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Beneficial Long-Term Effects of Combined Oral/Topical Antioxidant Treatment with the Carotenoids Lutein and Zeaxanthin on Human Skin: A Double-Blind, Placebo-Controlled Study

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Key Words
Carotenoid · Lutein · Zeaxanthin · Antioxidant · Premature aging

Abstract
Background: The skin is exposed to numerous environmental assaults that can lead to premature aging. Of these agents, perhaps none is more ubiquitous than the ultraviolet (UV) wavelengths of sunlight. The primary immediate defense against environmental skin damage is the antioxidant capacity of the skin. However, this defense system can be compromised by moderate exposure to UV light. Therefore, bolstering the antioxidant defense system of the skin is a potentially important strategy for reducing environmentally induced skin damage. ***Aim of the Study:*** This clinical trial was designed to study the efficacy of lutein and zeaxanthin, two potentially important antioxidants found naturally in the skin, upon five skin physiology parameters (surface lipids, hydration, photoprotective activity, skin elasticity and skin lipid peroxidation – malondialdehyde) of human subjects. These xanthophyllic carotenoids were administered either orally, topically, or in combination (both oral and topical routes). ***Results:*** The results obtained indicate that the combined oral and topical administration of lutein and zeaxanthin provides the highest degree of antioxidant protection. However, oral and topical administration of these antioxidants individually also provides significant activity in the skin. In addition, oral administration of lutein may provide better protection than that afforded by topical application of this antioxidant when measured by changes in lipid peroxidation and photoprotective activity in the skin following UV light irradiation.

Introduction

Environmentally induced premature aging of the skin as well as the general aging of the population is of increasing importance. Irrespective of gender, the amount of ultraviolet (UV) exposure obtained is spread almost uniformly over a lifetime [1, 2]. However, skin cancer is only one of the effects that environmental exposure, including UV light exposure, can have on skin homeostasis [3–7]. Sunburn is the most common type of damage on the minds of consumers when they think about environmental exposures. Sun-induced skin damage, which ulti-

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mately becomes apparent in the form of premature aging of the skin, is believed to be primarily a result of UV-induced free radical generation in the skin [6, 7]. Although a significant portion of free radical-induced skin damage is attributed to UV exposure, free radicals can also be induced in the skin by other wavelengths of light, especially the shorter wavelengths of visible light [8]. Furthermore, pollutants in the atmosphere, e.g. ozone, can initiate free radical damage at least in the epidermis [3, 9, 10]. Protection against free radical-induced skin damage has developed into an important aspect of skin protection.

Human skin has an inherent antioxidant capacity to reduce the potential damage caused by free radicals. This inherent capacity can be significantly depleted by moderate UV light exposure [11, 12]. The loss of this natural epidermal and dermal antioxidant capacity may be counteracted by topical and systemic administration of antioxidants to the skin [13–53]. However, most of the data supporting the use of orally administered antioxidants are based on in vitro or animal studies. The majority of the studies involving orally ingested antioxidants in humans emphasize the photoprotective effects obtained from materials such as beta-carotene. However, several clinical studies are indicative of a broader range of skin-related benefits from orally ingested antioxidants. It has been shown that the oral ingestion of a mixture of antioxidants containing 6 mg of lutein and 0.3 mg of zeaxanthin per day induces an increase in skin surface lipids and skin hydration, while simultaneously reducing the amount of oxidized skin lipids following UV light exposure (lipid peroxidation) [43]. A complex mixture of orally administered antioxidants increased skin elasticity while simultaneously reducing the roughness of the skin [49]. Oral administration of a mixture of antioxidants resulted in a decrease in lipid peroxidation after 2 months compared to placebo [54]. This study also showed that the amount of lipid peroxidation had returned to pretreatment values within 5 months after discontinuance of the oral administration of this antioxidant mixture.

As a follow-up to the previous study from these laboratories [43], the present randomized, placebo-controlled, multicentered study was conducted. It compares the efficacy of antioxidant products containing lutein and zeaxanthin (xanthophyllic carotenoids, also commonly referred to as xanthophylls) administered both topically and orally twice daily to placebo control products. These test preparations were evaluated for their effect upon superficial skin lipids, skin hydration, lipid peroxidation, photoprotective activity and skin elasticity. This study was designed to allow for direct comparisons of lutein and zeaxanthin efficacy between different routes of administration and to demonstrate the efficacy obtained when these two routes of administration were combined.

Materials and Methods

This randomized, placebo-controlled, 12-week clinical was performed as a multicenter study to evaluate the effect of lutein and zeaxanthin administered orally and applied topically upon human skin. Forty healthy women (age 25–50 years; mean age 35.1 years) were enrolled. All subjects exhibited signs of premature skin aging and signed a written informed consent. The protocol for the study was reviewed and approved by appropriate ethics committees. The test subjects were randomized into one of the test groups shown in table 1 so that each group was balanced by average age. Experts at each test site evaluated subjects to ensure that the skin was free of any dermatological condition that might affect the test results and that the overall skin types and condition were uniformly represented across test groups.

