Journal of Dermatological Treatment

ISSN: 0954-6634 (Print) 1471-1753 (Online) Journal homepage: http://www.tandfonline.com/loi/ijdt20

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To cite this article: H Murad & Michael P Tabibian (2001) The effect of an oral supplement containing glucosamine, amino acids, minerals, and antioxidants on cutaneous aging: a preliminary study, Journal of Dermatological Treatment, 12:1, 47-51, DOI: 10.1080/095466301750163590

To link to this article: https://doi.org/10.1080/095466301750163590

Published online: 12 Jul 2009.

  ISSN 0954–6634

The effect of an oral supplement containing glucosamine, amino acids, minerals, and antioxidants on cutaneous aging: a preliminary study

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BACKGROUND: Alterations in collagen, elastin, and glycosaminoglycans contribute to cutaneous changes seen in aging skin.

METHODS: A randomized, controlled, single-blind study was conducted with 53 female volunteers who were supplied with an oral supplement containing glucosamine, amino acids, minerals, and various antioxidant compounds. Hydration properties of the skin as well as textural analysis of the women's fine lines and wrinkles were assessed following 5 weeks intake of the oral supplement and results were compared with those of a control group of 12 individuals who did not take the supplement

RESULTS: There was a statistically significant reduction (34%) in the number of visible wrinkles as measured by the silflo replicas ($P < 0.01$) and a reduction (34%) in the number of fine lines ($P < 0.06$) in the group of women who took the supplement. No significant changes in epidermal hydration were observed in either the control or study groups.

CONCLUSION: The use of an oral supplement containing glucosamine, minerals, and various antioxidant compounds can potentially improve the appearance of visible wrinkles and fine lines. It does not, however, affect epidermal hydration. (*J Dermatol Treat* (2001) **12:** 47–51)

Received 5th November 1999
Revised 27th June 2000
Accepted 10th July 2000

Keywords: *Aging — Antioxidant — Nutraceuticals — Wrinkles*

Introduction

The prevention or reversal of the process of cellular aging has been an enduring goal of biological science as well as the basis of claims of a myriad of over-the-counter and prescription cosmetic preparations. Free oxygen radicals have been implicated in the development of both intrinsic aging and photoaging. Ultraviolet-activated molecules cause oxidation of cellular components, inducing a chain reaction of lipid peroxidation in membranes rich in polyunsaturated fatty acids.[1] Furthermore, structural DNA changes such as strand breaks and dimer formations[2] as well as release of inflammatory mediators[3] occur. When the delicate balance between free radical generation and our defense mechanisms with antioxidants is disturbed, the consequences can range from natural aging to a variety of benign and precancerous lesions to ultimately neoplastic transformations. An elaborate system of extra- and intracellular antioxidants exists to protect biological tissues against such oxidative stress. Antioxidant activity is provided by naturally occurring substances including vitamin C, vitamin E, glutathione, β-carotene, and histadine. Furthermore, intracellular enzymes such as superoxide dismutase, glutathione peroxidase, thioredoxin reductase, and catalase also provide protection against activated oxygen radicals.[4]

The different clinical patterns of photoaging are well known and have been well described in the literature. Major age-related cutaneous changes include 'dryness', wrinkling, laxity and development of various benign neoplasms. The end result of both intrinsic aging and chronic exposure to ultraviolet light and other potential exogenous hazards is a skin that is wrinkled, yellow, lax, dry, and leathery. Furthermore, precancerous as well as can-

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cerous lesions develop as consequences of repeated solar radiation.[5]

Concomitant to the clinical features associated with cutaneous aging are the histological changes seen after prolonged exposure to intrinsic and extrinsic factors. In general, the chronically photodamaged skin is metabolically hyperactive with epidermal hyperplasia (and sometimes neoplasia), increased deposition of abnormal elastic fibers, collagen, and glycosaminoglycans (GAGs).[6] In contrast, intrinsic aging is characterized by epidermal atrophy,[7] retraction of rete pegs with the flattening of the dermoepidermal interface,[8] and deposition of pseudoxanthoma-elasticum-like papillary dermal elastolysis.[9]

