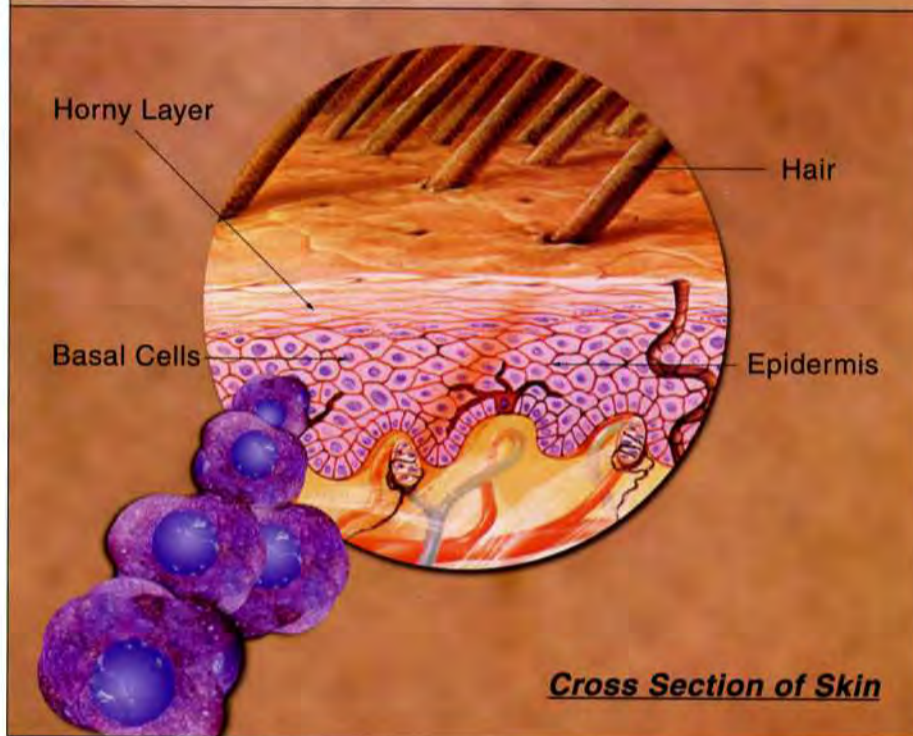


## NUTRACEUTICALS SUPPORT HEALTHY SKIN FUNCTION



### IN THIS EDITION

- Pilot Study: Orally-Administered Yeast  $\beta$ 1,3-glucan Prophylactically Protects Against Anthrax Infection and Cancer in Mice
- Results of a Study Evaluating the Use of a Dietary Supplement Formula in the Management of Age-Related Skin Changes in Women with Moderate to Severe Wrinkling of the Periorbital Area
- St. John's Wort for Depression: Weight of All the Evidence Still Favors Effectiveness of Herb for Mild to Moderate Depressive Disorders
- The Potential Application of *Spirulina* (*Arthrospira*) as a Nutritional and Therapeutic Supplement in Health Management

### AND MORE

**A Peer-Reviewed Journal on Nutraceuticals and Nutrition**

ISSN-1521-4524

# Results of a Study Evaluating the Use of a Dietary Supplement Formula in the Management of Age-Related Skin Changes in Women with Moderate to Severe Wrinkling of the Periorbital Area

Irwin Kantor, MD, FAAD,\*<sup>1</sup> Louise A. Donikyan, DO,<sup>1</sup>  
Randi Simon, BS,<sup>1</sup> Bernd Wollschlaeger, MD<sup>2</sup>

<sup>1</sup>RTL, Inc., Hackensack, New Jersey

<sup>2</sup>Clinical Assistant Professor of Medicine and Family Medicine,  
University of Miami School of Medicine, Miami, Florida

## ABSTRACT

### Context:

The relationship between dietary supplementation and the repair of aging skin is unclear.

### Objective:

To assess the safety and efficacy of a dietary formulation for the improvement of age-related degenerative skin changes in women.

### Methods:

Using a blinded, randomized, parallel design, a total of 40 women, ages 35-65 years were enrolled and divided into approximately balanced groups with 38 women completing this eight-week, four-visit study. Recommended daily doses of the study product, a dietary collagen formulation (Toki), were 7.5 g (given to Group A) and 8.5 g (given to Group B). Both investigator and subjects assessed skin wrinkling

and other skin characteristics in the periorbital area as well as overall facial aging.

### Main Outcome Measures:

Safety and efficacy of a dietary collagen formulation when used to improve periorbital wrinkling, aging, sagging and puffiness and periorbital overall facial aging.

### Results:

The consumption of the dietary collagen formulation resulted in a highly statistically significant improvement in periorbital wrinkling, in periorbital aging and in periorbital overall facial aging. The investigator's mean global improvement scores of overall facial aging as compared to baseline photographs were highly significant.

### Conclusion:

In women with age-related skin changes, a dietary collagen formulation (Toki) significantly improved periorbital wrinkling, periorbital aging, and periorbital overall facial aging with minimal adverse effects.

## INTRODUCTION

Women of all ages are increasingly seeking facial skin treatment for personal and professional reasons. The cosmetic and pharmaceutical industries are focusing their attention on product development and marketing for the prevention and management of skin aging.

### \* Correspondence:

Irwin Kantor, MD, FAAD  
RTL, Inc.

