by the Ethics Committee of TES Holdings Co. This study was conducted in compliance with the Declaration of Helsinki.

(1) Test drinks

Test drinks used in this study were two 350-ml beverage-type drinks containing 3 or 0 mg of AX along with other ingredients (Table 1). The latter with 0 mg of AX was used as control and contained the pigment from paprika to make the appearance indistinguishable from the AX-containing drinks. For 8 weeks, subjects consumed 1 drink daily as appropriate. Test drinks were stored in a cold place away from direct sunlight, high temperature, or high humidity.

Table 1. Composition of test drinks

AX-containing drink (350 ml)	Placebo drink (350 ml)
AX 3 mg	AX 0 mg
Crystalline fructose	Crystalline fructose
-	Paprika pigment
Citric acid	Citric acid
Sodium citrate	Sodium citrate
L-ascorbic acid	L-ascorbic acid
Sucralose	Sucralose
Flavoring	Flavoring

(2) Observation/measurement parameters and duration

Based on the measurement parameters of skin color, melanin/redness, water content, and viscoelasticity, physicians selected 20 out of 30 individuals who were not in conflict with the

exclusion criteria. Measurements were performed before and 4 and 8 weeks after the commencement of the study. Before each measurement, subjects cleaned the measurement sites, including the face, and acclimated to an environment for 20 min in a temperature (20–22°C) and humidity (50%±10%) controlled room. To ensure safety, we examined for adverse events when subjects visited the clinic.

i) Water content in skin

Using the Corneometer (CM825[®]; Courage+Khazaka electronic GmbH, Cologne, Germany), water content in the face was measured at the apex of the left cheek bone in an environmentally controlled room. Measurement was repeated 5 times at the same spot. After eliminating the highest and lowest values, 3 measurement values were averaged.

ii) Transepidermal water loss

Using the Tewameter (TM300[®]; Courage+Khazaka electronic GmbH, Cologne, Germany), transepidermal water loss was measured at the apex of the left cheek bone in an environmentally controlled room. Values with ≤ 0.5 standard deviation (SD) were used in this study.

iii) Skin color

Using a spectrophotometer (CM-2600d; Konica Minolta, Inc., Osaka, Japan), skin color was measured at the apex of the left cheek bone in an environmentally controlled room. Measurement was repeated 5 times at the same spot. After eliminating the highest and lowest values, 3 measurement values were averaged. Analysis was performed using L (Lightness) values.

iv) Melanin and erythema (redness)

Using the Mexameter (MX18[®]; Courage+Khazaka electronic GmbH, Cologne, Germany), measurement was repeated 5 times at the apex of the left cheek bone in an environmentally controlled room. After eliminating the highest and lowest values, 3 measurement values were averaged.

v) Viscoelasticity R2 and R7

Using the Cutometer MPA580[®] (Courage + Courage+Khazaka electronic GmbH, Cologne, Germany) on subjects in a supine position, viscoelasticity was measured at the center of a line connecting a point just below the left earlobe and the left corner of the mouth in an environmentally controlled room. Measurement was repeated 5 times at the same spot. After eliminating the highest and lowest values, 3 measurement values were averaged.

vi) Complexion analysis

The VISIA[™] Evolution Complexion Analysis System (Canfield Scientific Inc., New Jersey, USA) was

used to score brown spots, pores, porphyrins, red areas, spots, texture, UV spots, and wrinkles on the left side of the face.

vii) Skin surface replica analysis

Using a three-dimensional replica analysis system (ASA-03RXD; Asahi Biomed, Tokyo, Japan), skin samples for replica preparation were collected at the center of a line connecting a point below the left earlobe and the left corner of the mouth. Analysis was performed by illuminating a replica at 0 and 180 degrees to calculate the mean texture value using the number of skin ridges and the volume ratio of skin ridges.

(3) Number of subjects

The number of subjects was set based on the findings of previous clinical studies investigating the effect of AX-containing drinks on the skin.

(4) Analysis

In each measurement, mean \pm SD values were calculated, and the effect of test drinks was evaluated based on a percentage change in the after-intake value in relation to the before-intake value. For intergroup comparison, the F-test of equality of variance was performed to test whether the variances of two groups were equal. When equal, student t-test was performed, and when unequal, Aspin-Welch t-test was performed. Statistical significance was set at $p < 0.05$.

Results

1. Subjects' background

The mean age of participants was 41.1 ± 5.0 years in the AX group ($n = 10$) and 42.1 ± 5.3 years in the control group ($n = 10$), with no significant intergroup difference.