Previous studies have pointed out the beneficial effects of topical preparations such as tretinoin and alpha-hydroxy acids, as well as oral supplementation using vitamins and minerals in cutaneous aging.[6,10] Topical tretinoin has been shown to induce epidermal hyperplasia and compaction of the papillary dermis, and leads to deposition of a glycosaminoglycan-like substance in the dermis.[11] The rationale behind use of topical or oral formulations in cutaneous aging extends beyond cosmetic concerns. Cutaneous functions such as protection, secretion, absorption, and thermoregulation are detrimentally affected by as much as 60% by the structural changes in the skin due to aging and excessive sun exposure. There is also evidence for impairment of the barrier function, decreased turnover of epidermal cells, and a reduced number of fibroblasts and keratinocytes.[1]

In this study, the potential benefits of an oral nutraceutical supplement containing antioxidants, glucosamine, essential amino acids and minerals in reversing certain clinical features of cutaneous aging (i.e. fine lines and wrinkles) were assessed. By definition, nutraceuticals are substances that have health benefits, including the prevention and treatment of diseases. A discussion of the rationale and role of these ingredients in potentially improving the clinical signs of cutaneous aging will follow.

Patients and methods

A total of 72 female subjects (mean age 46.1 ± 6.7 years; range 39–56 years) was selected for the study. Patient's Fitzpatrick skin types ranged from type I to type IV. Prior to the study, the women were examined to determine eligibility. These subjects met the exclusion criteria of not using tretinoin or isotretinoin, or having had facial peels, dermabrasion or laser treatment performed within the last 12 months. The duration of the study, including the conditioning period was 6 weeks. The subject group of volunteers were supplied with the antioxidant supplement (Gemini pharmaceuticals, Bohemia, NY), which contained the following ingredients: *N*-acetyl D-glucosamine, glucosamine sulfate, L-proline, L-lysine, manganese, copper, zinc, quercetin, and grape seed extract.

The conditioning period began 7 days prior to the initiation of the study. At the time of the study (spring 1998) the average ambient temperature was 76°F (24°C). Subjects were instructed to discontinue use of any type of moisturizing products, including sunscreens, moisturizing soaps, and make-up products. The use of moisturizing products could have potentially affected textural analysis of the skin and given false positive results. The women were also instructed to avoid excessive ultraviolet (UV) exposure and to avoid visits to tanning salons.

After the 7-day conditioning period, the women returned for baseline measurements and instructions. A group of subjects who did not receive test material served as an untreated control group. The remaining subjects were supplied with test material. The investigators were blinded to the identity of the control vs treated subjects. Each subject from the treated group consumed two tablets of the test material in the morning and two tablets in the evening with meals. Those women in the untreated control group did not take any oral supplement but fully participated in baseline and subsequent skin analysis. After 2 weeks of product use, the women returned for 'mid-study' measurements and skin texture analysis. Final measurements were taken at the end of 6 weeks.

At each visit, three corneometer readings were taken, and one negative impression of the test site was made using silflo replicating material. The corneometer is a commercially available instrument (Courage and Khazaka, Germany) which is designed to measure changes in the capacitance of the skin resulting from changes in the degree of hydration. Corneometer measurements were performed on both sides of the face on the cheek area. Silflo replicating materials were used to make negative impressions of the texture of the skin, fine lines and wrinkles in the 'crow's feet' area. The resultant replicas were subsequently evaluated by a technique that combines image analysis and surface shadowing under grazing illumination. The replicas were illuminated at a precisely defined angle of 35° to create shadows, which were then analyzed according to shades of gray. The types of wrinkles or fine lines were determined on the basis of length, depth, and area. Fine lines were defined as such when the measured depth of the lines was *less* than 60 μm in depth. Wrinkles, on the other hand, were defined as such when the measured depth of the lines was *more* than 60 μm in depth. The percentage reduction in the number of wrinkles resulting from the treatment was then calculated. The replicas were taken from a randomly chosen site in the peri-orbital (crow's feet) area.