255 Great Neck Road,  
Great Neck, NY 11021

Phone: 516-773-7788 Fax: 516-773-7799

Email: info@rtlab.com

It is often forgotten that the predominant clinical and biochemical features of aged skin are mostly attributable to photoaging rather than chronological aging.

Chronologically aged, sun-protected skin is thin and has reduced elasticity, but is otherwise smooth and unblemished.<sup>1</sup>

Photoaging is a cumulative degenerative process induced by solar irradiation. Photoaging has well defined clinical and histological correlates, and is characterized clinically by wrinkles, mottled pigmentation, rough skin, and loss of skin tone.<sup>2,3,4</sup> The associated premature aging of the skin results largely from repeated exposure to ultraviolet (UV) radiation from the sun, including ultraviolet A (UVA) as well as ultraviolet B (UVB) radiation, reinforcing the need for sunscreens that block both.

The chronological, natural aging process decreases collagen synthesis and increases the expression of matrix metalloproteinases (*e.g.*, collagenase and elastase), whereas photoaging results in an increase of collagen synthesis and greater matrix metalloproteinase expression in human skin *in vivo*. Thus, the balance between collagen synthesis and degradation leading to collagen deficiency is different in photoaged and naturally-aged skin.<sup>5</sup> The major histologic alterations of photoaging lie in dermal connective tissue.<sup>6,7</sup>

The epidermis forms the outer protective layer of the skin of which the stratum corneum is the outermost layer. The dermis lies below and provides mechanical support for the epidermis. The extra cellular matrix in the dermis is composed primarily of type I collagen, with lesser amounts of type III collagen, elastin, proteoglycans, and fibronectin. The collagen fibrils are responsible for the strength and structural integrity of the skin.<sup>8</sup> Ultraviolet irradiation causes histologically a disorganization of collagen fibrils and the accumulation of abnormal elastin-containing material.<sup>9</sup> Such connective-tissue changes in photoaged skin include reduced levels of types I and III collagen precursors<sup>10</sup> and cross-links,<sup>11</sup> an increased ratio of type III to type I collagen,<sup>12</sup> and an increased level of elastin.<sup>13</sup> This reduction could result from increased degradation by metalloproteinases, a family of proteolytic enzymes that specifically degrade collagens, elastin, and other proteins in connective tissue and bone,<sup>14,15</sup> and/or from reduced procollagen synthesis. Recent findings indicate that fibroblasts from photoaged and sun-protected skin are similar in their capacities for growth and type I procollagen production; and that the accumulation of partially degraded collagen observed in photodamaged skin may inhibit, by an as yet unidentified mechanism, type I procollagen synthesis.<sup>16</sup>

In women, estrogen appears to slow the process of chronological skin aging in several ways. Estrogen 1) prevents a decrease in skin collagen in postmenopausal women; 2) increases the skin collagen content and therefore maintains skin thickness; and 3) maintains skin moisture by increasing acid mucopolysaccharides and hyaluronic acid in the skin

and possibly maintains stratum corneum barrier function.<sup>17</sup> The above beneficial effect cannot prevent the histopathological changes in photodamaged skin.

The periorbital region serves as a barometer of chronological and environmental age. Damaged skin can have significant psychological impact on affected men and women, who often seek its cosmetic rejuvenation, triggering the growing demand for effective treatments.

Minimally invasive soft tissue augmentation of the face with injectable substances has been performed for more than a century. During this period, many substances have been used to cosmetically improve soft-tissue defects and deficiencies.

Surgical approaches include facelift, dermabrasion, chemical peeling, collagen and botulinum toxin injections, laser resurfacing and fat transfer. Pharmaceutical approaches to photoaged skin can be categorized as antioxidants, alpha-hydroxy acids, beta and polyhydroxy acids, sunscreen lotions and topical retinoids.<sup>18</sup> Of these three approaches only topical retinoids, particularly tretinoin (all-trans retinoic acid), have a well-documented ability to repair photoaged skin at the clinical, histological, and molecular level.<sup>19</sup> The use of topical retinoids may actually prevent photoaging.<sup>20</sup>

All of the above treatment modalities may be associated with adverse effects including skin irritation and scarring, chemical or laser-induced burns, allergic reactions and pigment problems.<sup>21</sup> Nutritional modification and/or dietary supplementation might offer a physiological and safe approach to ameliorate chronological or sun-induced skin damage. Considering these potential beneficial effects of dietary and nutritional supplements on the skin remodeling and repair function, we studied a dietary collagen formula whose ingredients were collagen peptide, oyster acid, seaweeds, hyaluronic acid, dermatan acid, glucosamine, grape sugar, fermented lactic acid, malt sugar, citric acid, lemon, vitamin C, lemon juice, and the sweetener acesulfame-k.

## OBJECTIVE

The objective of this study was to determine the safety, efficacy, and dose-response relationship of a dietary supplement formulation in attenuating age-related skin changes in women with at least moderate wrinkling of the periorbital area (crow's feet).

## STUDY DESIGN

This was originally a three-visit, four-week, controlled clinical trial which was extended to a total of four visits over eight weeks. A sufficient number of females with at least moderate wrinkling of the periorbital area (crow's feet), who were between 35 and 65 years of age, were screened and qualified such that 40 subjects were enrolled in the study.

