

# Skin Aging-Scientific Protocols

## Skin Aging

### ABSTRACTS

[Abdel-Galil AM., 1984. Prevention of 3-methylcholanthrene-induced skin tumors in mice by simultaneous application of 13-cis-retinoic acid and retinyl palmitate \(vitamin A palmitate\).](#)

[Araneo BA., 1995. Dehydroepiandrosterone reduces progressive dermal ischemia caused by thermal injury.](#)

[Bangha E., 1997. Suppression of UV-induced erythema by topical treatment with melatonin \(N-acetyl-5-methoxytryptamine\). Influence of the application time point.](#)

[Becker-Wegerich P., 2001. Botulinum toxin A in the therapy of mimic facial lines.](#)

[Brincat M., 1983. Sex hormones and skin collagen content in postmenopausal women.](#)

[Chapellier B., 2002. Physiological and retinoid-induced proliferations of epidermis basal keratinocytes are differently controlled.](#)

[Darr D., 1996. Effectiveness of antioxidants \(vitamin C and E\) with and without sunscreens as topical photoprotectants.](#)

[Dreher F., 2001. Protective effects of topical antioxidants in humans.](#)

[Dunn LB., 1997. Does estrogen prevent skin aging?: Results from the first national health and nutrition examination survey \(NHANES I\).](#)

[Fischer T., 1999. \[Melatonin in dermatology. Experimental and clinical aspects\]](#)

[Fisher GJ., 1997. Pathophysiology of premature skin aging induced by ultraviolet light.](#)

[Fitzpatrick RE., 2002. Double-blind, half-face study comparing topical vitamin C and vehicle for rejuvenation of photodamage.](#)

[Gensler HL., 1987. Effects of dietary retinyl palmitate or 13-cis-retinoic acid on the promotion of tumors in mouse skin.](#)

[Giacomoni PU., 2000. Aging of human skin: review of a mechanistic model and first experimental data.](#)

[Hastings LA., 1988. Dehydroepiandrosterone and two structural analogs inhibit 12-O-tetradecanoylphorbol-13-acetate stimulation of prostaglandin E2 content in mouse skin.](#)

[Hong JT., 2001. Inhibitory effect of glycolic acid on ultraviolet-induced skin tumorigenesis in SKH-1 hairless mice and its mechanism of action.](#)

[Kim SJ., 1998. The effect of glycolic acid on cultured human skin fibroblasts: cell proliferative effect and increased collagen synthesis.](#)

[Kostarelos K., 2000. Double-blind clinical study reveals synergistic action between alpha-hydroxy acid and betamethasone lotions towards topical treatment of scalp psoriasis.](#)

[Koussoulakos S., 1990. Effect of vitamin A on wound epidermis during forelimb regeneration in adult newts.](#)

[Kozarev J., 1998. \[Use of photoprotective measures in relation to actual exposure to solar rays\]](#)

[Labrie F., 2000. Intracrinology and the skin.](#)

[Pashko LL., 1985. Inhibition of 7,12-dimethylbenz\(a\)anthracene-induced skin papillomas and carcinomas by dehydroepiandrosterone and 3-beta-methylandro-5-en-17-one in mice.](#)

[Pashko LL., 1991. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-promoted skin tumor formation in mice by 16 alpha-fluoro-5-androsten-17-one and its reversal by deoxyribonucleosides.](#)

[Podda M., 2001. Low molecular weight antioxidants and their role in skin ageing.](#)

[Nyirady J., 2001. Tretinoin cream 0.02% for the treatment of photodamaged facial skin: a review of 2 double-blind clinical studies.](#)

[Ridge BD., 1988. The dansyl chloride technique for stratum corneum renewal as an indicator of changes in epidermal mitotic activity following topical treatment.](#)

[Saliou C., 2001. Solar ultraviolet-induced erythema in human skin and nuclear factor-kappa-B-dependent gene expression in keratinocytes are modulated by a French maritime pine bark extract.](#)

[Scharffetter-Kochanek K., 2000. Photoaging of the skin from phenotype to mechanisms.](#)

[Schwartz AG., 1986. Food restriction inhibits \[3H\] 7,12-dimethylbenz\(a\)anthracene binding to mouse skin DNA and tetradecanoylphorbol-13-acetate stimulation of epidermal \[3H\] thymidine incorporation.](#)

[Schwartz AG., 1986. Inhibition of tumor development by dehydroepiandrosterone and related steroids.](#)

[Scott DE., 1986. Hypothalamic neuroendocrine correlates of cutaneous burn injury in the rat: I. Scanning electron microscopy.](#)

[Shah MG., 2001. Estrogen and skin. An overview.](#)

[Sorg O., 2001. Cutaneous vitamins A and E in the context of ultraviolet- or chemically-induced oxidative stress.](#)

[Tangpricha V., 2002. Vitamin D insufficiency among free-living healthy young adults.](#)

[Tixier JM., 1984. Evidence by in vivo and in vitro studies that binding of pycnogenols to elastin affects its rate of degradation by elastases.](#)

[Traikovich SS., 1999. Use of topical ascorbic acid and its effects on photodamaged skin](#)

[topography.](#)

[Trang HM., 1998. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2.](#)

[Uhoda I., 2002. Split face study on the cutaneous tensile effect of 2-dimethylaminoethanol \(deanol\) gel.](#)

[Varani J., 1998. Molecular mechanisms of intrinsic skin aging and retinoid-induced repair and reversal.](#)

[Varani J., 2000. Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin.](#)

[Vieth R., 2001. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it.](#)

[Yamamoto I., 2002. Synthesis and characterization of a series of novel monoacylated ascorbic acid derivatives, 6-O-acyl-2-O-alpha-D-glucopyranosyl-L-ascorbic acids, as skin antioxidants.](#)

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**Prevention of 3-methylcholanthrene-induced skin tumors in mice by simultaneous application of 13-cis-retinoic acid and retinyl palmitate (vitamin A palmitate).**

Abdel-Galil AM, Wrba H, El-Mofty MM.

Exp Pathol. 1984;25(2):97-102.

Two retinoids (13-cis-retinoic acid and retinyl palmitate ) have been shown to exert a good preventive effect in chemically induced papillomas and carcinomas of the skin in female Swiss mice; this effect was investigated over a period of 23 weeks. The tumors were induced by repeated topical application of 3-methylcholanthrene (0.3% MCA, dissolved in acetone; 14 applications). Retinyl palmitate (RP; 6 mg in 0.1 ml acetone/mouse; 10 applications) and 13-cis-retinoic acid (RA; 3 mg in 0.1 ml acetone/mouse; 10 applications) were also administered topically for the 3rd to 9th week from the start of the experiment. This investigation gave evidence for the fact that both the retinoids did not only inhibit the development of skin papillomas but had also a marked effect on skin carcinomas.

**Dehydroepiandrosterone reduces progressive dermal ischemia caused by thermal injury.**

Araneo BA, Ryu SY, Barton S, Daynes RA Department of Pathology, University of Utah School of Medicine, Salt Lake City 84132, USA.

J Surg Res 1995 Aug;59(2):250-62

Progressive ischemia and necrosis of the skin following thermal injury are reduced by postburn administration of the steroid hormone dehydroepiandrosterone (DHEA). Thermally injured animals were provided with a subcutaneous injection of DHEA, or a related species of steroid hormone, at various times after burning. During the 96 hr following administration of the scald burn, tissue necrosis was closely monitored. Subcutaneous administration of DHEA at

approximately 1 mg/kg/day achieved optimal protection against the development of progressive dermal ischemia. DHEA, 17 alpha-hydroxy-pregnenelone, 16 alpha-bromo-DHEA, and androstenediol each demonstrated, a similar level of protection. Other forms of steroids, including DHEA sulfate, androstenedione, 17 beta-estradiol, or dihydrotestosterone, exhibited no protective effect under the conditions tested. Additionally, intervention therapy with DHEA could be initiated up to 4 hr, but not 6 hr, after burn without a marked reduction in therapeutic benefit. Examination of the microvasculature of thermally injured dorsal skin suggested that postburn intervention with DHEA, either directly or indirectly, maintained a normal architecture in most of the dermal capillaries and venules within burn-exposed tissue. These findings suggest that systemic intervention therapy of burn patients with DHEA or a similar acting steroid hormone may be useful in preventing the progressive tissue destruction caused by progressive ischemia.

### **Suppression of UV-induced erythema by topical treatment with melatonin (N-acetyl-5-methoxytryptamine). Influence of the application time point.**

Bangha E, Elsner P, Kistler GS. Department of Dermatology, University of Zurich, Switzerland.

Dermatology 1997;195(3):248-52

**BACKGROUND:** In a previous study, we reported a significant and dose-dependent suppression of UV-induced erythema in human skin by a topically applied melatonin preparation.

**OBJECTIVE:** The present double-blind randomized study was designed to examine the influence of the application time point of topical melatonin on this antierythema effect.

