STUDY

Improvement of Naturally Aged Skin With Vitamin A (Retinol)

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Objective: To evaluate the effectiveness of topical retinol (vitamin A) in improving the clinical signs of naturally aged skin.

Design: Randomized, double-blind, vehicle-controlled, left and right arm comparison study.

Setting: Academic referral center.

Patients: The study population comprised 36 elderly subjects (mean age, 87 years), residing in 2 senior citizen facilities.

Intervention: Topical 0.4% retinol lotion or its vehicle was applied at each visit by study personnel to either the right or the left arm, up to 3 times a week for 24 weeks.

Main Outcome Measures: Clinical assessment using a semiquantitative scale (0, none; 9, most severe) and biochemical measurements from skin biopsy specimens obtained from treated areas.

Results: After 24 weeks, an intent-to-treat analysis using the last-observation-carried-forward method revealed that there were significant differences between retinol-treated and vehicle-treated skin for changes in fine wrinkling scores (–1.64 [95% CI, –2.06 to –1.22] vs –0.08 [95% CI, –0.17 to 0.01]; $P<.001$). As measured in a subgroup, retinol treatment significantly increased glycosaminoglycan expression ($P=.02$ [n=6]) and procollagen I immunostaining ($P=.049$ [n=4]) compared with vehicle.

Conclusions: Topical retinol improves fine wrinkles associated with natural aging. Significant induction of glycosaminoglycan, which is known to retain substantial water, and increased collagen production are most likely responsible for wrinkle effacement. With greater skin matrix synthesis, retinol-treated aged skin is more likely to withstand skin injury and ulcer formation along with improved appearance.

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WRINKLES AND BROWN spots that represent aged features are accentuated in the habitually sun-exposed face and the back of the hands. In this premature skin aging (ie, photoaging), matrix degradation triggered by solar radiation is critical in causing the wrinkled phenotype,[1,2] and topical use of retinoids can offer clinical improvement.[3,4] Human skin not exposed to the sun also ages but less dramatically. In intrinsic, natural, or chronologic aging, skin loses its youthful appearance by becoming thinner, laxer, and more finely wrinkled.[5,6] These changes are readily appreciated by inspecting the upper inner arm. Clinically evident atrophy of aged skin correlates histologically with thinner epidermis and dermis, with reduced numbers of keratinocytes and fibroblasts, respectively.[7,8] Reduced dermal thickness is a natural consequence of documented reduction in procollagen synthesis and constitutively elevated matrix metalloproteinases in naturally aged human skin.[7] In addition to these quantitative changes, there is a qualitative fragmentation of dermal collagen fibers.[9] These senescent changes partly explain the notably poor wound healing and propensity for chronic skin ulcerations seen in the elderly population. Such fragile skin is becoming a major public health issue as the population grows older. Safe and effective therapies to reverse the atrophy of natural skin aging do not exist currently.

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This reflects our limited understanding of the natural skin aging process, as well as significant logistical challenges in conducting clinical studies in the geriatric population.

Among the clinical features of photoaging, wrinkles have been a focus of extensive studies over the past decade. Compared with sun-protected skin, such as the buttock, photoaged skin has less procollagen formation.[10] Well-established treatments for photoaging, such as carbon dioxide laser resurfacing[11] or topical retinoic acid ,[12,13] stimulate procollagen synthesis and increase the mature collagen band in the high dermis (papillary dermis).[14] This increase in dermal collagen is associated with wrinkle effacement. Because accelerated skin aging due to excessive sun exposure has marked collagen deficiency and effective treatments for photoaging promote procollagen synthesis, we hypothesized that similar therapies might also improve the collagen deficiency found in intrinsic aging. For intrinsically aged skin, however, use of ablative lasers such as carbon dioxide is not feasible because it induces significant wounding that takes several weeks to heal. Topical retinoic acid and tazarotene, both approved for the treatment of photoaging, are not suitable for use in geriatric populations either because they consistently induce skin irritation at application sites.[4,12,15]

All-*trans*-retinol is a precursor to retinoic acid. When applied to human skin, it penetrates and is sequentially oxidized to retinoic acid, causing retinoic acid–like effects.[16] However, compared with retinoic acid, the ability of retinol to induce skin irritation is notably less, at least according to a 4-day patch test (an occlusive treatment).[16,17] Thus, retinol has the potential to deliver retinoic acid–like effects to human skin with improved tolerability. We report herein a geriatric clinical study (patients 80 years or older only) assessing the effectiveness of topical retinol in effacing fine wrinkling as a clinical end point for reversal of skin atrophy. This clinical observation is complemented by molecular measurements of procollagen and glycosaminoglycan (GAG).

METHODS

SUBJECTS

The protocol was approved by the University of Michigan Medical School institutional review board, and written informed consent was obtained from all subjects prior to enrollment. Eligible patients were 80 years or older, in relatively good health, and without active skin diseases involving the upper extremities. Forty-four subjects were screened for the study from March 2001 until December 2002. The last visit by a subject occurred in August 2002. A total of 36 subjects with a minimum age of 80 years from 2 senior citizen centers were enrolled in the study, and 23 subjects completed the entire study. The mean age was 87 years (range, 80-96 years), and the male-female ratio was 1:2.5. Exclusion criteria included topical corticosteroid or other topical drug use 2 weeks prior to study entry and hormone therapy for women 6 months prior to the study.