Subjects were randomized at the study site to one of two treatment groups; 50% of the subjects were assigned to use the product at a dose level of 7.5 g (Group A) and 50% at a dose level of 8.5 g (Group B). Dosing during study weeks 1 and 2 was three times daily (morning, mid-day, and evening) and during the remaining six weeks of the study, twice daily (morning and mid-day).

Determination of effectiveness was based on clinical perception of benefit, and safety on the incidence and type of adverse experience.

The study was initiated at the Hackensack facility of RTL, Inc., after review and approval of the study protocol, informed consent, product information, advertising and investigator's CV by the IRB/RTL on May 2, 2001. Amendment 1 to the protocol (dated June 6, 2001) and a Consent Form Addendum providing for the extension of the study to four visits over a total of eight weeks was approved by the IRB/RTL on June 12, 2001.

## STUDY POPULATION

Approximately 40 females between 35 and 65 years of age with at least moderate wrinkling in the periorbital area (crow's feet) were recruited at the single study site.

Prospective participants were excluded when they were currently involved in another investigational drug study or had been so involved within a period of 30 days prior to admission into this study. They were excluded when pregnant or nursing, suffering from known allergies or sensitivities to protein or other product ingredients, had pre-existing or dormant dermatological conditions (*e.g.*, psoriasis, atopic dermatitis, advanced skin cancer, *etc.*), had history of a disease/condition (*e.g.*, malabsorption), or a concurrent illness that could interfere with the outcome of the study or increase risk to the subject, had a history of GI surgery (at the discretion of the investigator), or were using any topical or systemic medication which could interfere with the outcome of the study. Ongoing oral medications (*e.g.*, birth control pills, oral or injectable insulin, *etc.*) were acceptable provided subject was on a stable regimen.

Prospective female participants were eligible for study entry at age 35 to 65 years when in good health and free of any facial skin problems (*e.g.*, scarring, rashes, irritation, chronic acne, rosacea, *etc.*) that would impair evaluations of the study area, had a score of 4-9 for the appearance of aging skin in the periorbital area, had discontinued systemic retinoids (other than normal recommended daily allowance of vitamin A) at least 12 weeks prior to first visit and during the study, had discontinued use of topical retinoids and/or topical facial alpha-hydroxy acids or other "face lifting," "anti-aging" products (including hydro-

quinone) 14 days prior to first visit and during the study, had not applied any topical products (*e.g.*, emollients, sunscreens) nor any cosmetics to the face one hour prior to study evaluations at Visit 1 and had a negative urine pregnancy test at the first visit.

Furthermore, prospective subjects of childbearing capacity had to satisfy one of the following three criteria:

1. Be currently engaging in (and/or during the study period planned to engage in) sexual activity that could lead to pregnancy and be willing to use an acceptable method of birth control (*e.g.*, oral contraceptive tablets, implanted contraceptive hormones, Depo-Provera® contraceptive injections, intrauterine devices, prophylactic condoms with spermicide, contraceptive diaphragms with spermicide, cervical caps with spermicide), as per the discretion of the investigator, or be in a monogamous relationship with a partner who had a vasectomy,
2. be sexually abstinent and intend to continue such practice, for the duration of the study,
3. be in a monogamous, same-sex relationship and have no intention to engage in sexual activity capable of producing pregnancy during the study period.

Additionally, prospective participants were not currently receiving or planning to receive any assisted reproductive technologies capable of producing pregnancy (whether in a single and abstinent, subfertile/infertile, or same-sex relationship), was post-menopausal or was congenitally, physiologically, or surgically sterile, was willing and able to follow all study directions and to commit to all visits for the duration of the study.

Before screening, prospective subject had read and signed the approved informed consent form after the nature of the study had been fully explained.

## METHODOLOGY

Subjects were randomized at the study site, in the order they appeared, to one of two treatment groups. Fifty percent of subjects were assigned to use a product dose level of 8.5 g and 50% were assigned to use a product dose level of 7.5 g. Each 8.5 g packet of product supplied the following: 600 mg activated calcium mineral, 3500 mg activated collagen, 250 mg activated complex mucopolysaccharide, 600 mg glucosamine and 200 mg vitamin C.

Both treatment groups were instructed to empty the contents of one packet in 8.5 ounces of liquid and to shake it vigorously until dissolved. Group A was to pour off one ounce of solution and drink the contents of the shaker bottle (7.5 g). Group B was to drink the entire contents of the shaker bottle (8.5 g). During Weeks 1 and 2 these packets had to be taken three times daily (morning, mid-day and evening) and during Weeks 3-8 two times daily (morning and mid-day).

The use of cosmetics was minimized. No approved emollients or cosmetics were applied to the face for one hour prior to study visits. Subjects were permitted to continue to use the sunscreen/s they ordinarily used, but not within one hour prior to study visits. If the administration of any medication became necessary, it had to be reported on the appropriate page of the case report form. The study monitor was to be notified in advance (or as soon as possible thereafter) of any such instances when appropriate.

Efficacy evaluations were conducted by the investigator and the study participants. At each visit to the study site, the investigator performed a clinical evaluation on a scale of 0 (none) to 9 of the periorbital skin area for sagging, puffiness, wrinkles, and overall aging skin, as well as the overall appearance of aging skin of the entire face with respect to wrinkles, puffiness and sagging. For qualification, the subject had to have an overall aging skin score of 4-9 in the periorbital area.