**METHODS:** Defined small areas on the lower back of 20 volunteers were treated with 0.6 mg/cm<sup>2</sup> melatonin dissolved in a nanocolloid gel carrier either 15 min before or 1, 30 or 240 min after UV irradiation with twice the individual minimal erythema dose delivered by a Multiport Solar UV Simulator (UVA and UVB). The erythemata induced were evaluated by visual scoring and chromametry 24 h after irradiation.

**RESULTS:** Treatment of the skin with melatonin 15 min before UV irradiation proved to almost completely suppress the development of an UV-induced erythema. In contrast, no significant protective effects of melatonin were observed when it was applied after UV irradiation.

**CONCLUSION:** Topically applied melatonin has a clear-cut protective effect against UV-induced erythema. Free radical scavenging of UV-generated hydroxyl radicals and interference with the arachidonic acid metabolism are possible mechanisms of the melatonin action.

### **Botulinum toxin A in the therapy of mimic facial lines.**

Becker-Wegerich P, Rauch L, Ruzicka T. Department of Dermatology, Heinrich Heine University Dusseldorf, Germany. [Petra.Becker-Wegerich@uni-duesseldorf.de](mailto:Petra.Becker-Wegerich@uni-duesseldorf.de)

Clin Exp Dermatol 2001 Oct;26(7):619-30

In aesthetic medicine, many different methods of skin rejuvenation are available. At the end of the 1980s, the neurotoxin Botulinum toxin A (BT-A) led to a revolution in aesthetic-corrective dermatology for the treatment of mimic facial wrinkles. The toxin is produced by *Clostridium botulinum* and causes a reversible, selective muscle relaxation that leads to a temporary flattening of the mechanical part of wrinkling without the stigmata of invasive surgery. After two decades of experience in different medical disciplines, there has been remarkable clinical development and progress in research, the identification of new botulinum toxin serotypes, and also innovation in indications and combined modalities. These lead to new and interesting questions. BT-A offers the experienced, critical dermatologist a time-saving, effective, cosmetically satisfactory, non-invasive treatment for mimic facial wrinkles and neck and decollete lines, with only minor side effects. Dermatologists should have a profound anatomical knowledge and should be able to perform all injection techniques to meet the needs of ever more demanding patients and to ensure optimization of patient satisfaction. The following review summarizes the historical development and the mechanism of action of both frequently and rarely used injection techniques with BT-A for the treatment of wrinkles and lines of the upper face, neck and decollete, and gives an update of different experiences encountered.

#### **Sex hormones and skin collagen content in postmenopausal women.**

Brincat M, Moniz CF, Studd JW, Darby AJ, Magos A, Cooper D.

Br Med J (Clin Res Ed) 1983 Nov 5;287(6402):1337-8

Skin biopsy specimens were taken from 29 postmenopausal women who had not been given hormone replacement therapy and from 26 women who had been treated with oestrogen and testosterone implants for two to 10 years. The mean hydroxyproline content and therefore the mean collagen content in the skin was found to be 48% greater in the treated than the untreated women, who were matched for age. This difference was significant ( $p$  less than 0.01). The implication of this finding is that oestrogen or testosterone, or both, prevents the decrease in skin collagen content that occurs with aging and protects skin in the same way as it protects bone in postmenopausal women.

#### **Physiological and retinoid-induced proliferations of epidermis basal keratinocytes are differently controlled.**

Chapellier B, Mark M, Messaddeq N, Calleja C, Warot X, Brocard J, Gerard C, Li M, Metzger D, Ghyselinck NB, Chambon P. Institut de Genetique et de Biologie Moleculaire et Cellulaire, CNRS/INSERM/ULP, College de France, BP 10142, 67404 Illkirch Cedex, CU de Strasbourg, France.

EMBO J. 2002 Jul 1;21(13):3402-13.

To investigate the roles of retinoic acid (RA) receptors (RARs) in the physiology of epidermis that does not express RAR beta, conditional spatio-temporally controlled somatic mutagenesis

was used to selectively ablate RAR alpha in keratinocytes of RAR gamma-null mice. Keratinocyte proliferation was maintained in adult mouse epidermis lacking both RAR alpha and RAR gamma, as well as in RAR beta-null mice. All RAR-mediated signalling pathways are therefore dispensable in epidermis for homeostatic keratinocyte renewal. However, topical treatment of mouse skin with selective retinoids indicated that RXR/RAR gamma heterodimers, in which RXR transcriptional activity was subordinated to that of its RAR gamma partner, were required for retinoid-induced epidermal hyperplasia, whereas RXR homodimers and RXR/RAR alpha heterodimers were not involved. RA-induced keratinocyte proliferation was studied in mutant mice in which RXR alpha, RXR alpha and RAR alpha, RAR gamma, or RXR alpha and RAR gamma genes were specifically disrupted in either basal or suprabasal keratinocytes. We demonstrate that the topical retinoid signal is transduced by RXR alpha/RAR gamma heterodimers in suprabasal keratinocytes, which, in turn, stimulate proliferation of basal keratinocytes via a paracrine signal that may be heparin-binding EGF-like growth factor.

### **Effectiveness of antioxidants (vitamin C and E) with and without sunscreens as topical photoprotectants.**

Darr D, Dunston S, Faust H, Pinnell S. North Carolina Biotechnology Center, Raleigh, N.C., USA.

Acta Derm Venereol. 1996 Jul;76(4):264-8.

Considerable interest has been recently generated concerning the use of natural compounds, antioxidants in particular, in photoprotection. Two of the best known anti-oxidants are vitamins C and E, both of which have been shown to be somewhat effective in different models of photodamage. Very little has been reported, however, on the effectiveness of a combination of the two (known to be biologically the more relevant situation); nor have there been detailed studies on the ability of these antioxidants to augment commercial sunscreen protection against UV damage. We report that (in swine skin) vitamin C is capable of additive protection against acute UVB damage (sunburn cell formation) when combined with a UVB sunscreen. A combination of both vitamins E and C provided very good protection from a UVB insult, the bulk of the protection attributable to vitamin E. However, vitamin C is significantly better than vitamin E at protecting against a UVA-mediated phototoxic insult in this animal model, while the combination is only slightly more effective than vitamin C alone. When vitamin C or a combination of vitamin C and E is formulated with a commercial UVA sunscreen (oxybenzone), an apparently greater than additive protection is noted against the phototoxic damage. These results confirm the utility of anti-oxidants as photoprotectants but suggest the importance of combining the compounds with known sunscreens to maximize photoprotection. **Protective effects of topical antioxidants in humans.**

Dreher F, Maibach H. Department of Dermatology, University of California, San Francisco, Calif., USA.

Curr Probl Dermatol. 2001;29:157-64.

Human studies have convincingly demonstrated pronounced photoprotective effects of 'natural'

and synthetic antioxidants when applied topically before UVR exposure. Particularly with respect to UVB-induced skin damage such as erythema formation, the photoprotective effects of antioxidants are significant when applied in distinct mixtures in appropriate vehicles. Topical application of such combinations may result in a sustained antioxidant capacity of the skin, possibly due to antioxidant synergisms. And, since UVA-induced skin alterations are believed to be largely determined by oxidative processes [26], topical administration of antioxidants might be particularly promising [27, 28]. In fact, topical application of antioxidants or antioxidant mixtures resulted in a remarkable increase in the minimal dose to induce immediate pigment darkening after UVA exposure [18, 23] and diminished the severity of UVA-induced photodermatoses [22] in humans. In conclusion, regular application of skin care products containing antioxidants may be of the utmost benefit in efficiently preparing our skin against exogenous oxidative stressors occurring during daily life. Furthermore, sunscreens may also benefit from combination with antioxidants resulting in increased safety and efficacy of such photoprotective products [11, 29]. **Does estrogen prevent skin aging?: Results from the first national health and nutrition examination survey (NHANES I)**

Dunn L.B.; Damesyn M.; Moore A.A.; Reuben D.B.; Greendale G.A. USA

Archives of Dermatology (USA), 1997, 133/3 (339-342)

**Objective:** To evaluate the relation between noncontraceptive estrogen use and skin wrinkling, dryness, and atrophy. **Design:** Cross-sectional analysis of a national probability sample-based cohort study. **Setting:** Multiple community sites throughout the United States. **Participants:** Postmenopausal women (n=3875) aged 40 years and older at baseline.

**Measurements:** Skin conditions (wrinkling, dryness, and atrophy) were ascertained using a uniform clinical examination by trained dermatology resident physicians. Self-reported use of estrogen before the baseline examination, sunlight exposure, and smoking history were obtained by standardized interview. Body mass index, a measure of weight in kilograms divided by the square of the height in meters, was evaluated in uniform examination clothing.