Diet

All subjects were requested to maintain a balanced Mediterranean diet containing not more than 0.5 mg of beta-carotene per day for the entire study period. This diet was initiated 15 days prior to the start of the study in order to maintain a serum level of beta-carotene at approximately 0.33 ± 0.8 μmol/l. This was confirmed by serum blood levels of beta-carotene taken on two separate occasions (data not shown).

Test Products

A 20% dispersion of FloraGLO® Lutein in Safflower Oil (Kemin Health, L.C., Des Moines, Iowa, USA) was employed as the source of lutein and zeaxanthin for the oral soft gelatin capsules. The optically identical placebo capsule contained safflower oil in complete replacement for the lutein/zeaxanthin/safflower dispersion. The oral soft gel capsules were manufactured by Gelkaps GmbH (Pritzwalk, Germany). The lutein and zeaxanthin topical preparation contained FloraGLO Lutein 5% Oil-Free Liquid (5% lutein dispersed in butylene glycol; Kemin Health L.C.) and the placebo contained butylene glycol in replacement for the active compounds. The topical preparations were manufactured by Mavi Sud S.r.l. (Aprilia, Italy). The concentration of lutein and zeaxanthin contained in the oral and topical test products are shown in table 1.

Test subjects ingested one capsule (active or placebo as appropriate) in the morning and in the evening with meals. Similarly, they applied the assigned topical product (active or placebo) twice per day. The topical test products were applied to the face, neck, and the right arm after cleansing these skin areas with Mavigen Idroschiuma skin cleanser (Mavi Sud).

Measurement of Skin Hydration and Superficial Skin Lipids

Skin hydration and superficial skin lipids were evaluated using the 3C System methodology described by Cardillo and Morganti [55]. This instrument (Dermotech Italy S.r.l.) has a separate probe for each of these test parameters. The probes of this com-

Table 1. Description of groups and lutein and zeaxanthin concentrations in the oral and topical preparations

Test group	Test product	
	oral	topical
A (placebo)	placebo 2 × per day	placebo 2 × per day
B (topical)	placebo 2 × per day	lutein 50 ppm/zeaxanthin 3 ppm 2 × per day
C (oral)	lutein 5 mg/zeaxanthin 0.3 mg 2 × per day	placebo 2 × per day
D (combined)	lutein 5 mg/zeaxanthin 0.3 mg 2 × per day	lutein 50 ppm/zeaxanthin 3 ppm 2 × per day

puterized instrument collect up to 15 separate readings over a 25-second sampling period. On the days of laboratory evaluations, the skin was cleansed in the morning before measurements were taken and then left undisturbed until after these evaluations were completed. The topical test product was only applied after measurements were completed. These individual readings were taken between the nose and cheek and automatically averaged together. The resulting mean value was stored in the computer after standardization for environmental conditions (at a relative humidity of 25% and a temperature of 22°C). The probe employed in the 3C System for the measurement of skin hydration specifically assesses the total capacitance of the epidermis. The values, expressed in arbitrary units by the computer-controlled system, are automatically reported as a percentage increase from baseline values measured within the 15 days prior to initiation of the study. All skin hydration measurements were taken under standardized conditions [56]. The probe employed in the 3C System for the measurement of superficial skin lipids employs a 1-cm^2 frosted plastic foil surface that is brought into contact with the surface of the skin. Upon contact with the lipids on the surface of the skin, this frosted foil becomes transparent in direct proportion to the amount of lipids present on the skin. The change in the light transmission of the foil is automatically recorded by the 3C System and converted to milligrams of lipid per square centimeter of skin surface. These converted values are automatically reported as a percentage increase in superficial skin lipids from baseline values measured within the 15 days prior to initiation of the study by the computer-controlled system.

Skin Elasticity

Skin elasticity was assessed on the right forearm using a Dermaflex A instrument (Cortex Technology, Hadsund, Denmark) [57]. This instrument measures the extension of the skin in response to a suction vacuum induced above the skin test site with a 300-mbar vacuum; 20-second exposure period and 5 cycles per measurement. The relative elastic retraction was calculated from the equation described by Gniadecka and Serup [57]. The values obtained were calculated as percentage increase from baseline measured in the 15 days prior to the initiation of the study.

