

Dietary/Nutritional Supplements: The New Ally to Topical Cosmetic Formulations?

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The skin is an organ that creates a boundary between our internal physiological system and the environment, thereby protecting against external insults such as UV and cosmic radiation, reactive oxygen species, air pollutants and mechanical damages. Skin is also an important part of the immune system¹ and prevents against excessive water loss through the epidermis.

Located externally, the skin bears the signs of its daily contacts with environmental stresses, which translates into the appearance of fine lines, wrinkles, uneven skin tone and decreased skin hydration. Ultimately, the deleterious consequence of UV radiation - actinic aging - conjugates with the inevitable chronological aging in the appearance of signs of aging.²

At the molecular level, constant exposure of skin to the environment may trigger the formation of free radicals, depletion of endogenous antioxidants, suppression of immune functions and the activation of specific matrix metalloproteinases (MMPs).³ From a physiological standpoint, these molecular reactions may be associated with oxidative stress, membrane lipid peroxidation, weakened immune surveillance, inflammation and the progressive dismantlement of the extra cellular matrix (ECM) 3-D structure. The collapse of the ECM is believed by many to represent a key event in the loss of skin elasticity and hydration and in the appearance of signs of aging.⁴

Biotechnology-based cosmetic companies have developed numerous topical formulations aimed at alleviating the appearance of signs of aging by acting directly on the underlying molecular mechanisms. In some cases, these topical formulations have proven to be efficacious in improving the skin appearance of consumers. However, applying active ingredients on the surface of the skin may not be the only mode to provide benefits to the skin.

Dietary/Nutritional Supplements

Dietary/nutritional supplements can be used to make active nutrients available to all organs of the body. As mentioned earlier, skin is an organ and may therefore benefit from

active nutrients conveyed by dietary/nutritional supplements. The repercussions of nutrition on skin health are well exemplified by the fact that some skin disorders are directly linked to nutritional deficiencies.⁵

Conversely, skin plays a major role in maintaining bone health through the synthesis of vitamin D.⁶ The interrelation between skin and the nutritional homeostasis has been recently highlighted⁷ and calls upon the understanding of the cellular and molecular processes in play.

We have performed a clinical trial in which a topical cream formulation and a dietary/nutritional supplement were concomitantly administered. The dietary/nutritional supplement provided proteoglycans, collagen, glucosamine, carotenoid pigment (astaxanthin esters) and omega-3 essential fatty acids (EPA and DHA). The efficacy of this regimen was demonstrated on the visual appearance of signs of aging as well as by the amelioration of functional properties of the skin.

Study design: This study was an evaluator-blinded, parallel group design clinical trial consisting of three evaluation visits over a period of 12 weeks. Three regimens were randomized among approximately 100 subjects in order to complete the study with about 30 subjects per cohort. Efficacy of regimen was assessed through visual evaluations for fine lines, unevenness of skin tone, sallowness and tactile roughness. In addition, instrumental measurements

Key words

Dietary/nutritional supplements, skin tone, sallowness, tactile roughness, skin elasticity, skin hydration

Abstract

The authors demonstrate that combining the efficacy of a topical cream with a dietary/nutritional supplement improved the skin's appearance. In the trial conducted, parameters associated with aged skin appearance were improved.

using a Dermal Torque Meter^a and Corneometer^b tested for skin elasticity and skin hydration, respectively. Self-evaluation questionnaires were administered to assess sensory and acceptance parameters.

Visual and instrumental evaluations were used to assess the efficacy of the test articles in supporting specific claims: diminution of fine lines was assessed visually, by the subjects and by the evaluator; improved skin elasticity was measured with the Dermal Torque Meter^a; skin tone was assessed visually by the evaluator and by the subjects; improvements in skin tone (sallowiness) were assessed visually by the evaluator and by the subjects, skin hydration was assessed visually by the evaluator, measured through Corneometer readings and by the subjects.