At Visits 2 to 8 the investigator evaluated the global improvement of the subject's facial appearance as compared to a baseline (Visit 1) photograph and scored such improvement as: -1= worse; 0 = no change; 1=slightly improved; 2= improved; 3 = much improved.

Forty-eight hours after initiation of treatment and at each visit subsequent to Visit 1, subjects self-evaluated improvement in their own skin condition by drawing a vertical line through a 160 mm horizontal visual assessment scale (VAS) with the left end labeled with a "0" and the right end with a "9". The parameters evaluated were as follows:

- peri-orbital skin sagging: 0 = more sagging and 9 = more lifted;
- peri-orbital skin puffiness: 0 = much more puffy and 9 = much less puffy;
- peri-orbital skin wrinkles: 0 = more/deeper wrinkles and 9 = less/smooth wrinkles;
- entire face/overall appearance of aging skin: 0= worse/more aging and 9 = much improved/looks younger.

## PROCEDURES

Each study participant underwent at baseline (Visit 1) the following procedures: medical history was obtained, the concomitant medication form and Skin Product Usage Questionnaire was completed, a urine pregnancy test was administered (when required), and weight was measured and recorded.

The above-described investigator's clinical assessments were conducted and when a subject met all inclusion/exclusion criteria she was enrolled as a study participant.

All participants were instructed to complete subject self-assessments 48 hours post-treatment. At the same time, blood was drawn and archived/frozen at -20°C for analysis of col-

lagen at a later date, and photographic images of the face were obtained using standardized equipment and procedures.

At 48 hours post-study enrollment, a Self-Assessment Questionnaire was completed by subjects at home and the form returned to the clinic via postal service.

On subsequent visits at Weeks 2, 4, and 8, participants were interviewed for adverse experiences, concurrent events or illness, use of concurrent therapy (including their daily skin care products), changes in medication, and missed dosing and/or changes in regimen of the test product, weight was measured and recorded and self-assessments were completed.

An investigator's clinical assessment was conducted that included a global evaluation of the subject's facial appearance compared to baseline photographs, blood was drawn and archived/frozen at -20°C for analysis of collagen, and photographic images were obtained. The test product was examined to record the use rate and ensure adequate compliance with the treatment regimen or collected and dispensed as needed. Any suspected compliance problems were discussed with the subject and resolved. At the end of the study, all participants completed a marketing questionnaire.

## RESULTS

Forty subjects were enrolled in the study and 38 completed it as of July 12, 2001. Subject No. 22 and Subject No. 35 discontinued after both withdrew consent.

The average age of the A (7.5 g) dosage group was 54 (range 38-65) years and of the B (8.5 g) dosage group 55 (range 39-65) years. With the exception of one Hispanic subject in the B group, all other subjects were Caucasian.

Over the course of the study eight subjects missed ten evaluation visits. All evaluation data obtained were included in Tables 1 and 4 which displayed the investigator's mean clinical evaluation scores and the subject's mean self-evaluation visual assessment scale (VAS) scores, respectively. For statistical treatments of differences between evaluation periods, only data from subjects at each of the two periods were used (correlated [non-independent] samples).

The mean values for the investigator's clinical evaluation scores and the range of values are reported in Table 1. The changes in mean score values over time (Visit 1 [baseline] through Visit 4) were not unidirectional except for Periorbital Wrinkles for both Groups A and B and Overall Periorbital Skin Aging for Group B, which showed improvement over the entire course of the study. The range of values at any time period for Groups A and B, and over the course of the study, generally overlapped. Occasional preliminary statistical comparisons between Group A and Group B values at the same time periods (data not shown) were not statistically significant. These findings were not surprising considering the similar dosage levels in Groups A and B.

**Table 2.** Overall changes in investigator's combined mean clinical evaluation<sup>a</sup>

	<u>Dosage/(n)<sup>b</sup></u>	<u>Visit 1</u>	<u>Visit 4</u>	<u>Δ<sup>c</sup></u>	<u>t<sup>d</sup></u>	<u>p<sup>e</sup></u>
<b>Periorbital Sagging</b>	A +B (33)	$\frac{3.12 \pm^f}{0.96^g}$	$\frac{3.33 \pm}{1.19}$	$\frac{+0.21 \pm}{1.54}$	0.79	<0.434
<b>Periorbital Puffiness</b>	A +B (33)	$\frac{3.30 \pm}{1.13}$	$\frac{3.67 \pm}{1.27}$	$\frac{0.36 \pm}{1.58}$	1.32	<0.195
<b>Periorbital Wrinkling</b>	A +B (33)	$\frac{5.03 \pm}{1.16}$	$\frac{4.06 \pm}{1.27}$	$\frac{-0.97 \pm}{1.21}$	4.6	<0.001
<b>Periorbital Aging</b>	A +B (33)	$\frac{4.82 \pm}{1.07}$	$\frac{3.76 \pm}{0.90}$	$\frac{-1.06 \pm}{1.27}$	4.8	<0.001
<b>Overall Facial Aging</b>	A +B (33)	$\frac{4.33}{1.16}$	$\frac{3.76 \pm}{0.87}$	$\frac{-0.58 \pm}{1.12}$	3.0	<0.006

- a. Scores: 0 = None; 1-3 = Mild; 4-6 = Moderate; 7-9 = Severe  
b. Combined values from A (7.5 g) and B (8.5 g) dosages with (n) equal to total number of values.  
c. Δ = the difference between visit 1 and visit 4 values.  
d. t = the t value determined for correlated (non-independent) samples.  
e. p = significance of the difference by two-tailed t test.  
f. mean score value (± standard deviation).  
g. standard deviation.