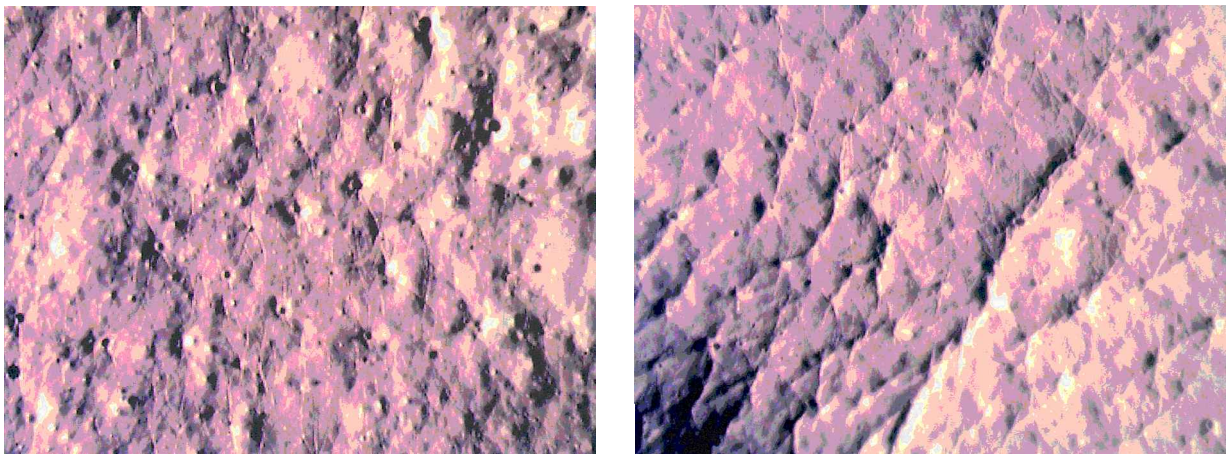
Results

A total of 65 women (12 controls) completed the study. Seven women discontinued the study for reasons unrelated to product use. As indicated in Table I, there was a statistically significant reduction (34%) in the number of visible wrinkles as measured by the silflo replicas

	Number of wrinkles				Number of fine lines			
	Mid-baseline		Final baseline		Mid-baseline		Final baseline	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Average	−3	−7	−3	−15	−5	−4	−6	−12
Standard deviation	9	13	13	12	6	10	14	10
P-value	$P < 0.41$		$P < 0.01$		$P < 0.96$		$P < 0.06$	
% Difference from baseline	−11%	−19%	−6%	−40%	−14%	−24%	−9%	−43%
Total % difference (T − C)	−8%		−34%		−10%		−34%	

T = treated; C = control.

Table I
Number of wrinkles and fine lines



Subject 7 Baseline Subject 7 Final

Figure 1
Photomicrograph of patient no. 7 at baseline and at the end of the study period (6 weeks).

($P < 0.01$) and a clinically significant reduction (34%) in the number of fine lines ($P < 0.06$) in the group of women who took the supplement (Figure 1). No significant changes occurred in the control group. Of importance, there were no significant changes involving the degree of hydration of the stratum corneum as measured by the corneometer in either group (Table II).

Discussion

In this study, an objective textural analysis of fine lines and wrinkles, as well as changes in cutaneous hydration, were performed before and after 5 weeks intake of an oral supplement containing glucosamine, essential amino acids, minerals, and antioxidants. At the end of 5 weeks

	Skin hydration			
	Mid-baseline		Final baseline	
	Control	Treated	Control	Treated
Average	−5	−7	−8	−4
Standard deviation	6	7	5	7
P-value	$P < 0.84$		$P < 0.67$	
% Difference from baseline	−7%	−10%	−12%	−6%
Total % difference (T − C)	−3%		−6%	

T = treated; C = control.

Table II
Corneometer readings

there were significant improvements in the number of wrinkles and fine lines based on image analysis of silflo replicating materials. No improvements were noted in the control group of women who did not take the supplement. Furthermore, no changes in the degree of hydration of the skin occurred as measured by the corneometer. The latter finding is significant since there can be an illusion of a reduction in superficial lines after 'sufficient' cutaneous hydration.