Subject selection: Inclusion criteria for subjects were as follows: female, age 35 to 60, inclusive; completion of a minimum 3-day conditioning period immediately prior to study enrollment with the presence of fine lines on the face as assessed by a trained evaluator; motivated and willing to comply with test procedures; willing to refrain from changing cosmetic use habits for the duration of the study; and the signature of an agreed informed consent document after the features of the study had been fully explained. The use of oral retinoid medications or topical retinoids on the face within the last three months (e.g. Retin-A or Retin-A type preparations, Accutane) stood as exclusion criteria.

Methodology: The regimen for cohort A consisted of 12 weeks home use of a topical active cream and a dietary/nutritional supplement. Efficacy parameters monitored for cohort A were: fine lines, unevenness of skin tone, sallowness, tactile roughness, skin elasticity and skin hydration. Cohort A subjects completed a self-evaluation questionnaire at week 0 and 12 as well.

The regimen for cohort B and cohort

C consisted of 12 weeks home use of the dietary/nutritional supplement on top of a placebo cream (cohort B) and sole use of the topical active cream (cohort C). Efficacy parameters monitored for cohort B and C were: skin elasticity and skin hydration.

The topical active cream^c provided a highly potent MMP inhibitor (INCI: Glycosaminoglycans) and was included in a cream base. The placebo cream consisted of the exact same cream base in which the cosmetic active ingredient was omitted.

As for the dietary/nutritional supplement^d, each 250 mg #1 clear gelatine capsule supplied 125 mg of a patented glycosaminoglycan extract as well as 125 mg of hydrolyzed extract powder providing, amongst others, highly potent MMP inhibitor, complex proteoglycans, collagen, glucosamine, carotenoid pigment (astaxanthin esters) and omega-3 essential fatty acids (EPA and DHA).

Procedure: Three to five days preceding the baseline visit (week 0), the candidates were required to engage in a conditioning period. Candidates replaced their facial cleanser with a regular consumer soap^e and refrained from the use of moisturizers. Use of facial cosmetics such as foundation, powder, eye makeup and remover and mascara was permitted.

Candidates showed to the test locations with faces free of facial makeup, with the exception of any lipstick and eye makeup. Candidates were screened for entrance into the study through completion of an inclusion/exclusion form and through visual examination by the evaluator. Baseline visual evaluations were conducted, and Dermal Torque Meter^a and Corneometer^b readings were performed. Subjects were randomly assigned to either cohort A, B or C. Subjects applied the topical active cream (cohort A and C) or placebo cream (cohort B), twice daily both morning and evening, for the entire period. Subjects of cohort A and B, in addition to applying their respective topical procedure, took the dietary/nutritional supplement once daily in the morning (e.g., two 250 mg capsules with breakfast). Subjects returned after approximately 1 and 12 weeks of using the assigned regimen.

Visual assessments and compliance checks were completed at week one. Test areas were examined for irritation, which in due case was noted and followed-up. All test products were collected, weighed and reissued. At week 12, visual assessments and instrumental readings were taken from the same locations as at the baseline. Subjects of cohort A completed a self-assessment questionnaire. All products of the regimen were collected.

Visual Evaluation: The facial area of each subject was visually evaluated by a trained evaluator using a standard light source and, if necessary, a magnifying glass to view the area.

- Fine lines were defined as shallow indentations or superficial wrinkling. Generally, these lines were

^aDermal Torque Meter is a product of Dia-Stron Ltd., Hampshire, U.K.

^bCorneometer is a product of Courage&Khazaka, Cologne, Germany

^cMRT_{EX} is a product of Atrium Biotechnologies Inc., Quebec City, Canada

^dMRT_N is a product of Atrium Biotechnologies Inc., Quebec City, Canada

^eIn this study, Neutrogena was used. Neutrogena is a product of Neutrogena Corporation, Los Angeles, California, USA.