**Table 3.** Investigator's mean overall facial global improvement scores<sup>a</sup>

<u>Dosage/(n)<sup>b</sup></u>	<u>2</u>	<u>3</u>	<u>4</u>
A = 7.5g (18)	$\frac{0.83 \pm^c}{0.79^d}$ t <sup>e</sup> =4.5 P <sup>f</sup> <0.001	$\frac{1.33 \pm}{1.08}$ 5.2 <0.001	$\frac{1.83 \pm}{0.79}$ 9.9 <0.001
B = 8.5g (14)	$\frac{0.36 \pm}{0.50}$ t=2.69 P=0.019	$\frac{0.86 \pm}{0.66}$ 4.84 <0.001	$\frac{1.57 \pm}{0.65}$ 9.10 <0.001
A +B (32)	$\frac{0.63 \pm}{0.71}$ t=5.00 p<0.001	$\frac{1.13 \pm}{0.94}$ 6.76 <0.001	$\frac{1.72 \pm}{0.73}$ 13.34 <0.001

- a. Scores: -1 = Worsened; 0 = No change; 1 = Slight improvement; 2 = improved; 3 = Much improved as compared to baseline (Visit 1) photograph = to Zero.  
b. (n) = number of correlated (non-independent) values.  
c. mean score value (± standard deviation).  
d. standard deviation.  
e. t = the t values determined for correlated (non-independent) values.  
f. p = significance of the difference by two-tailed t test.

Therefore, additional analyses were limited to the significance of the overall changes between values for Visit 1 and Visit 4 of the combined data from Group A and Group B.

The statistical findings of the overall changes in the investigator's combined (A + B) mean clinical evaluation scores between Visits 1 and 4 were based on correlated (non-independent) samples and are displayed in Table 2. For Periorbital Sagging ( $p < 0.434$ ) and Periorbital Puffiness ( $p < 0.195$ ) the changes were not statistically significant. However, Periorbital Wrinkling and Aging and Overall Facial Aging all show highly significant improvements between Visits 1 and 4 ( $p < 0.001$ ,  $0.001$ ,  $0.006$ , respectively).

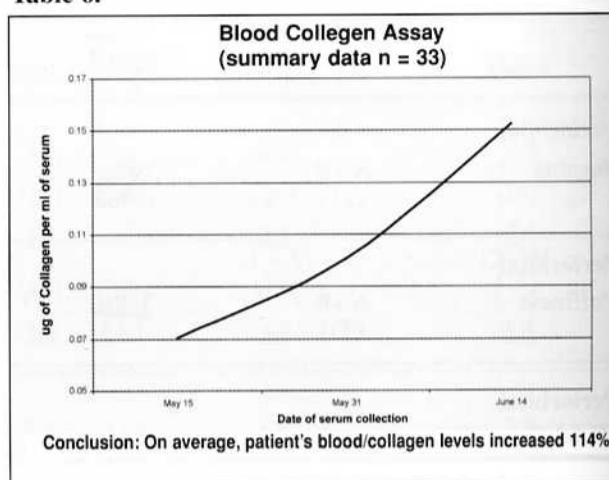
At Visits 2 through 4, the investigator evaluated the global improvement of each subject's facial appearance as compared to a baseline (Visit 1) photograph and scored any change as follows: -1 = worsened; 0 = no change; 1 = slight improvement; 2 = improved; 3 = much improved. The combined improvement score for each dosage (treatment) group at each of Visits 2 through 4 was the mean score per subject for the group (Table 3). As with the investigator's clinical evaluation scores, there was a general overlap between the ranges of values in the A and B groups at any time period and in this case mean Group A values were generally higher than Group B. Over time the mean global improvement scores increased, although there was no obvious linear correlation between improvement and the time course of the study. However, at all evaluation times and at both dosages (A or B) or the combined dosage groups (A+B) the mean global improvement scores were statistically significant ( $p < 0.019$  or better) as compared to the baseline photographs.

The subjects self-evaluated the improvement in their own skin condition by drawing a vertical line through a 160 mm horizontal visual assessment scale (VAS) marked on the left with a "0", indicating a worsening of their skin condition, and on the right with a "9", indicating improvement in skin condition. These mean self-evaluated VAS scores are displayed in Table 4.

At any time period, within any parameter evaluated, within both A and B treatment groups, there were wide variations in individual VAS values, with considerable overlap in the A and B group values. Mean VAS values for any single time period varied between 77.4 and 92.8 mm for the parameters evaluated in the A group and between 72.6 and 96.6 mm for the B group. These are displayed in Table 4. In all cases, there was an improvement in skin condition over the course of the study.

Using the "3 Day" mean values as baseline, the mean differences between the combined A + B "3 Day" values and end study (Visit 4) values for the parameters evaluated are presented in Table 5. For all four parameters (periorbital sagging, puffiness, wrinkling, and overall facial aging) self-evaluated by the subjects, the improvements over the course of the study were statistically significant ( $p < 0.002$ ,  $< 0.037$ ,  $< 0.002$ ,  $< 0.010$ , respectively).

**Table 6.**



Findings on blood collagen assays (Table 6) demonstrated an exponential increase in the blood collagen concentration ( $\mu\text{g/ml}$ ) during the course of the trial.