TREATMENT

Treatment areas consisted of upper inner (sun-protected) portions of the arms bilaterally (0.4% retinol [vitamin A] lotion was applied to one arm and its vehicle lotion was applied to the contralateral arm). Assignment of treatment was made through a computer-generated randomization code. Approximately 2 mL each of retinol and vehicle lotion were drawn via syringe and applied to the arm at each treatment session. These unoccluded topical retinol applications were performed up to 3 times per week (Monday, Wednesday, and Friday) for 24 weeks. The treated areas were not subsequently covered with clothing. Unblinded study personnel not involved in the evaluation of study subjects traveled to the 2 residential sites 3 times a week for 24 weeks to administer treatments in mid-afternoons. For subjects who experienced skin irritation or excessive dryness, treatments were discontinued for 1 or more treatment sessions until symptoms decreased. Subjects with unresolved irritation after 2 weeks without treatment were terminated from the study.

RETINOL FORMULATION AND BIOACTIVITY

Retinol was formulated in the laboratory of 1 of the investigators (G.J.F.) by combining a 41% retinol solution in 55% polysorbate 20 in sufficient proportions with fragrance-free Norwegian Formula Neutrogena Body Moisturizer (Ortho-Neutrogena Co, Los Angeles, Calif) to yield a 0.4% retinol lotion. The vehicle was similarly prepared with 55% polysorbate 20 solution in Norwegian Formula Neutrogena Body Moisturizer. There were no discernable differences in color, odor, or consistency between the active lotion with retinol and the placebo lotion control. A stability study in our laboratory using high-performance liquid chromatography demonstrated that more than 90% of 0.4% retinol remained in the moisturizer 3 months after preparation. Therefore, 0.4% retinol lotion and vehicle were formulated every 2 months to ensure that adequate amounts of the active ingredient were present throughout the study. Both 0.4% retinol lotion and its vehicle were placed in dark glass containers covered with foil to eliminate transmission of UV radiation and stored at 4°C.

A separate bioactivity study using this 0.4% retinol formulation was also conducted on 5 healthy nongeriatric volunteers. This protocol was also approved by our institutional review board, and written informed consent was obtained from the subjects prior to enrollment. The *CRABPII* gene contains a retinoic acid–responsive element.[18] Its messenger RNA (mRNA) is induced by topical application of retinoids dose-dependently, thus serving as a reliable reporter of retinoid action.[16,19] Retinol lotion, its vehicle, 0.1% retinoic acid in ethanol-propylene glycol (positive control), and ethanol-propylene glycol (negative control) were applied on buttock skin, and a biopsy from each treatment site was obtained after 24 hours. *CRABPII* mRNA levels were measured with quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR), as previously described.[20]

CLINICAL EVALUATIONS

Evaluations were performed by 2 blinded dermatologists (V.N.H. and S.K.) at baseline and then at weeks 2, 4, 8, 16, and 24. Clinical evaluations of the upper inner arms were based on (1) tactile roughness, (2) fine wrinkling, and (3) overall severity. Each of these parameters was graded on a semiquantitative scale from 0 to 9 (0, none; 1-3, mild; 4-6, moderate; and 7-9, severe). Fine wrinkling was the primary outcome measure, while the overall severity was secondary. At each visit, subjects were also evaluated for signs of cutaneous irritation such as erythema, peeling, pruritus, burning and/or stinging, and dryness on a similar 9-point scale. Photographs of treatment areas were obtained at

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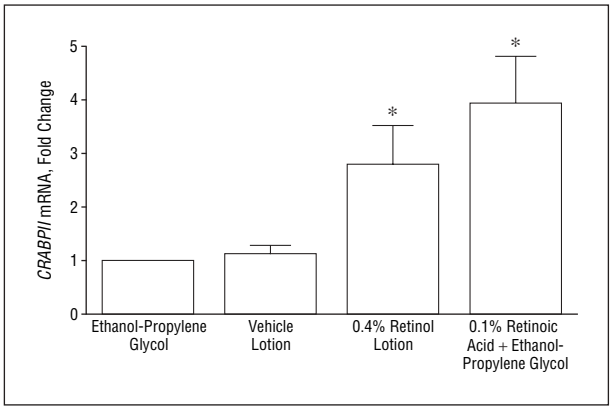

Figure 1. Induction of *CRABPII* messenger RNA by 0.4% retinol preparation in human skin in vivo (n=5). Error bars indicate standard error. *$P<.05$ vs control.