Table 1. Summary of cohort A visual evaluations

Visual Evaluation	Mean score	Responders ^a		Responders ^a						Efficacy	
	Baseline n=32	Week 1 n=32	Week 12 n=29	-1G ^b		-2G ^c		-3G ^d		Week 1	Week 12
				w1	w12	w1	w12	w1	w12		
Fine lines	4.63(4-6)	16/32	15/29	11	9	4	6	1	-	34%*	29%*
Unevenness of skin tone	2.22(1-4)	10/32	17/29	9	8	1	7	-	2	45%	58%*
Sallowness	3.13(1-4)	26/32	26/29	14	9	10	10	2	7	43%**	60%**
Tactile roughness	1.00(0-3)	12/17	10/16	7	5	5	4	-	1	89%	78%

^a Number of subjects that improved by at least one grade.

^b Number of subjects that improved by one grade from those who improved after 1 and 12 weeks of use

^c Number of subjects that improved by two grade from those who improved after 1 and 12 weeks of use

^d Number of subjects that improved by three grade from those who improved after 1 and 12 weeks of use

* p < 0.05

** p < 0.0005

eliminated by pulling the skin taut. The grades were as follows: 0 = no evidence of facial lines; 2 = occasional number of fine lines widely spaced; 4 = few number of discreet fine lines; 6 = moderate number of fine lines in close proximity; 8 = many fine lines densely packed.

- Unevenness of skin tone grades were as follows: 0 = even skin tone; 2 = slight differences in skin tone over small area(s); 4 = slight differences in skin tone involving moderate areas or moderate differences in skin tone involving small area(s); 6 = moderate differences in tone over moderate-sized area, slight differences over large areas, extreme differences in tone involving small area(s); 8 = extreme differences in skin tone over large areas, small areas of hyper pigmentation.
- Sallowness refers to the color of the skin tone. Sallow-ness grades were as follows: 0 = skin has very pink color; 2 = skin is pale; 4 = skin has slight suggestion of yellowness; 6 = skin is pale with moderate suggestion of yellowness; 8 = skin is quite pale with distinct suggestion of yellowness.
- Tactile roughness refers to the texture of cheek skin when gently palpated. Tactile roughness grades were as follows: 0 = skin is very smooth; 2 = skin is smooth with occasional rough area; 4 = mild roughness; 6 = moderate roughness; 8 = severe roughness.

Grades of 0, 2, 4, 6 or 8 reflect a generalized condition. Grades of 1, 3, 5, or 7 may be used to represent an intermediate condition or less than 50% of the test area having the next highest scoring condition. A score of 8 is the maximum grade assigned.

Instrumental Evaluation: The subjects equilibrated in a room maintained at 20±2°C and 35±5% relative humidity for at least 30 minutes prior to Corneometer^b readings. Triplicate readings were taken for each site. These readings were averaged for statistical analysis. Dermal Torque Meter^d measurements were conducted under ambient conditions. Subjects were in a prone position with the head approximately perpendicular to the supporting surface.

Statistical Analyses: Statistical analyses were conducted on all data collected, except the self-perception questionnaire. Within-regimen analyses were conducted evaluating the changes from baseline for each regimen. The method used for the visual evaluations was the signed rank test. The method used for the instrumental readings was the paired t-test. Between-regimen analyses were conducted using repeated measures analysis of variance technique comparing the changes from baseline among regimen. The model was a one-way comparison of cohorts with subject within cohort as the error stem. Significance-testing was performed at the alpha=0.05 level.

Results

Results reported in this study were obtained from 93 subjects: 29 in cohort A, 32 in cohort B and 32 in cohort C. The subjects' demographics of this clinical study ranged from 35 to 55. The mean and range of values for baseline visual evaluation parameters of cohort A (topical active cream and dietary/nutritional supplement) are reported in Table 1.

Subjects were diverse as shown by the range of values for each visual evaluation. Mean scores at baseline demonstrated that subjects had relatively moderate visible signs of skin aging. For each visual evaluation, the changes were unidirectional as per the grading scale.