Skin Lipid Peroxidation

Lipid peroxidation values were determined by the method described by Ohkido et al. [58]. The amount of peroxides in the skin lipids was measured in terms of the amount of malondialdehyde (MDA) generated in skin lipids following irradiation of the test site with a measured light exposure (5.6 erg/cm^2/min for 2 min) from a high-pressure UV light source (Osram 300-watt lamp operating in the wavelength region of 240 and 320 nm) equipped with a grating monochrometer and a photodetector (Model IL700 International Light, Newbury, Mass., USA). Ten minutes after irradiation, skin lipids were extracted from the surface of the skin by the cup method using two acetone extractions with a total volume of 10 ml. The extraction procedure and the MDA quantification have been described by Ohkido et al. [58]. In summary, an aliquot of the extracted lipids is added to sodium dodecyl sulfate in distilled water, adjusted to pH 4 with 20% acetic acid. Thiobarbituric acid is added to this medium and the entire mixture is heated to 95°C for 60 min. After cooling to room temperature, n-butanol is added and the sample is centrifuged. The absorption of the n-butanol layer is then measured on a spectrophotometer at 532 nm. The amount of skin lipid peroxidation is reported as nanograms of MDA per 100 mg of lipid.

Photoprotection

The photoprotective activity values were obtained by measuring the skin surface redness with a Minolta Chromameter CR-300 24 h after a 2-min exposure of the right forearm to 80 mJ/cm^2 UV light (240–320 nm) from a 300-watt Osram lamp [43]. The minimum erythemal UV dose (MED) for untreated skin was determined prior to the initiation of the study. The minimal erythemal UV dose for treated skin was determined regularly throughout the study period. These values were employed to calculate the photoprotective activity for each subject according to the following equation: photoprotective activity = MED for treated skin/MED for untreated skin.

Statistical Evaluations

All results are presented as the mean value ± standard deviation. The standard deviation values obtained in this study were similar to that obtained previously [43]. The baseline values were employed to calculate the percentage change values employed in the statistical evaluations where appropriate. Statistical evaluations were performed with GraphPad Prism® 4 (GraphPad Software Inc., San Diego, Calif., USA). All statistical evaluations were conducted as two-tailed analyses at a minimum of a 95% confidence interval ($p < 0.05$) using a repeated-measures ANOVA and a Tukey post-hoc test to determine statistically significant differences in the results. The statistical comparisons employed were between each of the three xanthophyll treatments (oral, topical, or combination, (oral and topical, lutein/zeaxanthin)) and the

Table 2. Qualitative measurement from the questionnaire regarding age, skin type and skin condition shows balanced distribution within the 4 groups

Parameter evaluated	Test group			
	A (placebo)	B (topical)	C (oral)	D (combined)
Average age, years	36.4 ± 6.5	36.5 ± 5.7	32.4 ± 4.1	35.5 ± 5.4
Skin condition[a]				
Good	1	–	1	1
Average	–	2	4	6
Needs some improvement	7	5	4	2
Poor	2	3	1	1
Skin type[a]				
Dry	5	4	5	3
Normal	2	1	2	2
Oily	–	1	1	1
Combination (dry and oily)	3	4	2	4

[a] Results obtained from subjective responses to questionnaires completed by subjects and not employed in the randomization process.

placebo treatment at the same week of evaluation to demonstrate the effect of the treatment or within the individual treatments (between the week 2 value and subsequent values) in order to demonstrate the continued effect of the individual xanthophyll treatments.

Results

The results of the randomization of subjects according to age are shown in table 2. No statistical differences in this parameter were found between study groups. The dermatological condition of the skin of test subjects was also found to be uniformly represented between test groups (data not shown). Table 2 also reports the subjective results of skin condition and skin type obtained from questionnaires completed by the test subjects. Because this information was obtained from subjective responses, it was not used in the randomization of subjects between test groups. It is provided for information only. All enrolled subjects completed the test.

Positive Effect of All Antioxidant Treatments upon Surface Lipids Most Prominent in the Combination Group (Oral and Topical)

Aside from the initial increase attributable to the lipids of the topical lotion, only a minor variability was detected in the placebo treatment (group A; fig. 1). All xanthophyll treatments indicated a positive and significant effect on superficial skin lipids compared to the placebo treatment. The topical treatment (group B) showed an immediate increase in superficial skin lipids at week 2. In comparison to the topical treatment, the increase for the oral treatment (group C) appeared to have a lower initial effect. Combined oral and topical treatment (group D) was similar to the topical treatment at week 2 and then increased in a similar manner to the oral treatment. The combined oral and topical treatment was approximately equivalent to the sum of the effects seen for the separate oral and topical treatments.

At each evaluation period, the combined oral and topical treatment resulted in a consistently greater increase in superficial skin lipids than either of the other two xanthophyll treatments. At weeks 2 and 4, the oral xanthophyll treatment resulted in a lower amount of superficial skin lipids as compared to the topical xanthophyll treatment group. However, from the week 6 evaluation to the end of the study, the oral xanthophyll treatment was found to result in consistently greater amounts of superficial skin lipids than the topical treatment. The maximum increase in skin lipids was seen at week 12: 63% for the combined oral and topical treatment, 46% for the oral treatment, and 23% for the topical treatment compared to 10% for the placebo treatment.