2. Measurement results

The measurement results for water content and transepidermal water loss are shown in Table 2. Water content was determined by measuring the electrical charge of water inside the stratum corneum. To determine transepidermal water loss, the cavity of a probe containing temperature and humidity sensors was placed against the subject's skin at a measurement site to analyze the water evaporating from their skin. After the 8-week intake of test drinks, water content in skin was 102.9 ± 4.5 in the AX group and 89.2 ± 12.2 in the control group, with a significant difference between the groups ($p < 0.01$). Also after the 8-week intake of test drinks, transepidermal water loss was 95.3 ± 9.6 in the AX group

and 120.0 ± 28.7 in the control group, with a significant intergroup difference ($p < 0.05$).

Table 3 shows the measurement results for skin color, melanin, erythema, viscoelasticity, complexion (VISIA analysis), and texture (replica analysis). Skin color was measured using a spectrophotometer, and the L values were evaluated. The level of melanin and the severity of erythema were determined by illuminating the skin with a constant amount of light and measuring the amount of light reflected from the skin. Viscoelasticity was determined by suctioning the skin at the measurement site for a predetermined period of time and measuring the height of the skin as it was returning to the original state. The results revealed a significant difference in erythema (83.8 ± 8.5 and 105.4 ± 12.2 , $p < 0.01$) and viscoelasticity (R7) (118.0 ± 15.5 and 101.9 ± 8.0 , $p < 0.05$) between the AX and control groups, respectively, after 8 weeks. Skin color, melanin, and viscoelasticity (R2) did not differ significantly between the groups.

The VISIA system was used to magnify the area of measurement for detailed observation. Complexion was evaluated based on the size, area, and concentration of individual measurement items in the preset analysis window. The results revealed a significant difference in texture between the AX and control groups (84.2 ± 20.7 and 133.3 ± 38.3 , respectively; $p < 0.01$) after 8 weeks. No significant intergroup difference was observed in brown spots, pores, porphyrins, red areas, spots, UV spots, or wrinkles. In addition, no significant intergroup difference was observed in texture volume ratio or numbers in the three-dimensional texture analysis using skin replicas.

Table 2. Water content and transepidermal water loss in the skin (percentage change in after-intake measurement value in relation to before-intake value)

Parameter	Group	4-week intake (%)	8-week intake (%)
Water content (CM units)	AX	101.3 ± 6.5	$102.9 \pm 4.5^{**}$
	Control	98.6 ± 12.5	89.2 ± 12.2
Transepidermal water loss (g/hm^2)	AX	99.7 ± 8.1	$95.3 \pm 9.6^*$
	Control	125.5 ± 46.4	120.0 ± 28.7

Mean \pm standard deviation

*, $p < 0.05$ Student's t test (vs control group)

***, $p < 0.01$ Student's t test (vs control group)

Table 3. Role of AX in the skin
(percentage change in after-intake measurement value in relation to before-intake value)

	Parameter	Group	4-week intake (%)	8-week intake (%)
	Skin color (L value)	AX	102.2±1.6	103.1±3.5
		Control	100.7±2.4	101.4±1.7
	Melanin (M value)	AX	92.7±7.6	83.5±8.3
		Control	91.8±7.2	86.4±7.0
	Erythema (E value)	AX	87.7±8.8**	83.8±8.5**
Control		111.4±15.9	105.4±12.2	
	Viscoelasticity R2 ^a	AX	101.9±2.5	102.1±2.9
		Control	101.7±2.4	100.3±3.1
	Viscoelasticity R7 ^b	AX	113.0±15.3	118.0±15.5*
		Control	102.9±6.2	101.9±8.0
Complexion analysis (scores)	Brown spots	AX	103.6±11.4	93.9±10.9
		Control	98.5±9.2	95.4±12.4
	Pores	AX	98.9±20.8	109.2±21.9
		Control	119.8±21.9	120.1±22.8
	Porphyrins	AX	118.8±84.7	115.1±54.7
		Control	106.6±67.7	126.4±64.8
	Red areas	AX	91.8±24.9	96.1±25.6
		Control	110.3±26.2	94.5±23.5
Spots	AX	108.5±16.8	83.6±10.9	
	Control	96.8±9.0	90.3±10.5	
Texture	AX	85.8±22.5*	84.2±20.7**	
	Control	131.0±43.0	133.3±38.3	
UV spots	AX	112.0±16.5	115.9±26.3	
	Control	113.9±32.7	120.2±36.1	
Wrinkles	AX	71.9±41.7	74.6±50.6	
	Control	101.0±65.5	109.2±57.1	
Texture analysis	Skin Ridge Volume Ratio ($\mu\text{m}^3/\text{mm}^2/100$)	AX	150.8±81.9	154.1±81.1
		Control	99.2±54.0	101.2±34.9
	Number of Skin Ridges (N/mm)	AX	166.3±91.9	176.1±131.5
		Control	104.6±48.9	110.5±30.4

Mean ± standard deviation

*, p < 0.05 Student's t-test (vs. control group); **, p < 0.01 Student's t-test (vs. control group)

^a Gross elasticity ^b Biological elasticity

3. Adverse events

No adverse events attributable to the test drinks were observed.