The rationale behind the constituents of the oral formulation rests on two premises:

- The mechanical properties of the skin, such as elasticity, are controlled by the density and geometry of collagen and elastic fibers. Consequently, damage to collagen and elastic fibers causes loss of their contractile properties, with resultant skin laxity and wrinkling. Therefore, it follows that supplementation of the skin with the normal constituents of collagen and elastin could help reverse these changes.
- Reactive oxygen species are produced by ultraviolet exposure and cause oxidative damage. Antioxidants have therefore been investigated for their defensive role against oxidative damage.

Proline and glycine comprised the primary amino acids in our supplement formulation because they constitute normal amino acid components of both collagen and elastic fibers.[12] *N*-acetylglucosamine and glucosamine sulfate, representing modified sugars found in GAGs, were the primary forms of glucosamine incorporated in to our supplement. The need for their supplementation is important in light of the documented age-related decrease in the content of GAGs (primarily hyaluronic acid) in aged skin.[13] The reduction in the levels of hyaluronic acid in the dermis appears to correspond to some of the most striking clinical features of aged skin, including wrinkling and altered elasticity.

Vitamins C and E were also included in our supplement because there is increasing evidence supporting the role of these powerful antioxidants in cutaneous and systemic disorders. Topical use of vitamin C is believed to prevent sun damage, reduce breakdown of connective tissues, and possibly promote collagen synthesis. Vitamin E (alpha-tocopherol) has been noted as the major naturally occurring lipid-soluble non-enzymatic antioxidant protecting skin from the adverse effects of oxidative stress including photoaging. In vitro studies have also documented reduction of collagen degradation by vitamin E and selenium.[14] Moreover, alpha-tocopherol has been demonstrated to prevent the reactive oxygen species (ROS)-induced alterations of collagen and GAGs synthesis.[15]

The role of several minerals in cellular protection from both intrinsic and extrinsic factors has previously been documented. Selenium, copper, zinc and manganese were the minerals included in our formulation. Selenium, an essential trace mineral, is an essential component of glutathione peroxidase. Copper not only assists in the treatment of elastic tissue defects but also has a significant antioxidant effect on photosensitized skin.[16] Specifically, copper appears to reduce lipid photoperoxidation and consequent lysis in photosensitized skin.[17] Zinc acts as a cofactor with superoxide dismutase (SOD), which in turn acts in concert with copper SOD, mitochondrial manganese SOD, glutathione peroxidase and catalase as essential cellular antioxidants. Manganese, a transition metal, binds collagen fibers and inhibits the elastase enzyme, which breaks down both collagen and elastic tissue. Manganese also plays a key role in the synthesis of GAGs, collagen, and glycoproteins, and acts as a cofactor for catalyzing the conversion of the glucosamine into hyaluronic acid. In addition, manganese has been shown to protect cultured human skin fibroblasts against oxidative injury by UVA and hydrogen peroxide.[16]

There is also an increasing body of evidence illustrating the beneficial antioxidant properties of 'naturally' available herbal products. Quercetin, a unique bioflavanoid from blue-green algae, makes collagen resistant to collagenase and also possesses powerful antioxidant properties.[18] It has also been shown to increase production of collagen and extracellular fibronectin. Catechin-based preparations, including proanthanols and proanthocyanidins are also powerful antioxidants. The latter compound is the active ingredient in grape seed extract, a bioflavanoid shown to be 50 times more effective than vitamin E and 20 times more effective than vitamin C as an antioxidant.[19]

The results of this study point to the potential beneficial role of oral antioxidant supplementation in cutaneous aging, providing a basis for future studies whereby quantitative changes in collagen and glycosaminoglycan are made and subsequently correlated with the degree of clinical improvement. Importantly, we found no significant changes in the degree of hydration of the skin as measured by the corneometer – suggesting that oral supplementation with antioxidants and essential constituents of the dermal matrix does *not* provide significant epidermal hydration. This latter finding is also significant in that it has important therapeutic implications. It illustrates that oral supplementation with antioxidants and the essential constituents of the dermal matrix does not provide significant epidermal hydration, and thus does not obviate the need for topical application of moisturizers.