Decrease in Lipid Peroxidation in All Treatment Groups except Placebo

Similar to the results for the superficial lipids, some variability was found in the data for the placebo treat-

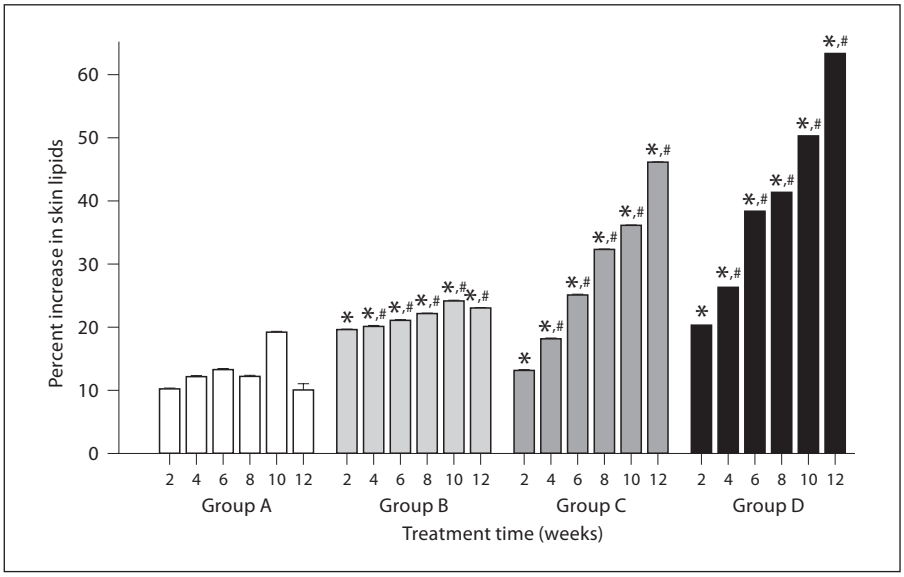

Fig. 1. Effect of lutein and zeaxanthin upon superficial skin lipids by treatment group over the study period. * $p < 0.05$ vs. placebo treatment at the same week; # $p < 0.05$ vs. the week 2 treatment value within the same treatment group.

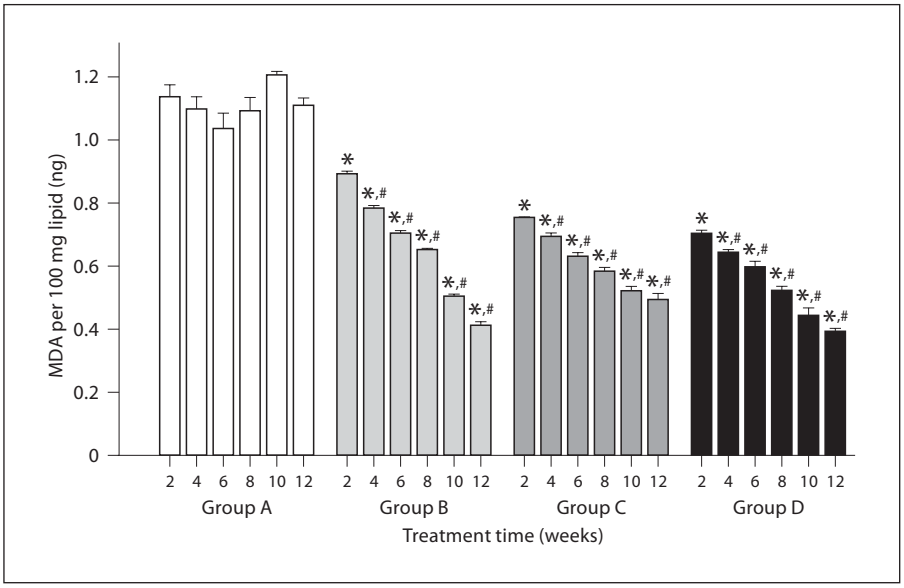

Fig. 2. Effect of lutein and zeaxanthin upon lipid peroxidation by treatment group over the study period. See figure 1 for explanation of statistical differences.

ment (fig. 2). Each of the three xanthophyll treatments induced statistically significant reductions in skin lipid peroxidation as measured by the amount of MDA throughout the study period in comparison to the placebo treatment. The combined oral and topical treatment resulted in the greatest decrease in skin lipid peroxidation at each evaluation time point. Through week 8 of the study, the oral treatment resulted in a greater reduction in skin lipid peroxidation compared to the topical treatment. The maximum change was seen at week 12 for all three xanthophyll treatments.