**Results:** Mean (plus or minusSD) age of the participants was 61.6 (plus or minus9.0) years and mean (plus or minusSD) number of years since menopause was 15.6 (plus or minus9.4). Most were white (83.7%), the remainder being African American (15.9%) or another race (0.4%). Atrophy was present in 499 (16.2%), dry skin in 1132 (36.2%), and wrinkled skin in 880 women (28.2%). The prevalence of all 3 skin conditions was lower in African American women compared with white women. Information on hormone use was available for 3403 participants (88%). Among all women, after adjustment for age, body mass index, and sunlight exposure, estrogen use was associated with a statistically significant decrease in the likelihood of senile dry skin (odds ratio, 0.76; 95% confidence interval, 0.60-0.97). The odds of wrinkling were substantially lower in estrogen users, adjusted for age, body mass index, and sun exposure (odds ratio, 0.68; 95% confidence interval, 0.52-0.89) and additionally for smoking (odds ratio, 0.67; 95% confidence interval, 0.44-1.01). In multivariable models, estrogen use was not associated with skin atrophy.

**Conclusion:** These results strongly suggest that estrogen use prevents dry skin and skin

wrinkling, thus extending the potential benefits of postmenopausal estrogen therapy to include protection against selected age- and menopause-associated dermatologic conditions.

**[Melatonin in dermatology. Experimental and clinical aspects]** [Article in German]

Fischer T, Wigger-Alberti W, Elsner P. Klinik für Hautkrankheiten, Friedrich-Schiller-Universität Jena.

Hautarzt 1999 Jan;50(1):5-11

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone with multiple functions in humans, produced by the pineal gland and stimulated by beta-adrenergic receptors. Serum melatonin levels exhibit a circadian rhythm with low levels during the day, rise in the evening and maximum levels at night between 2 and 4 a.m. Melatonin participates in the regulation of several physiological processes such as seasonal biological rhythm, daily sleep induction, aging and modulation of immunobiological defence reactions. Furthermore, melatonin has a highly lipophilic molecular structure facilitating penetration of cell membranes and serving as an extra- and intracellular free radical scavenger. Melatonin seems to quench mainly hydroxyl radicals, the most damaging of all free radicals. Melatonin may play a role in the etiology and treatment of several dermatoses e.g. atopic eczema, psoriasis and malignant melanoma. The influence of melatonin on hair growth is another aspect. Topical application of melatonin inhibits the development of UV-erythema. Penetration through skin after topical application and oral bioavailability await further investigations on the pharmacokinetic and pharmacodynamic actions of melatonin.

**Pathophysiology of premature skin aging induced by ultraviolet light.**

Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Department of Dermatology, University of Michigan Medical School, Ann Arbor 48109-0609, USA.

N Engl J Med. 1997 Nov 13;337(20):1419-28.

**BACKGROUND:** Long-term exposure to ultraviolet irradiation from sunlight causes premature skin aging (photoaging), characterized in part by wrinkles, altered pigmentation, and loss of skin tone. Photoaged skin displays prominent alterations in the collagenous extracellular matrix of connective tissue. We investigated the role of matrix-degrading metalloproteinases, a family of proteolytic enzymes, as mediators of collagen damage in photoaging.

**METHODS:** We studied 59 whites (33 men and 26 women, ranging in age from 21 to 58 years) with light-to-moderate skin pigmentation, none of whom had current or prior skin disease. Only some of the participants were included in each of the studies. We irradiated their buttock skin with fluorescent ultraviolet lights under standard conditions and obtained skin samples from irradiated and nonirradiated areas by keratome or punch biopsy. In some studies, tretinoin and its vehicle were applied to skin under occlusion 48 hours before ultraviolet irradiation. The expression of matrix metalloproteinases was determined by in situ hybridization, immunohistology, and in situ zymography. Irradiation-induced degradation of skin collagen was

measured by radioimmunoassay of soluble cross-linked telopeptides. The protein level of tissue inhibitor of matrix metalloproteinases type 1 was determined by Western blot analysis.

**RESULTS:** A single exposure to ultraviolet irradiation increased the expression of three matrix metalloproteinases -- collagenase, a 92-kd gelatinase, and stromelysin -- in skin connective tissue and outer skin layers, as compared with nonirradiated skin. The degradation of endogenous type I collagen fibrils was increased by 58 percent in irradiated skin, as compared with nonirradiated skin. Collagenase and gelatinase activity remained maximally elevated (4.4 and 2.3 times, respectively) for seven days with four exposures to ultraviolet irradiation, delivered at two-day intervals, as compared with base-line levels. Pretreatment of skin with tretinoin (all-trans-retinoic acid) inhibited the induction of matrix metalloproteinase proteins and activity (by 70 to 80 percent) in both connective tissue and outer layers of irradiated skin. Ultraviolet irradiation also induced tissue inhibitor of matrix metalloproteinases-1, which regulates the enzyme. Induction of the inhibitor was not affected by tretinoin.

**CONCLUSIONS:** Multiple exposures to ultraviolet irradiation lead to sustained elevations of matrix metalloproteinases that degrade skin collagen and may contribute to photoaging. Treatment with topical tretinoin inhibits irradiation-induced matrix metalloproteinases but not their endogenous inhibitor.

### **Double-blind, half-face study comparing topical vitamin C and vehicle for rejuvenation of photodamage.**

Fitzpatrick RE, Rostan EF. Dermatology Associates of San Diego County, Inc. 92024, USA.  
[fitzskin@pacbell.net](mailto:fitzskin@pacbell.net)

Dermatol Surg. 2002 Mar;28(3):231-6.

**BACKGROUND:** Aging of the population, in particular the "baby boomers," has resulted in increased interest in methods of reversal of photodamage. Non-invasive treatments are in high demand, and our knowledge of mechanisms of photodamage to skin, protection of the skin, and repair of photodamage are becoming more sophisticated and complex.

**OBJECTIVE:** The objective of this study is to determine if the topical use of a vitamin C preparation can stimulate the skin to repair photodamage and result in clinically visible differences, as well as microscopically visible improvement.

**METHODS:** Ten patients applied in a double-blind manner a newly formulated vitamin C complex having 10% ascorbic acid (water soluble) and 7% tetrahexyldecyl ascorbate (lipid soluble) in an anhydrous polysilicone gel base to one-half of the face and the inactive polysilicone gel base to the opposite side. Clinical evaluation of wrinkling, pigmentation, inflammation, and hydration was performed prior to the study and at weeks 4, 8, and 12. Two mm punch biopsies of the lateral cheeks were performed at 12 weeks in four patients and stained with hematoxylin and eosin, as well as in situ hybridization studies using an anti-sense probe for mRNA for type I collagen. A questionnaire was also completed by each patient.

**RESULTS:** A statistically significant improvement of the vitamin C-treated side was seen in the decreased photoaging scores of the cheeks ( $P = 0.006$ ) and the peri-oral area ( $P = 0.01$ ). The peri-orbital area improved bilaterally, probably indicating improved hydration. The overall facial improvement of the vitamin C side was statistically significant ( $P = 0.01$ ). Biopsies showed increased Grenz zone collagen, as well as increased staining for mRNA for type I collagen. No patients were found to have any evidence of inflammation. Hydration was improved bilaterally. Four patients felt that the vitamin C-treated side improved unilaterally. No patient felt the placebo side showed unilateral improvement.

**CONCLUSION:** This formulation of vitamin C results in clinically visible and statistically significant improvement in wrinkling when used topically for 12 weeks. This clinical improvement correlates with biopsy evidence of new collagen formation.

### **Effects of dietary retinyl palmitate or 13-cis-retinoic acid on the promotion of tumors in mouse skin.**

Gensler HL, Watson RR, Moriguchi S, Bowden GT.

Cancer Res. 1987 Feb 15;47(4):967-70.

The present study was designed to determine the effects of dietary 13-cis-retinoic acid and retinyl palmitate on mouse skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA). Female CD-1 mice were initiated with 150 nmol of 7,12-dimethylbenz(a)anthracene and promoted twice weekly with 8 nmol of TPA. Diets supplemented with retinyl palmitate to yield 60,000 or 200,000 IU or 700,000 for 5 wk followed by 350,000 IU per kg of diet (700,000/350,000) fed to mice during tumor promotion resulted in 9%, 37%, and 65% inhibition of the papilloma yield, respectively, at 21 wk of promotion. Although topical applications of 13-cis-retinoic acid have been almost as effective as retinoic acid in preventing the appearance of mouse skin tumors, dietary 13-cis-retinoic acid at 200,000 or 700,000 IU per kg of diet resulted in no reduction in papilloma yield but did result in a dose-dependent decrease in the tumor burden (weight of tumors per mouse). Therefore, dietary retinyl palmitate yielded a dose-dependent inhibition of the number and weight of tumors promoted by TPA, whereas dietary 13-cis-retinoic acid resulted in a decrease in weight but not in number of tumors promoted by TPA.

**Aging of human skin: review of a mechanistic model and first experimental data.**

Giacomini PU, Declercq L, Hellemans L, Maes D. Clinique Research Laboratories, Melville, NY, USA. [pgiacomo@estee.com](mailto:pgiacomo@estee.com)

IUBMB Life. 2000 Apr;49(4):259-63.