Acknowledgement

Study conducted in collaboration with Clinical Research Laboratory, an independent clinical laboratory at Piscataway, New Jersey.

Statement of conflict of interest

The nutraceutical compound used in this study was granted US patent protection (patent No. 5804954) on 8 September 1998.

References

1. Cerimele D, Celleno L, Serri F, Physiological changes in ageing skin. *Br J Dermatol* (1990) **122**: 13–20.
2. Plathak MA, Carbonare MD, Reactive oxygen and free radical reactions in mammalian skin induced by ultraviolet radiation. *Photochem Photobiol* (1988) **47**: 74S.
3. Grange RW, Acute effects of ultraviolet radiation in the skin. In: Fitzpatrick TB, Eisen AZ, Wolff K et al (eds) *Dermatology in general medicine*. McGraw-Hill: New York, 1993, p. 1451.
4. Sies H, Antioxidant activity in cells and organs. *Am Rev Respir Dis* (1987) **136**: 478–80.
5. Kligman AM, Kligman LH, Photoaging. In: Fitzpatrick TB, Eisen AZ, Wolff K et al (eds) *Dermatology in general medicine*. McGraw-Hill: New York, 1993, p. 2972.
6. Kligman LH, Photoaging: manifestations, prevention, and treatment. *Clin Geriatr Med* (1989) **5**: 235–51.
7. Fenske NA, Lober CW, Structural and functional changes of normal aging skin. *J Am Acad Dermatol* (1986) **15**: 571–85.
8. Montagna W, Carlisle K, Structural changes in aging human skin. *J Invest Dermatol* (1979) **73**: 47–53.
9. Rongioletti F, Rebora A, Fibroelastolytic patterns of intrinsic skin aging: pseudoxanthoma-elasticum-like papillary dermal elastolysis and white fibrous papulosis of the neck. *Dermatology* (1995) **19**: 19–24.
10. Ditre CM, Griffin TD, Murphy GF et al, Effects of alpha-hydroxy acids on photoaged skin: a pilot clinical, histologic, and ultrastructural study. *J Am Acad Dermatol* (1996) **34**: 187–95.
11. Voorhees J, Clinical effects of long-term therapy with topical tretinoin and cellular mode of action. *J Int Med Res* (1990) **18**: 26–8.
12. Rosenbloom J, Elastic fiber in health and disease. *Dermatol Foundation* (1996) **30**: 1–6.
13. Ghersetich I, Lotti T, Campanile G et al, Hyaluronic acid in cutaneous intrinsic aging. *Int J Dermatol* (1994) **33**: 119–22.
14. Asman B, Wijkander P, Hjerpe A, Reduction of collagen degredation in experimental granulation tissue by vitamin-E and selenium. *J Clin Periodontol* (1994) **21**: 45–7.
15. Tanaka H, Okada T, Konishi H et al, The effect of reactive oxygen species on the biosynthesis of collagen and glycosaminoglycans in cultured human dermal fibroblasts. *Arch Dermatol Res* (1993) **285**: 352–5.
16. Parat M, Richard MJ, Leccia MT, Does manganese protect cultured human skin fibroblasts against oxidative injury by UVA dithranol and hydrogen peroxide? *Free Radic Res* (1995) **23**: 339–51.
17. Costanzo LL, De-Guidi G, Guiffrida S et al, Antioxidant effect of copper (II) on photosensitized lipid peroxidation. *J Inorg Biochem* (1995) **57**: 115–25.
18. Oyama Y, Fuchs PA, Katayama N et al, Myricetin and quercetin, the flavanoid constituents of Ginkgo bilboa extract, greatly reduces oxidative metabolism in both resting and Ca^{2+}-loaded brain neurons. *Brain Res* (1994) **635**: 125–9.
19. Masquelier J, Oligomeres procyanidoliques. *Parfums, cosmetiques et aromes* (1990) **95**: 89–95.