A substantial number of subjects displayed improvements as soon as one week of use. After 12 weeks of use and

Table 2. Self-assessment questionnaire cohort A*

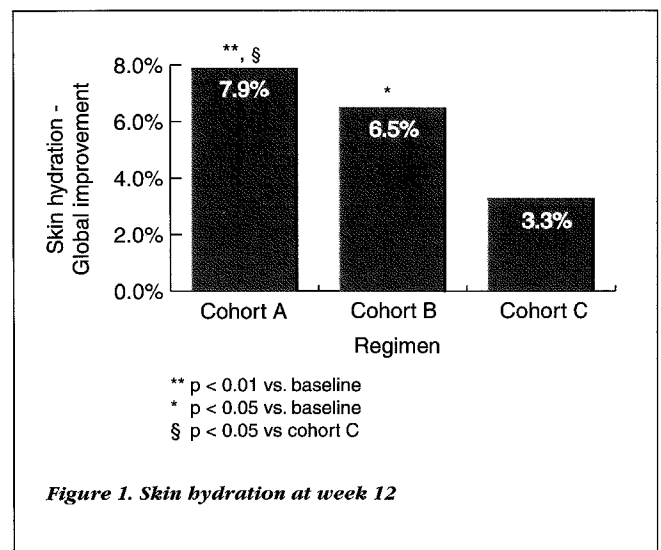
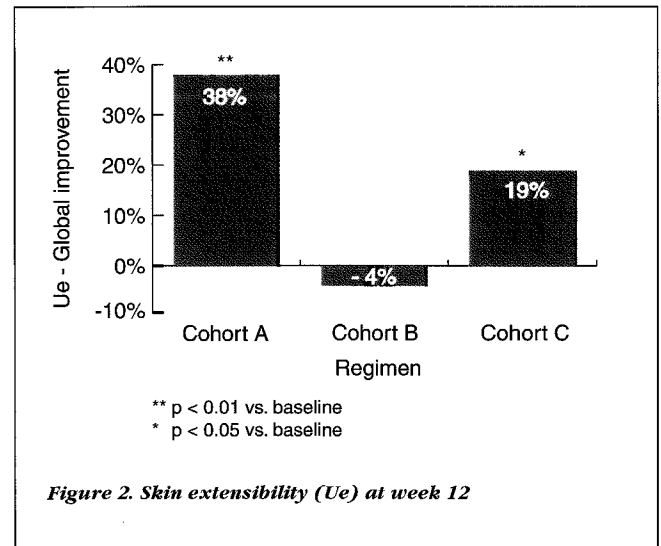
Fine lines	48%
Wrinkles	35%
Firmness	31%
Elasticity	41%
Global quality of skin	59%
Nutritive effect	79%
Telangiectasia	38%
Tone homogeneity	52%
Imperfections Improvements	41%
Silky aspect	83%
Skin's overall comfort	76%
Signs of fatigue	31%
Tone	45%
Hydratation	83%
Did the treatment meet your expectations	86%
Global evaluation of regimen: Excellent	17%
Brown spots	45%

* percentage of cohort A subjects who selected among notes excellent, very effective and effective for each parameter after twelve weeks of regimen use

for all visual evaluations, more than half of all subjects in cohort A had at least improved by one grade with a more pronounced response in the case of sallowness. We observed a noticeable progression between week one and 12 in the actual number of grades gained by the subjects who improved (Table 1).

Of a particular interest was the widespread range of improvements surveyed. All visual evaluations had three grade improvements after 12 weeks of use except for fine lines. In the case of sallowness, striking improvements were noticed with 7 improvements of three grades out of 26 subjects who experienced positive changes in this specific visual evaluation performed by investigators. The subjects who were given a grade 0 at baseline and also after 12 weeks of use for tactile roughness were not considered in the total number of subjects when calculating the ratio of subjects who improved.