The physical, chemical, and biochemical factors that accelerate skin aging have been proposed to activate a self-maintained microinflammatory process, one of the expected end results of which is an imbalance in the turnover of macromolecules in the dermis. Surface peroxides are recognized as controllable factors of skin aging, and their accumulation is attributed to environmentally induced impairment of defense enzymes. Topical application of antioxidants

decreases the rate at which skin elasticity and skin thickness are modified.

**Dehydroepiandrosterone and two structural analogs inhibit 12-O-tetradecanoylphorbol-13-acetate stimulation of prostaglandin E2 content in mouse skin.**

Hastings LA, Pashko LL, Lewbart ML, Schwartz AG. Department of Microbiology, Temple University Medical School, Philadelphia, PA 19140.

Carcinogenesis 1988 Jun;9(6):1099-102

Dehydroepiandrosterone, a naturally occurring adrenal steroid, is a highly effective tumor chemopreventive agent in laboratory mice and rats, inhibiting spontaneous breast cancer and chemically induced tumors of the lung, colon, skin, liver and thyroid. Dehydroepiandrosterone blocks three processes that have been implicated in experimental tumorigenesis: (i) carcinogen activation through the mixed-function oxidases, (ii) 12-O-tetradecanoylphorbol-13-acetate stimulation of superoxide anion production in neutrophils, and (iii) 12-O-tetradecanoylphorbol-13-acetate stimulation of [3H]thymidine incorporation in mouse epidermis. All of these effects of dehydroepiandrosterone very likely result from glucose-6-phosphate dehydrogenase inhibition and a lowering of the NADPH cellular pool. It is now reported that oral administration of dehydroepiandrosterone (0.2% in the diet) for two weeks inhibits the stimulation in prostaglandin E2 content in mouse epidermis produced by topical application of 12-O-tetradecanoylphorbol-13-acetate. Two synthetic steroids, 16 alpha-fluoro-5-androsten-17-one and 16 alpha-fluoro-5 alpha-androstan-17-one, which are more potent inhibitors of the above three processes in tumorigenesis and are also more effective than dehydroepiandrosterone in inhibiting skin papilloma development in the mouse, are more active in suppressing prostaglandin E2 induction by 12-O-tetradecanoyl-phorbol-13-acetate. These two structural analogs, which also lack specific side-effects associated with dehydroepiandrosterone treatment, may find application as cancer chemopreventive drugs in humans.

**Inhibitory effect of glycolic acid on ultraviolet-induced skin tumorigenesis in SKH-1 hairless mice and its mechanism of action.**

Hong JT, Kim EJ, Ahn KS, Jung KM, Yun YP, Park YK, Lee SH. Department of Toxicology, National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul, Korea.

Mol Carcinog 2001 Jul;31(3):152-60

Glycolic acid, an alpha-hydroxy acid derived from fruit and milk sugars, has been used commonly as a cosmetic ingredient since it was discovered to have photoprotective and anti-inflammatory effects and antioxidant effects on ultraviolet (UV)B-irradiated skin. Little is known, however, about the functional role of glycolic acid on UV-induced skin tumorigenesis. In the present study, we examined the effect of glycolic acid on UV (UVA + UVB)-induced skin tumorigenesis and assessed several significant contributing factors in SKH-1 hairless mice. Inbred hairless female mice (15 animals/group) were irradiated for 5 d/wk at a total dose of 74.85 J/cm<sup>2</sup> UVA and 2.44 J/cm<sup>2</sup> UVB for 22 wk. Glycolic acid was applied topically twice

a week at a dose of 8 mg/cm<sup>2</sup> immediately after UV irradiation. Glycolic acid reduced UV-induced skin tumor development. The protective effect of glycolic acid was a 20% reduction of skin tumor incidence, a 55% reduction of tumor multiplicity (average number of tumors/mouse), and a 47% decrease in the number of large tumors (larger than 2 mm). Glycolic acid also delayed the first appearance of tumor formation by about 3 wk. The inhibitory effect of glycolic acid on UV-induced tumor development was accompanied by decreased expression of the following UV-induced cell-cycle regulatory proteins: proliferating cell nuclear antigen (PCNA), cyclin D1, cyclin E, and the associated subunits cyclin-dependent kinase 2 (cdk2) and cdk4. In addition, the expression of p38 kinase, jun N-terminal kinase (JNK), and mitogen-activated protein kinase kinase (MEK) also was lower in UV + glycolic acid-treated skin compared with expression in UV-irradiated skin. Moreover, transcription factors activator protein 1 (AP-1) and nuclear factor kappaB (NF-kappaB) activation was significantly lower in UV + glycolic acid-treated skin compared with activation in UV-irradiated skin. These results show that glycolic acid reduced UV-induced skin tumor development. The decreased expression of the cell-cycle regulatory proteins PCNA, cyclin D1, cyclin E, cdk2, and cdk4 and the signal mediators JNK, p38 kinase, and MEK may play a significant role in the inhibitory effect of glycolic acid on UV-induced skin tumor development. In addition, the inhibition of activation of transcription factors AP-1 and NF-kappaB could contribute significantly to the inhibitory effect of glycolic acid. Copyright 2001 Wiley-Liss, Inc.

### **The effect of glycolic acid on cultured human skin fibroblasts: cell proliferative effect and increased collagen synthesis.**

Kim SJ, Won YH Department of Dermatology, Chonnam University Research Institute of Medical Science, Chonnam National University Medical School, Kwangju, Korea.

J Dermatol 1998 Feb;25(2):85-9

Glycolic acid peeling is known to improve photoaging processes such as wrinkling and roughness, but this effect has not been clearly defined, even though functional activation of fibroblasts has been suggested. The study was aimed to determine the effects of glycolic acid and malic acid (AHA: alpha hydroxy acid) on cultured dermal fibroblasts. Whether it directly increases cell proliferation may be an important factor influencing the production of extracellular matrix such as type I collagen. Cultured human skin fibroblasts were treated for 24 hours with glycolic acid and malic acid at different concentrations (10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup> M), and cell proliferation was measured by MTT assay. Then quantitative analysis of collagen synthesis was performed by PICP (Procollagen Type I C-peptide) enzyme immunoassay and radioisotope (<sup>3</sup>H-proline) labeled collagen assay. The results showed increased cell proliferation and collagen production in response to glycolic acid in a dose dependent manner. The range of cell proliferation and collagen production were significantly higher with glycolic acid treatment than with malic acid or control. It is suggested that the favorable effects of glycolic acid treatment on aging skin were mediated by increased cell proliferation in addition to functional activation of fibroblasts.

### **Double-blind clinical study reveals synergistic action between alpha-hydroxy acid and**

### **betamethasone lotions towards topical treatment of scalp psoriasis.**

Kostarelos K, Teknetzis A, Lefaki I, Ioannides D, Minas A Research and Development Section, Farmeco Co., Athens, Greece.

J Eur Acad Dermatol Venereol 2000 Jan;14(1):5-9

**OBJECTIVE:** A double-blind, single-site, split-face clinical study was organized and carried out in order to evaluate the efficacy, tolerability, and safety of a glycolic acid containing scalp lotion in conjunction with a betamethasone (as the 17-valerate) scalp application against conditions of psoriasis.

**BACKGROUND:** Alpha-hydroxy acids (AHA) have been proposed as therapeutic modalities against skin exfoliative conditions such as ichthyosis, xeroderma, and psoriasis. AHAs are hereby clinically investigated as therapeutic modalities adjuvant to corticosteroids in order to diminish systemic and topical adverse side-effects most frequently associated with use of the latter.

**METHODS:** Twenty patients suffering from scalp psoriasis and other psoriatic conditions were included in a double-blind, split-face clinical study, using combinations of a 10% (w/w) glycolic acid scalp lotion, placebo lotion (excipients only), and a 0.1% (w/w) betamethasone scalp application, applied twice daily without any bandage for a period of 8 weeks. Clinical assessments were carried out by highly experienced physician evaluations based on a four-grade scale, prior to treatment and after 2, 4, 6 and 8 weeks.

**RESULTS:** Improvement was observed in all cases included in the study following treatment with the 10% glycolic acid lotion. However, when equal parts of the 0.1% betamethasone lotion were combined, most of the treated sites were healed. Moreover, the duration of treatment required for healing was in this case reduced to approximately half of that needed when the glycolic acid or the betamethasone lotions were used separately for treatment.

**CONCLUSIONS:** The present clinical study demonstrates for the first time that the effective and well tolerated therapeutic efficacy of glycolic acid scalp lotions is enhanced when used in conjunction with a 0.1% betamethasone scalp application against scalp psoriasis. This potential offers the practising dermatologist with novel treatment modes against severe skin conditions by combining topical corticosteroid with exfoliative agent therapy.