### SAFETY

During the course of this study, a total of 13 subjects presented with 22 adverse reactions of all types (seven treatment subjects with nine, and six B treatment subjects with 13). All reactions were resolved.

Of the total number of adverse reactions, Group treatment Subject Nos. 2, 30, and 38 presented with three possibly product-related adverse reactions, and Group treatment Subject No. 17 presented with three possible product related reactions. With the exception of a mild pruritus of the extremities over 3 days (No. 17), all other possibly product-related reactions were gastrointestinal in nature (diarrhea and one case of nausea). One of the reactions was moderate in severity (No. 38), while all others were mild. Overall, under the conditions of this study the dietary collagen treatments were apparently safe to use.

### DISCUSSION

The results of our study suggest that a dietary collagen formulation is a safe and efficacious modality to manage facial aging in women.

The beneficial effects of dietary and nutritional supplements on aging skin have been reported.

Animal experiments<sup>22</sup> revealed that caloric restriction (CR) in rodents results in an increase in both mean maximum life span and that nonenzymatic glycation of body proteins and subsequent advanced glycation reactions contribute to the aging process. In such experiments CR reduces the extent of glycation of blood and tissue proteins and the age-related accumulation of glycoxidation products.

**Table 4.** Subjects' mean self evaluation vas scores<sup>a</sup>

	<u>Dosage</u>	<u>Day 3</u>	<u>Visit 2</u>	<u>Visit 3</u>	<u>Visit 4</u>
<b>Periorbital Sagging</b>	A = 7.5 g	<u>82.4</u> <sup>b</sup> (63 - 108) <sup>c</sup> [20] <sup>d</sup>	<u>87.1</u> (50 - 124) [19]	<u>88.6</u> (50 - 126) [20]	<u>92.8</u> (61 - 127) [18]
	B = 8.5 g	<u>77.0</u> (39 - 110) [19]	<u>83.7</u> (25 - 129) [20]	<u>92.7</u> (54 - 131) [19]	<u>94.6</u> (77 - 137) [15]
	A + B	<u>79.7</u>	<u>85.4</u>	<u>90.7</u>	<u>93.7</u>
<b>Periorbital Puffiness</b>	A	<u>79.6</u> (35 - 125)	<u>84.0</u> (51 - 112)	<u>90.2</u> (52 - 123)	<u>84.8</u> (53 - 121)
	B	<u>74.0</u> (07 - 116)	<u>82.1</u> (30 - 123)	<u>91.1</u> (62 - 136)	<u>92.7</u> (62 - 137)
	A + B	<u>76.8</u>	<u>83.0</u>	<u>90.6</u>	<u>88.8</u>
<b>Periorbital Wrinkling</b>	A	<u>77.4</u> (16 - 111)	<u>82.5</u> (49 - 109)	<u>89.8</u> (53 - 153)	<u>88.0</u> (53 - 116)
	B	<u>73.3</u> (10 - 106)	<u>80.7</u> (8 - 125)	<u>86.9</u> (45 - 114)	<u>95.1</u> (77 - 136)
	A + B	<u>75.3</u>	<u>81.6</u>	<u>88.4</u>	<u>91.5</u>
<b>Overall Facial Aging</b>	A	<u>79.8</u> (64 - 109)	<u>87.5</u> (61 - 140)	<u>85.0</u> (45 - 126)	<u>86.3</u> (47 - 129)
	B	<u>72.6</u> (14 - 113)	<u>83.2</u> (10 - 126)	<u>85.5</u> (72 - 124)	<u>96.6</u> (66 - 155)
	A + B	<u>76.2</u>	<u>85.3</u>	<u>85.2</u>	<u>91.4</u>

a. In millimeters (mm) from 160 mm horizontal visual assessment scale marked at left end as "0", indicating worsening of condition, and at right end with a "9", indicating improvement of condition  
b. Mean score in mm      c. Range of values      d. Number of values

**Table 5.** Overall changes in subjects' mean self evaluation vas scores<sup>a</sup> using day 3 as baseline

	<u>Dosage/(n)</u> <sup>b</sup>	<u>Day 3</u>	<u>Visit 4</u>	<u>Δ</u> <sup>c</sup>	<u>t</u> <sup>d</sup>	<u>p</u> <sup>e</sup>
<b>Periorbital Sagging</b>	A +B (32)	<u>79.8±</u> <sup>f</sup> 19.4 <sup>g</sup>	<u>93.9±</u> 18.7	<u>14.1±</u> 23.3	3.41	<0.002
<b>Periorbital Puffiness</b>	A +B (32)	<u>77.4±</u> 24.9	<u>88.5±</u> 20.0	<u>11.1±</u> 28.6	2.19	<0.037
<b>Periorbital Wrinkling</b>	A +B (32)	<u>74.6±</u> 23.9	<u>91.3±</u> 19.5	<u>16.8±</u> 27.5	3.45	<0.002
<b>Overall Facial Aging</b>	A +B (32)	<u>76.5±</u> 21.4	<u>91.8±</u> 22.3	<u>15.3±</u> 31.6	2.73	<0.010

a. In millimeters (mm) from 160 mm horizontal visual assessment scale marked at left end as "0", indicating worsening of condition, and at right end with a "9", indicating improvement of condition  
b. Combined values from A (7.5 g) and B (8.5 g) dosages with (n) equal to the number of correlated (non-independent) values included in the statistical determination.  
c. Δ = the difference between Day 3 and Visit 4 values.  
d. t = the t value determined for correlated (non-independent) values.  
e. p = significance of the difference by two-tailed t test.  
f. mean score value (± standard deviation).  
g. standard deviation.

in skin collagen.<sup>22</sup> Ascorbic acid (vitamin C) is a cofactor required for the function of several hydroxylases and monooxygenases. This vitamin has to be provided by diet or pharmacologic means as it is not synthesized in humans and some animal species. Its absence is responsible for scurvy, a condition related in its initial phases to a defective synthesis of collagen by the reduced function of prolylhydroxylase and production of collagen polypeptides lacking hydroxyproline. This defect prevents the formation into stable triple-helical collagen molecules.