In order to better quantify the effectiveness of the cohort A regimen within the subjects who improved, an efficacy measure was designed discerning between the various improvements as per the grading scale (shift of 1, 2 or 3 grades). The efficacy value after one and 12 weeks of use was

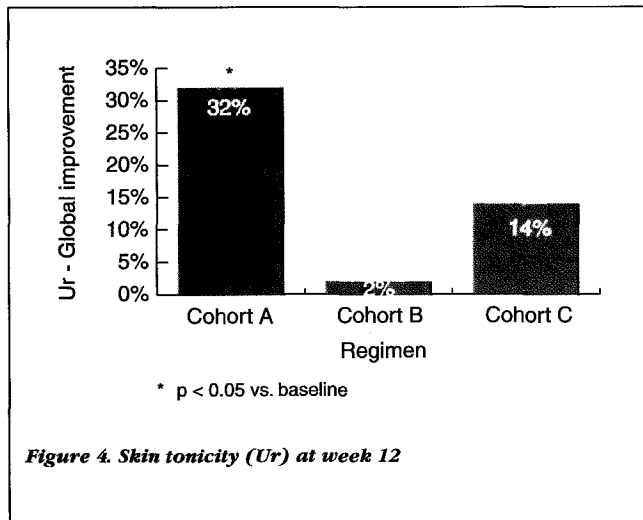
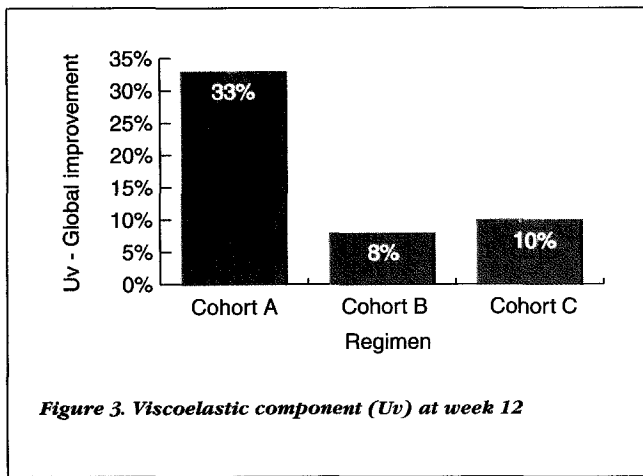
**Figure 1. Skin hydration at week 12****Figure 2. Skin extensibility (Ue) at week 12**

tested against baseline for statistical difference (Table 1). Sallowness demonstrated a clear significant statistical difference ($p < 0.005$) as soon as one week after use and after twelve weeks of regimen use.

Unevenness of skin tone and fine lines both significantly improved with regimen use ($p < 0.05$), as soon as one week after use in the case of fine lines. Regimen use did not produce any statistical difference against baseline in the case of tactile roughness. This is probably explained by the presence of subjects with grade 0 evaluation at baseline and therefore the lower number of subjects that could be entered in the statistical calculation.

The results from self-assessment questionnaires filled out by subjects of cohort A are shown in Table 2. Self-assessment was evaluated using a questionnaire that included 17 questions regarding skin quality and appearance parameters at baseline and after twelve weeks of use. For each question, subjects had to select between: excellent, very effective, effective, average, mediocre and not applicable in appreciating their skin.

The regimen met the expectations of 86% of cohort A subjects. Generally the subjects noted that the regimen



was effective in regards to all parameters, especially the hydrating and nutritive effects as well as the skin silky aspect. They were all clearly eminent with 83%, 79% and 83% of subjects from cohort A reporting the regimen was effective, very effective or excellent, respectively, in these particular cases. In some instances improvement for the texture of hair and nails were reported.