Photoprotective Activity Is Highest in the Combined Treatment Group

The topical treatment (group B) increased the photoprotective activity at week 2 and remained relatively constant throughout the remainder of the study period (fig. 3). In a similar manner, the oral treatment (group C) produced a slight increase in photoprotective activity at weeks 2, 4 and 8 and then remained relatively constant for the rest of the test period. The combined oral and topical treatment (group D) follows a similar trend to the oral treatment and exhibited the greatest efficacy on photoprotection. The efficacy exhibited by the combined

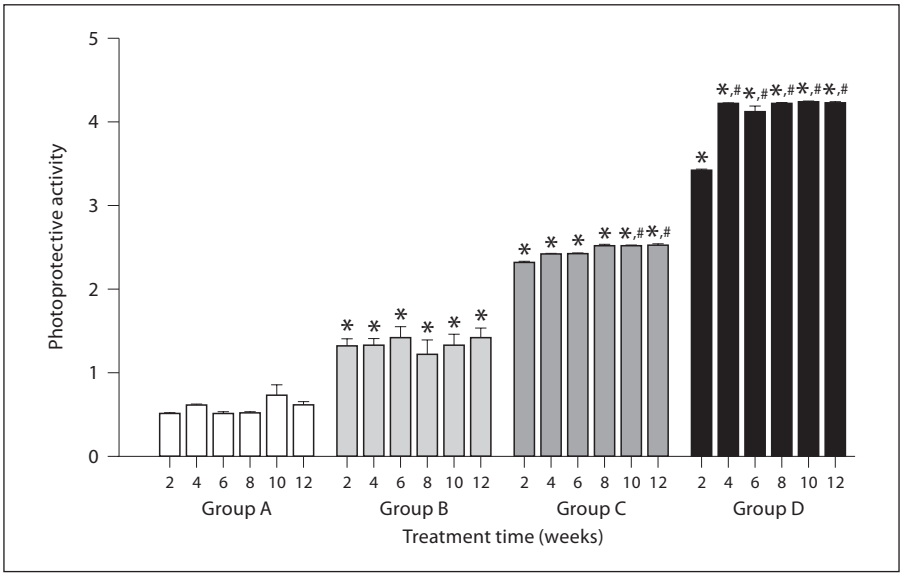

Fig. 3. Effect of lutein and zeaxanthin upon photoprotective activity by treatment group over the study period. See figure 1 for explanation of statistical differences.

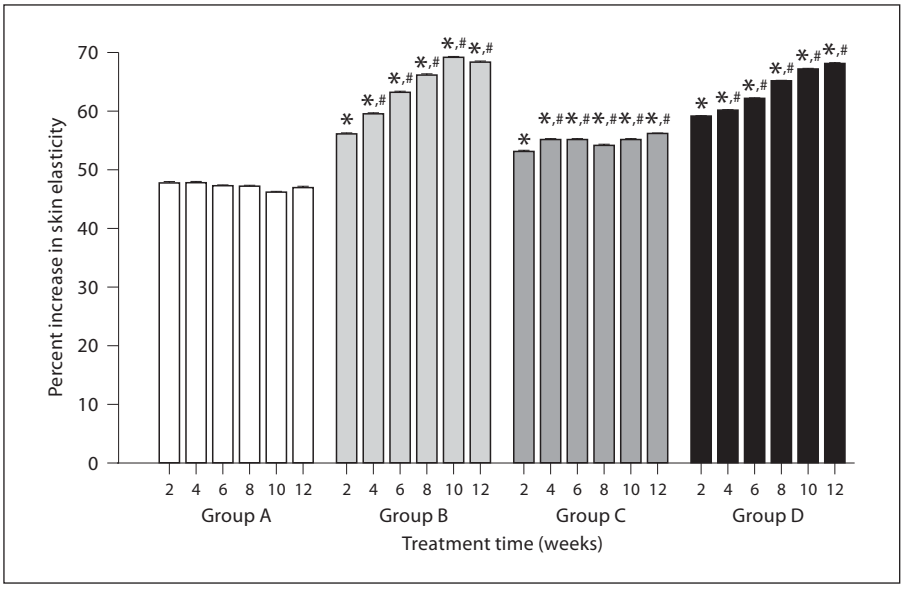

Fig. 4. Effect of lutein and zeaxanthin upon skin elasticity by treatment group over the study period. See figure 1 for explanation of statistical differences.

treatment was greater than the sum of the oral and topical treatments after accounting for the contribution of the placebo, indicating that there may be a synergistic effect from the combined treatment adjusted in the same manner (data not shown).

Skin Elasticity: Topical Treatment Is Most Effective

The results for the oral treatment (group C) exhibited an increase in skin elasticity at weeks 2 and 4, but remained relatively unchanged after week 4 (fig. 4). Topical and combined oral and topical treatments showed increases in skin elasticity throughout the entire study period in comparison to placebo. The most prominent increase was detectable in the topical treatment group (group B). All xanthophyll treatments resulted in statistically significant improvements in skin elasticity in comparison to the placebo at each week of evaluation.