In fibroblast cultures, vitamin C also stimulates collagen production by increasing the steady-state level of mRNA of collagen types I and III through enhanced transcription and prolonged half-life of the transcripts. Clinical studies<sup>23</sup> have shown that the stimulating activity of topical vitamin C was most conspicuous in the women with the lowest dietary intake of the vitamin and was unrelated to the level of actinic damage. This indicates that functional activity of the dermal cells is not maximal in postmenopausal women and can be increased.

Antioxidant supplementation is also recommended to facilitate wound healing due to the loss of different enzymatic and non-enzymatic free radical scavengers during the acute injury phase, which either partially or completely recover following healing.<sup>24</sup> Hyaluronic acid (HA) is a ubiquitous component of extracellular matrix and plays an important role in the homeostasis of connective tissues dur-

### Case Study 3. Patchy Discolorations



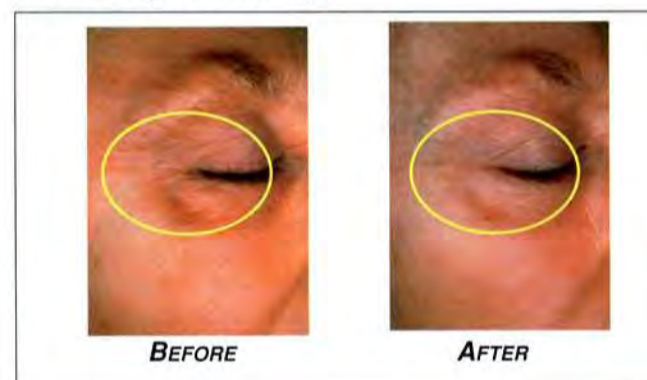
### Case Study 4. Fine Lines, "Crow's Feet"



### Case Study 1. Patchy Discolorations



### Case Study 2. Under Eye



ing embryogenesis, aging, and tissue repair.<sup>25</sup> After tissue injury, HA appears in greater abundance during the inflammatory response and the clearing phase of cell and matrix debris, before collagen production and matrix degradation.<sup>26</sup> Proteins associated with hyaluronan may be critical determinants of tissue remodeling after injury.<sup>27</sup>

Chitosan is a polymeric beta (1,4) glucosamine (2-amino-2-deoxy-D-glucose) and N-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose) and has been reported as a wound-healing accelerator.<sup>28</sup> Animal studies suggest chitosan functions in the acceleration of infiltration of polymorphonuclear (PMN) cells at the early stage of wound healing, followed by the production of collagen by fibroblasts.<sup>28</sup> A decrease in glycosaminoglycans (GAGs) in the skin is a result of a decrease in GAG biosynthesis, which can impair wound healing.<sup>29</sup>

In this study a combination of several components of the above discussed nutritional supplements was used and studied. One limitation of the study was the fact that the study had no control arm (no placebo).

The clinical study demonstrates the efficacy and safety of this dietary collagen formulation on aging skin.

Based on these initial results, additional well-designed clinical studies with larger patient populations are required

to determine whether the findings are reproducible in an expanded female population.

In summary, a dietary formulation (Toki) had beneficial effect on periorbital wrinkling and overall facial aging with minimal adverse effects.

## ACKNOWLEDGEMENT

This study was supported through an unrestricted educational grant from Lane Labs, Allendale, New Jersey. None of the authors have any financial interest in the study product or the company supporting this study.