### **Effect of vitamin A on wound epidermis during forelimb regeneration in adult newts.**

Koussoulakos S, Sharma KK, Anton HJ. Zoological Institute, University of Cologne, Germany.

Int J Dev Biol. 1990 Dec;34(4):433-9.

The effects of vitamin A on blastemal epidermis were studied during the early postamputational period of forelimb regeneration in *Triturus alpestris*. Vitamin A was administered through oral intubation at a dose of 250 IU per gram of body weight per day. The results were evaluated by

morphometry, histology, and autoradiography. After 7, 11 and 14 days of treatment, several alterations were observed in the wound epidermis: a) reversal of keratinization; fewer keratinized cells were counted in sections from vitamin A-treated limbs; b) decrease in the incorporation of tritiated thymidine, as judged by estimation of labeling indices; c) increased mitotic activity in the cells of the stratum germinativum, and in the middle layer of the epithelial cells, as well. The significance of these cellular effects is discussed against the relevant literature.

**[Use of photoprotective measures in relation to actual exposure to solar rays]** [Article in Serbo-Croatian (Roman)]

Kozarev J.

Med Pregl. 1998 Nov-Dec;51(11-12):555-8.

**OBJECTIVE:** There is evidence that in spite of worldwide campaigns against excessive sun exposure, children as well as adults still spend long periods in the sun. The purpose of this study was to evaluate sun exposure in a group of doctors of different specialties and to compare their knowledge about sun protection methods with regular use of sun protection products.

**METHODS:** 51 doctors of different specialties, volunteers, mean age 40.78, filled out questionnaires with 21 multiple choice questions about their skin type, sun exposure habits, sun protection habits and questions about meaning of the Sun Protection Factor.

**RESULTS:** Thirty-three percent of our study participants spent more than two peak ultraviolet hours outdoors every day, and additional 33.33% are sun exposed for longer than 5 hours, regularly. Only 39% of them utilized sunscreens. Majority of sunscreen users utilized less than 100 ml of commercial sunscreen products which is an inadequate amount for full body protection per year. Majority of study participants did not believe that sunscreens could prevent skin cancer, but 57% of them believed that these compounds can slow the process of skin aging. Meaning of the term Sun Protection Factor is not familiar to 84.3% study participants. The two most common reasons for not using sunscreens are time consuming application and high cost.

**CONCLUSION:** Results of the presented study confirm our statement that there is bad understanding of a need for sun protection which is in correlation with deficient application of sun protective measures. It should be stressed out that our study participants lack well formed sun protection habits.

### **Intracrinology and the skin.**

Labrie F, Luu-The V, Labrie C, Pelletier G, El-Alfy M. Oncology and Molecular Endocrinology Research Center, Laval University Medical Center (CHUL), Quebec City, Canada.  
fernand.labrie@crchul.ulaval.ca

Horm Res 2000;54(5-6):218-29

The skin, the largest organ in the human body, is composed of a series of androgen-sensitive

components that all express the steroidogenic enzymes required to transform dehydroepiandrosterone (DHEA) into dihydrotestosterone (DHT). In fact, in post-menopausal women, all sex steroids made in the skin are from adrenal steroid precursors, especially DHEA. Secretion of this precursor steroid by the adrenals decreases progressively from the age of 30 years to less than 50% of its maximal value at the age of 60 years. DHEA applied topically or by the oral route stimulates sebaceous gland activity, the changes observed being completely blocked in the rat by a pure antiandrogen while a pure antiestrogen has no significant effect, thus indicating a predominant or almost exclusive androgenic effect. In human skin, the enzyme that transforms DHEA into androstenedione is type 1 3beta-hydroxysteroid dehydrogenase (type 1 3beta-HSD) as revealed by RNase protection and immunocytochemistry. The conversion of androstenedione into testosterone is then catalyzed in the human skin by type 5 17beta-HSD. All the epidermal cells and cells of the sebaceous glands are labelled by type 5 17beta-HSD. This enzyme is also present at a high level in the hair follicles. Type 1 is the 5alpha-reductase isoform responsible in human skin for the conversion of testosterone into DHT. In the vagina, on the other hand, DHEA exerts mainly an estrogenic effect, this effect having been demonstrated in the rat as well as in post-menopausal women. On the other hand, in experimental animals as well as in post-menopausal women, DHEA, at physiological doses, does not affect the endometrial epithelium, thus indicating the absence of DHEA-converting enzymes in this tissue, and avoiding the need for progestins when DHEA is used as hormone replacement therapy. Copyright 2001 S. Karger AG, Basel

**Inhibition of 7,12-dimethylbenz(a)anthracene-induced skin papillomas and carcinomas by dehydroepiandrosterone and 3-beta-methylandrosterone in mice.**

Pashko LL, Hard GC, Rovito RJ, Williams JR, Sobel EL, Schwartz AG.

Cancer Res 1985 Jan;45(1):164-6

Topical application of the adrenal steroid, dehydroepiandrosterone, or the synthetic steroid, 3-beta-methylandrosterone, which unlike dehydroepiandrosterone is not demonstrably uterotrophic, inhibits 7,12-dimethylbenz(a)anthracene-induced skin papillomas and carcinomas in the CD-1 mouse.

**Inhibition of 12-O-tetradecanoylphorbol-13-acetate-promoted skin tumor formation in mice by 16 alpha-fluoro-5-androsterone and its reversal by deoxyribonucleosides.**

Pashko LL, Lewbart ML, Schwartz AG. Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140.

Carcinogenesis 1991 Nov;12(11):2189-92

The work of ourselves and others has demonstrated that dehydroepiandrosterone (DHEA) displays a broad spectrum of cancer preventive action in laboratory rodents, with little toxicity. In the two-stage skin tumorigenesis model in mice, topical application of the synthetic DHEA analog 16 alpha-fluoro-5-androsterone, a more potent preventive agent than DHEA without the sex-hormonal side-effects of the parent steroid, markedly inhibited promotion of 7,12-

dimethylbenz[a]anthracene (DMBA)-initiated tumor development by 12-O-tetradecanoylphorbol-13-acetate (TPA). DHEA is a powerful inhibitor of glucose-6-phosphate dehydrogenase (G6PDH), suggesting that its inhibiting effect in carcinogenesis may be due to a lack of NADPH and ribose-5-phosphate production for deoxyribonucleotide synthesis and subsequent DNA replication. Further evidence of a reduced NADPH and ribose-5-phosphate pool on the lowering of intracellular deoxyribonucleotide levels has been demonstrated in this paper by completely reversing the 16 alpha-fluoro-5-androsten-17-one-induced inhibition of tumor promotion by the addition of the four deoxyribonucleosides-deoxyadenosine, deoxycytidine, deoxyguanosine and thymidine--to the drinking water during the promotion period of tumorigenesis.

### **Low molecular weight antioxidants and their role in skin ageing.**

Podda M, Grundmann-Kollmann M. Department of Dermatology, J. W. Goethe University, Frankfurt, Germany. podda@em.uni-frankfurt.de

Clin Exp Dermatol 2001 Oct;26(7):578-82

There is increasing evidence that reactive oxygen species play a pivotal role in the process of ageing. The skin, as the outermost barrier of the body, is exposed to various exogenous sources of oxidative stress, in particular UV-irradiation. These are believed to be responsible for the extrinsic type of skin ageing, termed photo-ageing. It therefore seems reasonable to try to increase levels of protective low molecular weight antioxidants through a diet rich in fruits and vegetables or by direct topical application. Indeed, various in vitro and animal studies have proved that low molecular weight antioxidants, especially vitamins C and E, ascorbate and tocopherol, as well as lipoic acid, exert protective effects against oxidative stress. However, controlled long-term studies on the efficacy of low molecular weight antioxidants in the prevention or treatment of skin ageing in humans are still lacking.

### **Tretinoin cream 0.02% for the treatment of photodamaged facial skin: a review of 2 double-blind clinical studies.**

Nyirady J, Bergfeld W, Ellis C, Levine N, Savin R, Shavin J, Voorhees JJ, Weiss J, Grossman R. Johnson & Johnson Consumer Products Worldwide, Skillman, New Jersey 08558-9418, USA.

Cutis 2001 Aug;68(2):135-42

In extensive clinical studies and practical use since its US Food and Drug Administration approval in 1995, tretinoin emollient cream 0.05% has been shown to be safe and effective in the treatment of fine facial wrinkles, mottled hyperpigmentation, and skin roughness. To provide additional prescribing flexibility for various patient needs, a new lower concentration formulation, tretinoin cream 0.02% was chosen for further development. Two multicenter, randomized, double-blind, vehicle-controlled clinical, studies were conducted to evaluate the safety and efficacy of the lower concentration tretinoin formulation in the treatment of moderate-to-severe facial photodamage. Results indicate statistically significant improvement in fine wrinkling, coarse wrinkling, and yellowing with the use of tretinoin cream 0.02% at week-24 end

point, compared with placebo. Therapy with tretinoin cream 0.02% was well tolerated overall and demonstrated a favorable safety profile. Both studies demonstrated that tretinoin cream 0.02% is safe and effective for the treatment of moderate-to-severe photodamaged facial skin.