Skin Hydration

Each of the three xanthophyll treatments resulted in a statistically significant increase in skin hydration versus the placebo (fig. 5). The combined oral and topical xanthophyll treatment showed a greater effect than either of the individual (oral or topical) treatments throughout the

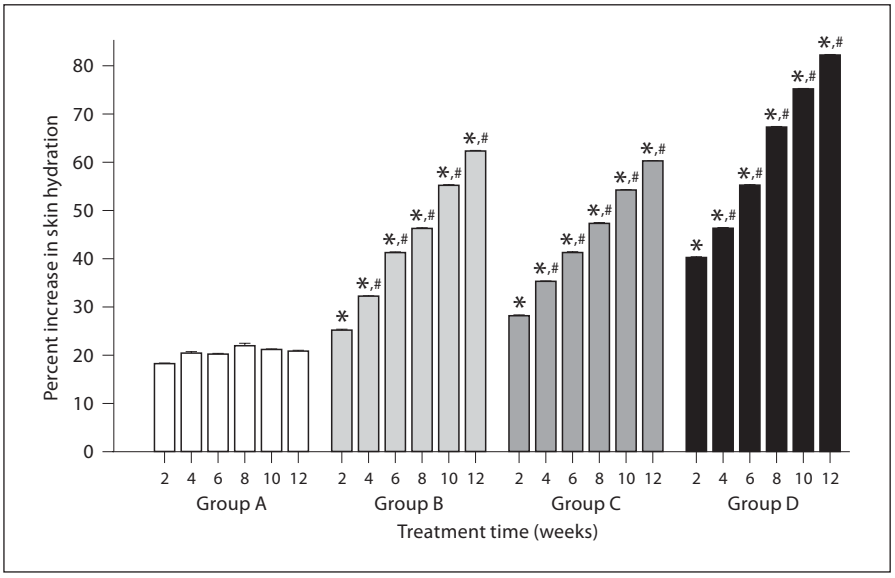

Fig. 5. Effect of lutein and zeaxanthin upon skin hydration by treatment group over the study period. See figure 1 for explanation of statistical differences.

Table 3. Pearson correlation (p value) calculated over all 5 parameters for each of the 4 treatment groups

	Skin surface lipids				Photoprotection				Lipid peroxidation				Skin elasticity			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Skin hydration	<0.05	<0.05	<0.05	<0.05	n.s.	n.s.	<0.05	n.s.	n.s.	<0.05	<0.05	<0.05	n.s.	<0.05	n.s.	<0.05
Skin elasticity	<0.05	<0.05	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.	<0.05				
Lipid peroxidation	<0.05	n.s.	<0.05	<0.05	n.s.	n.s.	<0.05	n.s.								
Photoprotection	n.s.	n.s.	<0.05	n.s.												

Group A = Placebo, oral and topical; group B = placebo oral and lutein topical; group C = lutein oral and placebo topical; group D = lutein, oral and topical.

entire study period. Through week 4, the oral xanthophyll treatment produced a greater increase in skin hydration than the topical xanthophyll treatment. Over the remainder of the study the topical and oral treatments resulted in approximately equal increases in skin hydration.

Correlation

A Pearson correlation analysis was conducted in order to reveal the existence of any correlations between the five separate measures of the xanthophyll treatment-related skin physiology parameters evaluated in this clinical study (table 3). Skin lipids and skin hydration values showed a significant correlation ($p < 0.05$) for all four treatments. Superficial skin lipids and skin lipid peroxidation were negatively correlated for two of the xanthophyll treatments. A positive slope was found for the placebo treatment, indicating that the amount of skin lipid peroxidation increased as the amount of skin lipids increased during the course of the study for this group (data not shown). Superficial skin lipids and skin elasticity were correlated for the placebo, topical xanthophyll, and combined oral and topical xanthophyll treatments. The correlation indicates that the topical treatment effect was the most prominent. Skin hydration and skin lipid peroxidation were negatively correlated for the topical, oral, and the combined oral and topical xanthophyll treatments, indicating that increased skin hydration is associated with decreasing lipid peroxidation (data not shown). Additionally, the slopes of these lines and the intercepts are similar, indicating that each of the three treatments may be a result of a similar effect, possibly induced by xanthophyll treatment. This is reinforced by an absence

of a similar correlation for the placebo treatment group. Skin elasticity was correlated with both skin hydration and skin lipid peroxidation for the same two treatment groups, namely the topical xanthophyll and the combined oral and topical xanthophyll treatments. This effect was not consistent in all treatment groups and needs careful interpretation. Correlations involving photoprotective activity were only observed for the oral xanthophyll treatment.

Discussion

The administration of lutein and zeaxanthin resulted in statistically significant positive directional changes in all of the evaluated skin parameters. These statistically significant changes relative to the placebo treatment were detected regardless of the route of administration of xanthophylls. The combined oral and topical xanthophyll treatment showed the largest change in all parameters and a greater change than either route of individual administration. Although the separate oral and topical administration of lutein and zeaxanthin produced positive changes, the magnitude of those changes was dependent upon the particular test parameter, e.g. the topical xanthophyll treatment resulted in a greater initial change in skin lipids, but the oral xanthophyll treatment produced larger changes in the later stage of the study.