Discussion

After the 8-week intake of test drinks, water content in the skin, transepidermal water loss, erythema (redness), viscoelasticity (R7), and texture scores improved significantly in the AX group compared with the control group. Several previous studies investigated the effect of AX on the skin in humans. In a study of women in their 20s and 30s, UV-induced skin pigmentation was significantly reduced after intake of 3 mg of AX once daily for 4 weeks⁶. In addition, wrinkles, skin moisture, and viscoelasticity were improved in healthy adults who took 4 mg of AX once daily for 8 weeks⁷. Furthermore, wrinkles, texture, and water content in skin improved significantly in healthy women who took 6 mg of oral and topical AX daily for 8 weeks, whereas a significant improvement of wrinkles, viscoelasticity, and transepidermal water loss was observed among healthy adults who took 6 mg of oral AX daily for 8 weeks⁸. These studies used AX in capsules, while in this study, we developed an AX-containing drink to investigate how it improves skin conditions. As in the studies using capsules, our study also showed the beneficial effects of AX on water content in skin, transepidermal water loss, and viscoelasticity.

The stratum corneum, 0.02 mm in thickness, plays the role of a protective barrier, but when this skin barrier function is compromised by various stimuli, such as UV rays and foreign objects, transepidermal water loss increases. Transepidermal water loss is defined as the amount of water evaporation from the body via the stratum corneum, and is regarded as an indicator of skin barrier function. In this study, AX intake improved skin viscoelasticity and texture presumably because AX preserved skin barrier function and thereby suppressed water loss from the skin. Treatment of erythema, which is an UV-induced reddening of the skin, is of great importance because erythema may trigger further skin damage if left untreated.

A study using human epidermal corneocytes has shown that AX suppresses the UVB-induced secretion of PGE2 and the inflammatory cytokine IL-8³. Also, in a study using human dermal fibroblasts, addition of AX suppressed the UVA-induced expression of matrix-metalloproteinase-1, skin fibroblast elastase, and secretion of the inflammatory cytokine IL-6, suggesting that this is how AX suppresses photoaging effects such as sagging skin and wrinkles⁹. These studies suggest that AX acts on cytokines and protein enzymes involved in the skin barrier mechanism to improve/preserve the barrier function of the skin. Because AX inhibits the activation of the transcription factor NFκ-B, which plays a key role in pro-inflammatory and pro-aging reactions, AX is expected to function as a potent anti-aging agent in the skin¹⁰. In addition, studies investigating the role of AX in the expression

of aquaporin 3, a water channel protein, are currently underway and attracting attention.

— We conducted the present study in early winter, and our findings suggest that AX preserves skin moisture while preventing skin deterioration, dryness, and other bad conditions that are attributed to changes in outside environments, such as UV irradiation and cold or dry air.

Conclusion

Our findings suggest that AX suppresses damage to the barrier mechanism, preserves water-retention capacity, and alleviates dry skin, while also improving erythema, viscoelasticity, and texture. No adverse events attributable to the test drinks were observed in this study. Taken together, these findings suggest that AX-containing drinks are safe and beneficial to the skin.

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Abstract

Effects of Intake of Astaxanthin Contained Drink on Skin Condition

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Objectives:

To study the effect of astaxanthin contained drink to skin condition

Method:

The study was conducted to the Japanese females between age over thirty to less than fifty, who had weakening of skin (including aging, sag and dry skin) and skin dullness.

In order to conduct the objective evaluation, the comparison between the groups by the double-blind test was taken.

Twenty of subjects were randomly allocated to the intake group of astaxanthin contained drink (astaxanthin 3mg contained) and the placebo group.

After eight weeks intake of the drink, each group was evaluated with skin water contents, transepidermal water loss, skin elasticity, VISIA and skin texture etc.

Result:

In between the groups, the intake group of astaxanthin contained drink was greatly excellent in the categories of skin moisture, transepidermal water loss, skin elasticity, erythema dose and skin texture.

Conclusion:

Astaxanthin has protecting effect of skin barrier and is considered to increase the water retention capability to reduce skin dryness. Astaxanthin is also effective to erythema dose, skin elasticity and skin texture. As no adverse events resulting from the test drink was seen,

such food containing astaxanthin is considered as a safe and useful health functional food material to skin.

Key word : astaxanthin、 skin moisture、 skin barrier、 skin water contents、
transepidermal water loss