**The dansyl chloride technique for stratum corneum renewal as an indicator of changes in epidermal mitotic activity following topical treatment.**

Ridge BD, Batt MD, Palmer HE, Jarrett A. Beecham Products Research Department, Weybridge, Surrey, U.K.

Br J Dermatol. 1988 Feb;118(2):167-74.

Using a hypomitotic agent, triamcinolone acetonide, and a hypermitotic agent, retinyl propionate, we investigated the relationship between epidermal mitotic activity and stratum corneum renewal time of topically treated skin as determined by the dansyl chloride staining technique. Treatment with the base cream resulted in a reduction in renewal time compared with an untreated control site. The predicted increase in renewal time with the hypomitotic agent and reduction with the hypermitotic agent was only observed when daily treatment was commenced 2 weeks prior to and continued after dansyl chloride staining and not when treatment was started after staining. These results indicate that in order to use cell renewal methods to demonstrate changes in mitotic activity brought about by topical treatments, it is necessary to pre-treat the skin with the test material to establish full epidermal equilibrium at the changed mitotic state before labelling with dansyl chloride. Meaningful claims for effects on cell renewal of specific cosmetic ingredients should only be made after comparison with a base cream treated site, both having been allowed to equilibrate, rather than on the basis of comparison with untreated skin.

**Solar ultraviolet-induced erythema in human skin and nuclear factor-kappa-B-dependent gene expression in keratinocytes are modulated by a French maritime pine bark extract.**

Saliou C, Rimbach G, Moini H, McLaughlin L, Hosseini S, Lee J, Watson RR, Packer L. Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA.

Free Radic Biol Med. 2001 Jan 15;30(2):154-60.

The procyanidin-rich French maritime pine bark extract Pycnogenol (PBE) has been investigated for its effect in protecting human skin against solar UV-simulated light-induced erythema. Twenty-one volunteers were given an oral supplementation of Pycnogenol: 1.10 mg/kg body weight (b. wt.)/d for the first 4 weeks and 1.66 mg/kg b. wt./d for the next 4 weeks. The minimal erythema dose (MED) was measured twice before supplementation (baseline MED), once after the first 4 weeks of supplementation, and a last time at the end of the study. The UVR dose necessary to achieve 1 MED was significantly increased during PBE supplementation. Since the activation of the pro-inflammatory and redox-regulated transcription factor NF-kappaB is thought to play a major role in UVR-induced erythema, the effect of PBE was also investigated in the human keratinocyte cell line HaCaT. PBE, added to the cell culture medium, inhibited UVR-induced NF-kappaB-dependent gene expression in a concentration-dependent manner. However, NF-kappaB-DNA-binding activity was not prevented, suggesting that PBE affects the

transactivation capacity of NF-kappaB. These data indicate that oral supplementation of PBE reduces erythema in the skin. Inhibition of NF-kappaB-dependent gene expression by PBE possibly contributes to the observed increase in MED.

### **Photoaging of the skin from phenotype to mechanisms.**

Scharffetter-Kochanek K, Brenneisen P, Wenk J, Herrmann G, Ma W, Kuhr L, Meewes C, Wlaschek M. Department of Dermatology, University of Cologne, Joseph-Stelzmann-Str. 9, 50931, Cologne, Germany. Karin.Scharffetter@uni-koeln.de

Exp Gerontol. 2000 May;35(3):307-16.

The skin is increasingly exposed to ambient UV-irradiation thus increasing its risk for photooxidative damage with longterm detrimental effects like photoaging, which is characterized by wrinkles, loss of skin tone, and resilience. Photoaged skin displays prominent alterations in the cellular component and the extracellular matrix of the connective tissue with an accumulation of disorganized elastin and its microfibrillar component fibrillin in the deep dermis and a severe loss of interstitial collagens, the major structural proteins of the dermal connective tissue. The unifying pathogenic agents for these changes are UV-generated reactive oxygen species (ROS) that deplete and damage non-enzymatic and enzymatic antioxidant defense systems of the skin. As well as causing permanent genetic changes, ROS activate cytoplasmic signal transduction pathways in resident fibroblasts that are related to growth, differentiation, senescence, and connective tissue degradation. This review focuses on the role of UV-induced ROS in the photodamage of the skin resulting in biochemical and clinical characteristics of photoaging. In addition, the relationship of photoaging to intrinsic aging of the skin will be discussed. A decrease in the overall ROS load by efficient sunscreens or other protective agents may represent promising strategies to prevent or at least minimize ROS induced photoaging.

### **Food restriction inhibits [3H] 7,12-dimethylbenz(a)anthracene binding to mouse skin DNA and tetradecanoylphorbol-13-acetate stimulation of epidermal [3H] thymidine incorporation.**

Schwartz AG, Pashko LL.

Anticancer Res 1986 Nov-Dec;6(6):1279-82

It has been known for many years that reducing the food intake of laboratory mice and rats inhibits the development of a broad spectrum of chemically induced and spontaneous tumors, but the mechanism of this effect is poorly understood. Food restriction of A/J mice for two weeks is now shown to inhibit the binding of topically applied [3H]7,12-dimethylbenz(a)anthracene (DMBA) to skin DNA by 50% and to abolish the stimulation of [3H]-thymidine incorporation in the epidermis produced by topical application of the tumor promoter tetradecanoylphorbol-13-acetate (TPA). Similar effects on the actions of DMBA and TPA are observed following topical application of the adrenal steroid, dehydroepiandrosterone (DHEA), a potent glucose-6-phosphate dehydrogenase (G6PDH) inhibitor, while food restriction for two weeks depresses epidermal G6PDH activity by 60%. It is suggested that both the inhibition of [3H]DMBA

binding to skin DNA and the TPA stimulation in epidermal [3H]thymidine incorporation result from a reduction in the NADPH cellular pool as a result of G6PDH inhibition.

### **Inhibition of tumor development by dehydroepiandrosterone and related steroids.**

Schwartz AG, Pashko L, Whitcomb JM.

Toxicol Pathol 1986;14(3):357-62

The naturally occurring adrenal steroid, dehydroepiandrosterone (DHEA), is a potent non-competitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PDH). Oral administration of DHEA to mice inhibits spontaneous breast cancer and chemically induced tumors of the lung and colon. Topical application of DHEA to mouse skin inhibits 7,12-dimethylbenz(a)anthracene (DMBA)-initiated and tetradecanoylphorbol-13-acetate (TPA)-promoted papillomas and DMBA-induced carcinomas at both the initiation and promotion phase. Evidence is presented that critical steps in the initiation process (mixed-function oxidase activation of a carcinogen) and promotion process (enhanced rates of cell proliferation and superoxide formation) all require NADPH and may be inhibited by DHEA and structural analogs as a result of a lowering of the NADPH cellular pool. Results obtained by others with fibroblasts and lymphocytes from individuals with the Mediterranean variant of G6PDH deficiency also indicate that a reduction in the NADPH cellular pool confers resistance to benzo(a)pyrene. Preliminary data suggest that food restriction may depress G6PDH levels and this may contribute to the tumor preventive effect of underfeeding.

### **Hypothalamic neuroendocrine correlates of cutaneous burn injury in the rat: I. Scanning electron microscopy.**

Scott DE, Vaughan GM, Pruitt BA Jr.

Brain Res Bull. 1986 Sep;17(3):367-78

Rats were given a standard scald burn on 60% of the body surface or only a sham burn and were sacrificed at intervals from 6 hr to 14 days later. Serum thyroxine (T4), free thyroxine index (FT4I) and triiodothyronine (T3) were depressed compared to values in respective shams as early as 6 hr post-burn. T4 and FT4I were less depressed on post-burn days (PBD) 2-3 than on PBD 1 and then exhibited a further fall. T3 remained depressed through PBD 14. Pineal melatonin content was elevated at 6 hr and fell to the normal daytime range in subsequent samples. The ventral portion of the diencephalon was prepared for scanning electron microscopy. Only in the burned rats and beginning on PBD 2, large numbers of supraependymal neurons (SEN) appeared in the ventricular space attached to the inferior walls and floor of the third cerebral ventricle. Transmission electron microscopy was used to confirm the neuronal nature of the SEN. Viewed by scanning electron microscopy, these persisted through PBD 14. SEN were interconnected by cables of their neurites exhibiting varicosities on individual neurites as they passed over perikarya of other SEN. Some SEN were seen to be only partially emerged from the underlying tissue and others were seen to send a thick process into the hypothalamic tissue. These observations indicate that after peripheral injury there is marked plasticity of the brain in an area

thought to control the endocrine systems that show abnormalities after such a peripheral injury. The timing, location and nature of these anatomic changes indicate the possibility that at least some aspects of central nervous orchestration of the endocrine metabolic response to injury may be related to the emergence of a neuronal system receiving or sending messages through the cerebrospinal fluid and/or through new neurite circuits along the surface of the third ventricular wall. These structures may appear in response to initial primary hormonal changes and/or may play a role in maintaining the post-injury hormonal milieu manifested in part by a subsequent second fall in serum T4.