Superficial Skin Lipids

The initial increase in skin lipids observed for the placebo treatment shown in figure 1 is attributable to the application of lipids in the topical vehicle. However, the presence of lutein and zeaxanthin in each of the three xanthophyll treatments resulted in an additional increase in surface lipids beyond that observed for the placebo treatment. Both the oral and the combined oral and topical xanthophyll treatments resulted in a similar increase in skin lipids to that found for the topical xanthophyll treatment at the initial evaluation. The results for the combined oral and topical xanthophyll treatment then parallel the results for the oral xanthophyll treatment for the remainder of the study.

Increases in skin lipids have been reported from previous studies of orally ingested antioxidants [43, 49] without offering an explanation for this effect. Although it remains to be determined whether this increase is specifically related to increased sebaceous gland output, it has been shown that sebaceous glands are the primary delivery route for dietary sources of tocopherols to the skin surface [59]. Given the similarities in the solubility characteristics of tocopherols and carotenoids, the increases in superficial skin lipids resulting from the oral xanthophyll treatment may be a result of this route of delivery with subsequent stimulation of the sebum output by oral administration of lutein and zeaxanthin. However, this explanation does not account for the increased skin surface lipids observed for the topical xanthophyll treatment. In this instance, the increase in measured skin surface lipids may be the result of an increased flow of sebaceous lipids from the reservoir of these lipids in the follicles to the skin surface stimulated by the application of lutein and zeaxanthin to the skin surface.

An alternate explanation is that lutein and zeaxanthin affect the lipids present on the surface of the skin in a manner that results in an increase in the adherence of the lipids present on the surface of the skin to the frosted plastic film employed in this measurement. Since the results obtained from questionnaires completed at the conclusion of the study fail to indicate subject dissatisfaction with the treatments in relation to significant increases in skin oiliness (data not shown), the increases in superficial skin lipids observed may be only partially attributable to actual increases in sebaceous lipid output. The remainder of the increase seen from these measurements must then be accounted for by other factors.

Effect of Treatment upon Skin Lipid Peroxidation

All three lutein and zeaxanthin treatments resulted in a statistically significant decrease in lipid peroxidation versus placebo over the entire course of the study (fig. 2). The greatest decrease was found for the combined treatment. The fact that the topical xanthophyll treatment produces a smaller decrease in lipid peroxidation than the other xanthophyll treatments through week 8 can potentially be attributed to two separate effects. First, lutein and zeaxanthin applied to the surface of the skin are constantly exposed to the environment. This exposure has the potential to reduce the amount of these xanthophylls on the skin's surface through photodegradation. Furthermore, the amount of lutein and zeaxanthin present on the surface of the skin could establish a gradient that allows these xanthophylls to penetrate the stratum corneum. If a sufficient amount of the topically administered xanthophylls penetrates beyond the depth of the lipids removable by the organic solvent employed to extract the oxidized lipids from the skin in this assay procedure, then the protection afforded by these topically applied xantho-

phylls could appear to be diminished. Since skin penetration by tocopherols from the skin surface has been demonstrated [59] and since these molecules have similar solubility characteristics to those of carotenoids, such skin penetration for the xanthophylls employed in this study may also be possible.

The alternative explanation involves the rate of delivery of orally administered lutein and zeaxanthin to the surface of the skin. The presence of lutein and zeaxanthin in the skin following dietary and oral administration has been demonstrated [33, 39, 60–62]. These xanthophylls might be delivered to the cells at the dermal-epidermal junction via the bloodstream, in addition to delivery from the sebaceous gland route as described above for oral administration, thus establishing a xanthophyll concentration gradient. This combination of diffusion and sebaceous delivery could result in greater amounts of xanthophylls at or near the skin surface than might be achieved from either route alone. Additionally, the degree of penetration of the topically administered xanthophylls over time may explain how this route of administration resulted in a greater degree of protection in the latter weeks of this study as compared to the oral administration of these carotenoid molecules.

It is also important to understand that the oxidized skin lipids extracted from the skin in this particular evaluation are not solely attributable to the UV-induced oxidation of sebaceous lipids. The lipids extracted by the technique employed in this study include sebaceous lipids, corneocyte membrane lipids, and intercellular lipids. Additionally, since UV light has the potential to penetrate the skin to at least the dermal-epidermal junction [4], lipids from each of the above sources have the potential to be oxidized by free radicals induced by UV light. The lipid peroxidation activity could also, at least partially, be related to the effect of UV light on squalene since wavelengths of UVA light induce the formation of squalene monohydroperoxide isomers in vivo [63].

Treatment Effect on Free Radical-Related Photoprotective Activity Is Detectable in the Early Treatment Phase

All lutein and zeaxanthin treatments but not the placebo provide protection from UV radiation-induced damage regardless of their route of administration (fig. 3). The topical xanthophyll treatment provides a twofold increase in this activity in just 2 weeks of treatment, the oral treatment more than a fourfold increase and the combined treatment a sixfold increase in photoprotective activity compared to the placebo treatment.