Skin hydration measurements were performed after 12 weeks of use by subjects of cohort A, B and C. As shown in Figure 1 and demonstrated by Corneometer^b instrumental readings, the combined regimen (cohort A) was the most successful in improving the skin hydration by a mean of 7.9% ($p < 0.01$). Notice the greater proportion of cohort A subjects who improved their skin surface hydration (75%). The use of the dietary/nutritional supplement alone

(cohort B) still statistically improved the skin surface hydration opposite to baseline but to a lesser extent of 6.5% ($p < 0.05$). The use of topical active cream (cohort C) was unsuccessful to distinguish itself from baseline value nonetheless exhibiting a hydrating action. Between-regimen analysis showed a significant statistical difference between cohort A and C ($p < 0.05$).

Skin elasticity was evaluated based on average improvement for skin extensibility (Ue), viscoelastic component (Uv) and tonicity (Ur) parameters. As shown in Figure 2, Ue was best improved after 12 weeks of use by the combined regimen of cohort A with a global increase of 38%. This result was significantly statistically different against the baseline ($p < 0.01$). Cohort C also exhibited significant improvement ($p < 0.05$) but to a lesser extent (19%).

Cohort A revealed a noticeable but non-statistically significant 33% global improvement between baseline and 12 weeks of use values for Uv. Cohort A once again differentiated itself by displaying a statistical difference in the case of Ur ($p < 0.05$) by improving 32% after twelve weeks of use (Figure 4). Cohort C had a tendency toward improvement (14%) but not sufficient to be statistically significantly different. Cohort B only produced moderate efficacy in every skin elasticity parameter monitored when compared to baseline values.

Discussion

We have demonstrated that combining the efficacy of a topical cream with that of a dietary/nutritional supplement resulted in the improvement of skin appearance. In the clinical trial conducted, parameters associated with an aged skin appearance were improved: fine lines, sallowness, unevenness of skin tone and tactile roughness. Improvement of skin appearance was observed and judged by trained investigators, assessed directly by the subjects through a questionnaire and quantified through skin hydration and skin elasticity measurements using appropriate devices. Statistical significance was reached according to the parameters and the time point tested. In some instances (sallowness and tactile roughness), the extent of improvement attained 3 grades on a 0-8 scale.

The regimen used in this clinical study consisted of a topical cream containing an active ingredient endowed with a potent MMP inhibitory activity and a dietary/nutritional supplement providing proteoglycans, collagen, glucosamine, carotenoid pigment (astaxanthin esters) and omega-3 essential fatty acids (EPA and DHA). A MMP inhibitory activity is also present in the dietary/nutritional supplement. Based on the data reported by the hydration and elasticity measurements, the concurrent use of the topical cream and the dietary/nutritional supplement brings additive or synergistic benefits.

When used alone, the dietary/nutritional supplement (cohort B) created a significant hydration effect (Figure 1), however, its effect on skin elasticity when assayed by

the Dermal Torque Meter was moderate. Conversely, the topical active cream scored better in the extensibility parameter (Figure 2) and less for hydration (Figure 1). This demonstrates the complementary effect between the oral and topical regimen in acting on different features of skin functionality.

Conclusion

Despite its important functions, skin is too often seen as an inert enve-

lope. Indeed, skin is a living organ just as much as the heart, the kidneys and the liver. Therefore, skin highly relies on what we use topically as well as what we ingest orally to ensure its physiological homeostasis.

We have shown that combining a dietary/nutritional supplement and an active topical cream both formulated with selected ingredients provides benefits to the skin's appearance by reducing visual signs of aging. To elucidate the precise mechanisms underlying the clinical improvement of each parameter tested in this study would require further investigation. It is, however, tempting to suggest that all potential pathways involved: inhibition of MMP enzymatic action, chelation of reactive oxygen species and anti-inflammatory action, team up to prevent excessive oxidative damages and degradation activities to ultimately preserve the multifunctionality of the skin ECM leading to a better appearance.

Working from the "inside out" represents a new and exciting global cosmeceutical approach to supply the skin with biologically active ingredients that can act at the surface of the skin and through nutrition. In this way, synergistic actions can be expected at the molecular level of the skin's ECM.

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