### **Estrogen and skin. An overview.**

Shah MG, Maibach HI. University of California, San Francisco, School of Medicine, San Francisco, California, USA. mgshah@alumni.stanford.org

Am J Clin Dermatol 2001;2(3):143-50

As the population of postmenopausal women increases, interest in the effects of estrogen grows. The influence of estrogen on several body systems has been well-documented; however, one area that has not been explored is the effects of estrogen on skin. Estrogen appears to aid in the prevention of skin aging in several ways. This reproductive hormone prevents a decrease in skin collagen in postmenopausal women; topical and systemic estrogen therapy can increase the skin collagen content and therefore maintain skin thickness. In addition, estrogen maintains skin moisture by increasing acid mucopolysaccharides and hyaluronic acid in the skin and possibly maintaining stratum corneum barrier function. Sebum levels are higher in postmenopausal women receiving hormone replacement therapy. Skin wrinkling also may benefit from estrogen as a result of the effects of the hormone on the elastic fibers and collagen. Outside of its influence on skin aging, it has been suggested that estrogen increases cutaneous wound healing by regulating the levels of a cytokine. In fact, topical estrogen has been found to accelerate and improve wound healing in elderly men and women. The role of estrogen in scarring is unclear but recent studies indicate that the lack of estrogen or the addition of tamoxifen may improve the quality of scarring. Unlike skin aging, the role of endogenous and exogenous estrogen in melanoma has not been well established.

### **Cutaneous vitamins A and E in the context of ultraviolet- or chemically-induced oxidative stress.**

Sorg O, Tran C, Saurat JH. Department of Dermatology, University Hospital, Geneva, Switzerland. olivier.sorg@hcuge.ch

Skin Pharmacol Appl Skin Physiol 2001 Nov-Dec;14(6):363-72

Vitamins A and E are present in mammalian skin. Although the main circulating form of vitamin A in the blood is retinol, the epidermis stores it as retinyl esters. The epidermis can be easily loaded with high amounts of vitamin A by topical application of either retinol or retinaldehyde, two well-tolerated precursors of the biologically active retinoic acid, while topical alpha-tocopherol loads the epidermis with vitamin E. The probable physiological function of epidermal

vitamin E is to contribute to the antioxidant defense of the skin, whereas that of epidermal vitamin A (retinol and retinyl esters) is not yet well understood. Besides being a precursor for retinoic acid, vitamin A also has a free radical scavenging potential. Due to their physical properties, vitamins A and E absorb ultraviolet (UV) light in the region of solar spectrum that is responsible for most of the deleterious biological effects of the sun. In the mouse, topical vitamin A has been shown to prevent the UV-induced epidermal hypovitaminosis A, while topical vitamin E prevents oxidative stress and cutaneous and systemic immunosuppression elicited by UV. Thus constitutive epidermal vitamins A and E appear complementary in preventing UV-induced deleterious cutaneous and systemic effects, and these properties can be reinforced by topical application of retinol or retinaldehyde and topical alpha-tocopherol. Copyright 2001 S. Karger AG, Basel

### **Vitamin D insufficiency among free-living healthy young adults**

Tangpricha, V., Pearce, E.N., Chen, T.C., Holick, M.F.

Am. J. Med. 2002 Jun 1; 112(8): 659-62.

No abstract available.

### **Evidence by in vivo and in vitro studies that binding of pycnogenols to elastin affects its rate of degradation by elastases.**

Tixier JM, Godeau G, Robert AM, Hornebeck W

Biochem Pharmacol 1984 Dec 15;33(24):3933-9

Procyanidol oligomers and (+) catechin bound to insoluble elastin markedly affect its rate of degradation by elastases. Insoluble elastin pretreated with procyanidol oligomers (PCO) was resistant to the hydrolysis induced by both porcine pancreatic and human leukocyte elastases. The quantitative adsorption of pancreatic elastase was similar on either untreated or PCO-treated elastin suggesting that the binding of this compound to elastin increases the non-productive catalytic sites of elastase molecules. (+) Catechin-insoluble elastin complexes were partially resistant to the degradation induced by human leukocyte elastase but were hydrolysed at the same rate as untreated samples by a constant amount of pancreatic elastase. In addition, the coacervation profile of kappa-elastin peptides as a function of temperature is greatly modified in presence of these flavonoids. We conclusively evidenced that PCOs bind to skin elastic fibres when injected intradermally into young rabbits. As a result, these elastic fibres were found more resistant to the hydrolytic action of porcine pancreatic elastase when injected to the same site. These in vivo studies further emphasized the potential effect of these compounds in preventing elastin degradation by elastase(s) as occurred in inflammatory processes.

### **Use of topical ascorbic acid and its effects on photodamaged skin topography.**

Traikovitch SS. Beeson Aesthetic Surgery Institute, Carmel, Ind., USA. AJLively@POL.NET

Arch Otolaryngol Head Neck Surg. 1999 Oct;125(10):1091-8.

**OBJECTIVE:** To determine the efficacy of topical ascorbic acid application in treating mild to moderate photodamage of facial skin using an objective, computer-assisted image analysis of skin surface topography and subjective clinical, photographic, and patient self-appraisal questionnaires.

**DESIGN:** A 3-month, randomized, double-blind, vehicle-controlled study.

**SETTING:** Facial plastic surgery private practice.

**PATIENTS:** Nineteen evaluable volunteer sample patients aged between 36 and 72 years with Fitzpatrick skin types I, II, and III who were in good physical and mental health with mild to moderately photodamaged facial skin were considered for analysis.

**INTERVENTION:** Coded, unmarked medications were randomly assigned to the left and right sides of each subject's face, one containing the active agent, topical ascorbic acid (Cellex-C high-potency serum; Cellex-C International, Toronto, Ontario), the other, the vehicle serum (Cellex-C International). Three drops (0.5 mL) of each formulation were applied daily to the randomly assigned hemifaces over the 3-month study period. Treatment assignments were not disclosed to subjects, clinicians, or personnel involved in analyzing skin replicas.

**MAIN OUTCOME MEASURES:** Specific clinical parameters were evaluated and graded on a 0- to 9-point scale (0, none; 1-3, mild; 4-6, moderate; and 7-9, severe). Reference photographs were used to standardize grading criteria. Overall investigator scores were compared with baseline and graded as excellent (much improved), good (improved), fair (slightly improved), no change, or worse. Patient self-appraisal questionnaires rated the degree of improvement (much improved, improved, slightly improved, no change, or worse) and reported adverse effects (burning, stinging, redness, peeling, dryness, discoloration, itching, and rash). Standard photographs were taken at baseline, including anteroposterior and left and right oblique views to facilitate subsequent clinical evaluations, and at the end of therapy for comparison. Optical profilometry analysis was performed on the skin surface replicas of the lateral canthal (crow's feet) region, comparing baseline to end-of-study specimens. Using this computer-based system, the resulting image was digitally analyzed, and numeric values were assigned to reflect surface features. The parameters obtained included Rz, Ra, and shadows. These values provided objective data that document pretreatment and posttreatment texture changes proportional to the degree of wrinkling, roughness, and other surface irregularities.

**RESULTS:** Optical profilometry image analysis demonstrated a statistically significant 73.7% improvement in the Ra and shadows north-south facial axis values with active treatment greater than vehicle control, as well as a trend for improvement in the Rz north-south facial axis parameter, showing a 68.4% greater improvement of active treatment vs vehicle control. Clinical assessment demonstrated significant improvement with active treatment greater than control for fine wrinkling, tactile roughness, coarse rhytids, skin laxity/tone, sallowness/yellowing, and overall features. Patient questionnaire results demonstrated statistically significant improvement overall, active treatment 84.2% greater than control. Photographic assessment demonstrated

significant improvement, active treatment 57.9% greater than control.

**CONCLUSIONS:** A 3-month daily regimen of topical ascorbic acid provided objective and subjective improvement in photodamaged facial skin. Skin replica optical profilometry is an objective method for quantification of the skin surface texture changes.

**Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2.**

Trang HM, Cole DE, Rubin LA, Pierratos A, Siu S, Vieth R. Department of Laboratory Medicine, University of Toronto, and The Wellesley Hospital, Canada.

Am J Clin Nutr. 1998 Oct;68(4):854-8.