Since lutein and zeaxanthin do not absorb light in the UV wavelengths [64], this efficacy is attributable to protection against UV-induced free radicals. Animal studies have demonstrated that these xanthophylls accumulate in the skin as a result of the oral ingestion of lutein and zeaxanthin [62]. Additionally, carotenoids are known to quench both the triplet state of photosensitizers and singlet oxygen that are responsible for the formation of free radicals [65]. This type of activity has previously been demonstrated for oral administration of carotenoids [35, 36, 40, 41]. Dietary intake of tomato paste, which contains a number of carotenoids including beta-carotene, lycopene, lutein, and zeaxanthin, can provide photoprotective activity [37]. Furthermore, animal studies have demonstrated that lutein and zeaxanthin may provide protection against inflammation, epidermal hyperplasia (skin thickening), formation of apoptotic (sunburn) cells, and immunosuppression in the skin [62, 66]. This type of activity has not been previously demonstrated for topical or for the combination of orally and topically administered xanthophylls in humans or animals. The rapid increase followed by only small changes in this activity may indicate that there are binding sites for carotenoids in the skin that may limit the amount of protection afforded by such molecules. The potential synergistic activity obtained from the combined oral and topical xanthophyll treatment might have an important impact on life habits in terms of improving the homeostasis of the skin of the general population.

Skin Elasticity

Oral treatment, but not placebo, induced an immediate improvement in skin elasticity (fig. 4). Conversely, the topical treatment resulted in a larger initial increase in skin elasticity and the combined oral and topical treatment resulted in the largest initial change.

Since the changes in this parameter were similar for the topical and combined oral and topical xanthophyll treatments and the fact that the combined xanthophyll treatment included the same formulation as the topical xanthophyll treatment, this result is probably attributable to the effects of topically applied lutein and zeaxanthin. The low density of sebaceous glands on the volar forearm probably restricts the amount of lutein and zeaxanthin delivered to the surface of the skin from the oral route of administration and thereby limits the efficacy observed from the skin elasticity measurement for this treatment. It is possible that the penetration of the lutein and zeaxanthin present on the surface of the skin and the uptake of these xanthophylls by the membranes of the corneo-

cytes and intercellular lipids affect the viscoelastic properties of the skin with an important impact on skin plasticity [67].

Highest Improvement of Skin Hydration by Combined Treatment

The placebo treatment caused an immediate increase in skin hydration probably related to the effect upon the lipid barrier and the addition of moisture to the stratum corneum (fig. 5). However, each of the xanthophyll treatments provided a statistically significant initial increase in stratum corneum hydration that continued to increase with further treatment. As anticipated, the combined oral and topical xanthophyll treatment provided the greatest degree of increase in skin hydration throughout the entire study.

The increased skin moisturization resulting from the xanthophyll treatments seemed to be related to the penetration of lutein and zeaxanthin into the membranes of the corneocytes and the intercellular lipids that constitute the barrier properties of the stratum corneum. The reduced lipid peroxidation may also have played an important role in increasing skin hydration. Although these effects have not previously been demonstrated from treatment of the skin with lutein and zeaxanthin, increases in skin hydration have previously been demonstrated for formulations containing topically applied antioxidants as well as for orally administered combinations of antioxidants [34, 42, 43, 68, 69].

Correlation of Different Parameters

The above explanation of the results obtained for each of the separate skin parameters takes into account the known effects of carotenoids and antioxidants on these closely related parameters. For instance, it is known that skin hydration is positively related to skin elasticity [56, 67]. Therefore, the Pearson correlation analysis was conducted on these data for each pair of evaluation parameters for each treatment (table 3).

The positive correlation of skin lipids and skin hydration supports the observation that the amount of surface lipids influences skin hydration. The degree of lipid peroxidation also had an effect on skin hydration for each of the xanthophyll treatments but not for the placebo treatment as shown in the correlation data. This correlation may indicate that xanthophyll-induced reduction in lipid peroxidation may be important since it is correlated to the skin hydration effects observed. The decrease in skin lipid peroxidation was correlated with the increase in skin lipids for the oral and combined oral and topical treatments. However, no such correlation was found for the topical treatment. Furthermore, although a correlation was found in the results for the placebo treatment associated with these two parameters, it is the inverse of that found for the xanthophyll treatments, indicating that the increase in skin lipids observed was associated with an increase in lipid peroxidation.

In conclusion, the present study indicates that the administration of lutein and zeaxanthin provides multiple benefits to the skin. In addition to the protection of the skin from the deleterious effects of UV light-inducible damage (increased free radical production) and decreased lipid peroxidation, these xanthophylls also increased the surface lipids, skin hydration, and skin elasticity. Although these benefits were obtained regardless of whether lutein and zeaxanthin were administered orally or topically, the study demonstrated that an additional benefit is achieved through the simultaneous administration of these xanthophylls by both routes. Furthermore, this study provides insights into the way that lutein and zeaxanthin may act in the skin as well as the effects that alternative routes of administration may have upon the benefits derived from these carotenoids.

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