## REFERENCES

1. Lavker RM. Cutaneous aging: chronologic versus photoaging. In: Gilchrist BA, ed. *Photoaging*. Cambridge, Mass: Blackwell Science; 1995;123-135.
2. Gilchrist BA, Yaar M. Ageing and photoageing of the skin: observations at the cellular and molecular level. *Br J Dermatol*. 1992;127(suppl 41):25-30.
3. Kang S, Fisher GJ, Voorhees JJ. Photoaging: pathogenesis, prevention, and treatment. *J Invest Dermatol*. 2001; Jun;116(6):853-859.
4. Helander SD. Treatment of photoaged skin; efficacy, tolerability and costs of available agents. *Ophthal Plast Reconstr Surg*. 1998 Jan;14(1):13-16.
5. Chung JH, Sco JY, Choi HR, Lee MK, Youn CS, Rhie G, Cho KH, Kim KH, Park KC, Eun HC. Modulation of skin collagen metabolism in aged and photoaged human skin *in vivo*. *Am J Pathol* 2001. 158(3):931-942.
6. Smith JG Jr, Davidson EA, Sams WM Jr, Clark RD. Alterations in human dermal connective tissue with age and chronic sun damage. *J Invest Dermatol*. 1962;39:347-350.
7. Warren R, Gartstein V, Kligman AM, Montagna W, Allendorf RA, Ridder GM. Age, sunlight, and facial skin: a histologic and quantitative study. *J Am Acad Dermatol*. 1991;25:751-760. [Erratum, *J Am Acad Dermatol*. 1992;26:558.]
8. Uitto J. Collagen. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. *Dermatology in general medicine*. 4th ed. Vol. 1. New York: McGraw-Hill; 1993;299-314.
9. Bernstein EF, Chen YQ, Kopp JB, et al. Long-term sun exposure alters the collagen of the papillary dermis: comparison of sun-protected and photoaged skin by Northern analysis, immunohistochemical staining, and confocal laser scanning microscopy. *J Am Acad Dermatol*. 1996;34:209-218.
10. Talwar HS, Griffiths CE, Fisher GJ, Hamilton TA, Voorhees JJ. Reduced type I and type III procollagens in photodamaged adult human skin. *J Invest Dermatol*. 1995;105:285-290.
11. Yamauchi M, Prisyanyh P, Haque Z, Woodley DT. Collagen cross-linking in sun-exposed and unexposed sites of aged human skin. *J Invest Dermatol*. 1991;97:938-941.
12. Schwartz E, Cruickshank FA, Christensen CC, Perlish JS, Leibold M. Collagen alterations in chronically sun-damaged human skin. *Photochem Photobiol*. 1993;58:841-844.
13. Calderone DC, Fenske NA. The clinical spectrum of actinic elastosis. *J Am Acad Dermatol*. 1995;32:1016-1024.
14. Griffiths CEM, Russman AN, Majumdar G, Singer RS, Hamilton TA, Voorhees JJ. Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). *N Engl J Med*. 1993;329:530-535.
15. Birkedal-Hansen H, Moore WG, Bodden MK, et al. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med*. 1993;4:197-250.
16. Varani J, Spearman D, Perone P, Fligiel SE, Datta SC, Wang ZQ, Shao Y, Kang S, Fisher GJ, Voorhees JJ. Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen *in vitro*. *J Invest Dermatol*. 2001; Nov;117(5):1212-1217.
17. Shah MG, Maibach HI. Estrogen and skin: an overview. *Clin Exp Dermatol*. 2001 Oct;26(7):613-618.
18. Klein AW. Skin filling. Collagen and other injectables of the skin. *Facial Plast Surg*. 2001 Aug;17(3):165-173.
19. Trelles MA, Benitez V, Garcia-Solana L. Controlled vaporization of the skin for the treatment of wrinkles. *Dermatol Clin*. 2001 Jul;19(3):453-466.
20. Helander SD. Treatment of photoaged skin. Efficacy, tolerability and costs of available agents. *Ophthal Plast Reconstr Surg*. 1998 Jan;14(1):13-16.
21. Ahn KY, Park MY, Park DH, Han DG. Botulinum toxin A for the treatment of facial hyperkinetic wrinkle lines in Koreans. *Dermatol Surg*. 2000 Feb;26(2):102-104.
22. Cefalu WT, Bell-Farrow AD, Wang ZQ, Sonntag WE, Fu MX, Baynes JW, Thorpe SR. Caloric restriction decreases age-dependent accumulation of the glycoxidation products, N epsilon-(carboxymethyl) lysine and pentosidine, in rat skin collagen. *J Gerontol A Biol Sci Med Sci*. 1995 Nov;50(6):B337-341.
23. Nusgens BV, Humbert P, Rougier A, Colige AC, Haftek M, Lambert CA, Richard A, Creidi P, Lapiere CM. Topically applied vitamin C enhances the mRNA level of collagens I and III, their processing enzymes and tissue inhibitor of matrix metalloproteinase 1 in the human dermis. *Plast Reconstr Surg*. 2000 Feb;105(2):778-784.
24. Shukla A, Rasik AM, Patnaik GK. Depletion of reduced glutathione, ascorbic acid, vitamin E and antioxidant defence enzymes in a healing cutaneous wound. *Free Radic Res*. 1997 Feb;26(2):93-101.
25. Croce MA, Dyne K, Boraldi F, Quaglino D Jr, Cetta G, Tiozzo R, Pasquali Ronchetti I. Hyaluronan affects protein and collagen synthesis by *in vitro* human skin fibroblasts. *Nephron*. 2001;88(4):347-353.
26. Pedagogos E, Hewitson TD, Nicholls KM, Becker GJ. Hyaluronan and rat renal fibroblasts: *in vitro* studies. *Matrix*. 1993 Nov;13(6):441-446.
27. Burd DA, Greco RM, Regauer S, Longaker MT, Siebert JW, Garg HG. Hyaluronan and wound healing: a new perspective. *Biomaterials*. 1999 Aug;20(15):1407-1414.
28. Ueno H, Yamada H, Tanaka I, Kaba N, Matsuura M, Okumura M, Kadosawa T, Fujinaga T. Accelerating effects of chitosan for healing at early phase of experimental open wound in dogs. *Exp Toxicol Pathol*. 1999 May;51(3):239-243.
29. Cechowska-Pasko M, Palka J, Bankowski E. Decreased biosynthesis of glycosaminoglycans in the skin of rats with chronic diabetes mellitus. *J Foot Ankle Surg*. 1999 Sep-Oct;38(5):333-338.