In all species tested, except humans, biological differences between vitamins D2 and D3 are accepted as fact. To test the presumption of equivalence in humans, we compared the ability of equal molar quantities of vitamin D2 or D3 to increase serum 25-hydroxyvitamin D [25(OH)D], the measure of vitamin D nutrition. Subjects took 260 nmol (approximately 4000 IU) vitamin D2 (n=17) or vitamin D3 (n=55) daily for 14 d. 25(OH)D was assayed with a method that detects both the vitamin D2 and D3 forms. With vitamin D3, mean (+/-SD) serum 25(OH)D increased from 41.3+/-17.7 nmol/L before to 64.6+/-17.2 nmol/L after treatment. With vitamin D2, the 25(OH)D concentration went from 43.7+/-17.7 nmol/L before to 57.4+/-13.0 nmol/L after. The increase in 25(OH)D with vitamin D3 was 23.3+/-15.7 nmol/L, or 1.7 times the increase obtained with vitamin D2 (13.7+/-11.4 nmol/L; P=0.03). There was an inverse relation between the increase in 25(OH)D and the initial 25(OH)D concentration. The lowest 2 tertiles for basal 25(OH)D showed larger increases in 25(OH)D: 30.6 and 25.5 nmol/L, respectively, for the first and second tertiles. In the highest tertile [25(OH)D >49 nmol/L] the mean increase in 25(OH)D was 13.3 nmol/L (P < 0.03 for comparison with each lower tertile). Although the 1.7-times greater efficacy for vitamin D3 shown here may seem small, it is more than what others have shown for 25(OH)D increases when comparing 2-fold differences in vitamin D3 dose. The assumption that vitamins D2 and D3 have equal nutritional value is probably wrong and should be reconsidered.

**Split face study on the cutaneous tensile effect of 2-dimethylaminoethanol (deanol) gel.**

Uhoda I, Faska N, Robert C, Cauwenbergh G, Pierard GE. Unit of Dermocosmetology, Department of Dermatopathology, University Medical Center of Liege, CHU Sart Tilman, B-4000 Liege, Belgium.

Skin Res Technol. 2002 Aug;8(3):164-7.

**BACKGROUND/AIMS:** Beyond subjective assessments, the effect of skin tensors is difficult to assess. The present 2-phase randomized double-blind split face study was designed to compare the effect of a gel containing 3% 2-dimethylaminoethanol (deanol, DMAE) with the same formulation without DMAE.

**METHODS:** In a first pilot study, sensorial assessments and measures of the skin distension under suction were performed in eight volunteers. In a second study conducted in 30 volunteers, shear wave propagation was measured.

**RESULTS:** Large interindividual variations precluded any significant finding in the first study. The DMAE formulation showed, however, a significant effect characterized by increased shear wave velocity in the direction where the mechanical anisotropy of skin showed looseness.

**CONCLUSION:** The DMAE formulation under investigation increased skin firmness.

### **Molecular mechanisms of intrinsic skin aging and retinoid-induced repair and reversal.**

Varani J, Fisher GJ, Kang S, Voorhees JJ. Department of Pathology, The University of Michigan, Ann Arbor 48109, USA.

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Past studies have shown that topical treatment of sun-exposed skin with all-trans retinoic acid improves the clinical and histologic appearance of the skin. This is associated with a reduction in matrix metalloproteinase elaboration and with expression of a newly synthesized collagenous matrix. Whether retinoid therapy might have a similar impact on the appearance of intrinsically aged skin is not known. This study, using human skin in organ culture and epidermal keratinocytes and fibroblasts in monolayer culture, show that retinoic acid stimulates growth of both keratinocytes and fibroblasts and stimulates extracellular matrix production by the fibroblasts. Adult skin from sun-exposed and sun-protected sites responds equally well to retinoic acid, whereas neonatal skin is much less responsive under the same conditions. The implications of this are (i) that retinoids may be able to repair intrinsically aged skin as well as photoaged skin, and (ii) that retinoids modulate human skin cell function in a manner that is age-related, and not simply a response to photodamage.

### **Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin.**

Varani J, Warner RL, Gharaee-Kermani M, Phan SH, Kang S, Chung JH, Wang ZQ, Datta SC, Fisher GJ, Voorhees JJ. Departments of Pathology and Dermatology, The University of Michigan, Medical School, Ann Arbor, MI 48109, USA. varani@umich.edu

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Damage to human skin due to ultraviolet light from the sun (photoaging) and damage occurring as a consequence of the passage of time (chronologic or natural aging) are considered to be distinct entities. Photoaging is caused in part by damage to skin connective tissue by increased elaboration of collagen-degrading matrix metalloproteinases, and by reduced collagen synthesis. As matrix metalloproteinase levels are known to rise in fibroblasts as a function of age, and as oxidant stress is believed to underlie changes associated with both photoaging and natural aging, we determined whether natural skin aging, like photoaging, gives rise to increased matrix

metalloproteinases and reduced collagen synthesis. In addition, we determined whether topical vitamin A (retinol) could stimulate new collagen deposition in sun-protected aged skin, as it does in photoaged skin. Sun-protected skin samples were obtained from 72 individuals in four age groups: 18-29 y, 30-59 y, 60-79 y, and 80+ y. Histologic and cellular markers of connective tissue abnormalities were significantly elevated in the 60-79 y and 80+ y groups, compared with the two younger age groups. Increased matrix metalloproteinase levels and decreased collagen synthesis/expression were associated with this connective tissue damage. In a separate group of 53 individuals (80+ y of age), topical application of 1% vitamin A for 7 d increased fibroblast growth and collagen synthesis, and concomitantly reduced the levels of matrix-degrading matrix metalloproteinases. Our findings indicate that naturally aged, sun-protected skin and photoaged skin share important molecular features including connective tissue damage, elevated matrix metalloproteinase levels, and reduced collagen production. In addition, vitamin A treatment reduces matrix metalloproteinase expression and stimulates collagen synthesis in naturally aged, sun-protected skin, as it does in photoaged skin.

**Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it.**

Vieth R, Cole DE, Hawker GA, Trang HM, Rubin LA. Mount Sinai Hospital, Toronto, Canada. rvieth@mtsinai.on.ca

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**OBJECTIVE:** We asked whether women self-reporting the recommended consumption of vitamin D from milk and multivitamins would be less likely to have low wintertime 25-hydroxyvitamin D (25(OH)D) levels.

**METHODS:** This cross-sectional study enlisted at least 42 young women each month (age 18-35 y, 796 women total) through one year. We measured serum 25(OH)D and administered a lifestyle and diet questionnaire. **RESULTS:** Over the whole year, prevalence of low 25(OH)D (<40 nmol/l) was higher in non-white, non-black subjects (25.6% of 82 women) than in the white women (14.8% of 702 white women,  $P < 0.05$ ). Of the 435 women tested during the winter half of the year (November-April), prevalence of low 25(OH)D was not affected by vitamin D intake: low 25(OH)D occurred in 21% of the 146 consuming no vitamin D, in 26% of the 140 reporting some vitamin D intake, up to 5 microg/day (median, 2.5 microg/day), and in 20% of the 149 women reporting vitamin D consumption over 5 microg/day (median, 10 microg/day).

**INTERPRETATION:** The self-reported vitamin D intake from milk and/or multivitamins does not relate to prevention of low vitamin D nutritional status of young women in winter. Recommended vitamin D intakes are too small to prevent insufficiency. Vitamin D nutrition can only be assessed by measuring serum 25(OH)D concentration.

**Synthesis and characterization of a series of novel monoacylated ascorbic acid derivatives, 6-O-acyl-2-O-alpha-D-glucopyranosyl-L-ascorbic acids, as skin antioxidants.**

Yamamoto I, Tai A, Fujinami Y, Sasaki K, Okazaki S. Department of Immunochemistry,

Faculty of Pharmaceutical Sciences, Okayama University, Okayama 700-8530, Japan.  
iyamamoto@pheasant.pharm.okayama-u.ac.jp

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A series of novel monoacylated vitamin C derivatives were chemically synthesized with a stable ascorbate derivative, 2-O-alpha-D-glucopyranosyl-L-ascorbic acid (AA-2G), and acid anhydrides in pyridine. Their solubility in organic phase, thermal stability, radical scavenging activity, and in vitro skin permeability was evaluated. These monoacylated derivatives were identified as 6-O-acyl-2-O-alpha-D-glucopyranosyl-L-ascorbic acids (6-Acyl-AA-2G) by UV spectra, elemental analyses, and nuclear magnetic resonance spectroscopy. The reactions afforded 6-Acyl-AA-2G in high yields (30-60%). 6-Acyl-AA-2G exhibited satisfactory stability in neutral solution comparable to that of a typical stable derivative, AA-2G, and also showed the radical scavenging activity. The lipid solubility of 6-Acyl-AA-2G was increased with increasing length of their acyl group. Increased skin permeability was superior to those of AA-2G and ascorbic acid (AsA). 6-Acyl-AA-2G that is susceptible to enzymatic hydrolysis by tissue esterase and/or alpha-glucosidase produces AA-2G and AsA, which is in the skin tissues. Thus, these findings indicate that the novel vitamin C derivatives presented here, 6-Acyl-AA-2G, may be effective antioxidants in skin care and medicinal use.