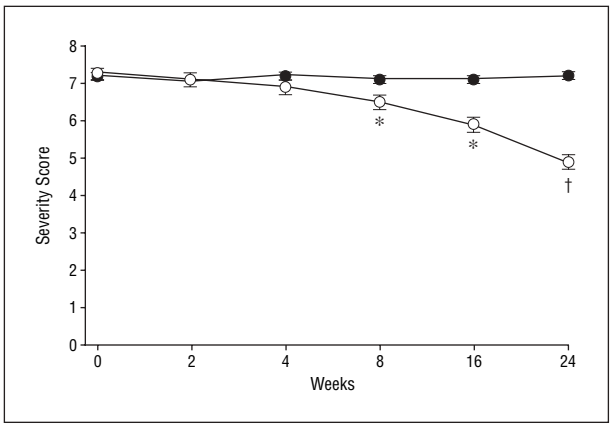

Figure 2. Topical retinol reduces the fine wrinkling in chronologically aged skin (n=23). Error bars indicate standard error. *$P<.01$ vs vehicle. †$P<.001$ vs vehicle.

baseline and weeks 2, 4, 8, 16, and 24. For standardization, subjects were asked to stand erect and extend their arms 90° from the trunk laterally and gently place their hands (palm surface down) on a vertically placed pole.

SKIN BIOPSY SPECIMENS

Four-millimeter punch biopsy specimens were taken from the upper inner arms on both retinol- and vehicle-treated arms at baseline and again at week 24. Biopsy specimens at week 24 were taken at least 2 cm away from the baseline biopsy sites to avoid inadvertent sampling of scar tissue. Biopsy specimens from each patient were assigned, through a randomization code, to one of several laboratory assays (immunohistologic and Western blot analyses, real-time RT-PCR, and transmission electron microscopy). Owing to a relatively small sample size in each category (≤6 subjects), significant differences in measurements were not observed in most. Only the results of statistically significant effects are reported herein. Biopsy specimens for immunohistochemical analysis and GAG quantification were embedded in optimal cutting temperature medium and stored at –70°C until use. For type I procollagen immunostaining, 7-µm sections were cut and reacted with SP1.D8 monoclonal antibody (1.8 µg/mL, from the University of Iowa Department of Biological Sciences, Iowa City), as previously described.[10] SP1 staining within the dermis was quantified using Image-Pro Plus software (Media Cybernetics, Silver Spring, Md). Data were expressed as the percentage of dermal area stained. For GAG determination, eight 50-µm sections were combined, washed with 50mM Tris (pH 8.0), digested with protease (500 µg/mL) (Sigma, St Louis, Mo), centrifuged for 15 minutes at 10 000*g*, and the supernatant assayed for GAG using a Blyscan Assay Kit (Biocolor Ltd, Belfast, Ireland) with the supplied GAG standard. Data were expressed as GAG (in micrograms) per volume (in microliters). *CRABPII* mRNA levels in skin samples were quantified by real-time RT-PCR, as previously described.[21,22] Forward and reverse primers and probe were 5′-CAAGACCTCGTGGACCAGAGA-3′, 5′-ACCCTGGTGCACACAACGT-3′, and 6FAM-TCCGCCGTCATGGTCAGGATCAGTTC, respectively.

STATISTICAL ANALYSIS

Comparisons of clinical end points between vehicle- and retinol-treated skin were made with the paired *t* test. Subjects who withdrew prior to completion of the study had their last available global evaluation carried forward to week 24. Summary data are represented as mean±SE. All *P* values are 2 tailed. The data were analyzed using SAS analytic software (version 8.2, SAS Institute Inc, Cary, NC).

A sample size of 36 provides a power level of 0.80 in detecting a difference of 0.5 units on the overall global response scale between treated and untreated skin, with a type I error rate of 0.05 for a 2-tailed hypothesis, assuming a standard deviation of differences to be 1.0. This assumption was based on previous data from similar studies in photoaging.

RESULTS

A total of 36 subjects were enrolled in the study. All were white with skin phototypes I or II. Eighteen were randomized to receive 0.4% retinol lotion on the right arm and 18 were randomized to receive lotion alone on the left arm. Among the 36 subjects, 23 completed the entire study and 13 withdrew prior to completion. Five subjects withdrew because of cutaneous irritation and/or pruritus (n=3), broken arm (n=1), and broken hip (n=1). Six subjects discontinued because of personal reasons, 1 withdrew owing to a protocol violation, and 1 subject died during the treatment period from a cause unrelated to the study.

RETINOL BIOACTIVITY RESULTS

Unoccluded application of 0.4% retinol lotion to normal human skin induced *CRABPII* mRNA 3-fold over its vehicle treatment ($P<.05$; n=5). This magnitude of *CRABPII* mRNA induction was comparable to that seen with 0.1% retinoic acid, indicating retinol's ability to penetrate human skin and cause retinoid induced molecular changes (**Figure 1**).

CLINICAL RESULTS

At baseline, clinical severity of the upper inner arms was similar between the left and right sides. For fine wrinkling, arms assigned to retinol treatment had a mean score of 7.25 (95% CI, 7.03 to 7.47) and those assigned to vehicle treatment, 7.22 (95% CI, 7.01 to 7.43). An analysis of all patients using the last-observation-carried-forward method revealed that retinol treatment

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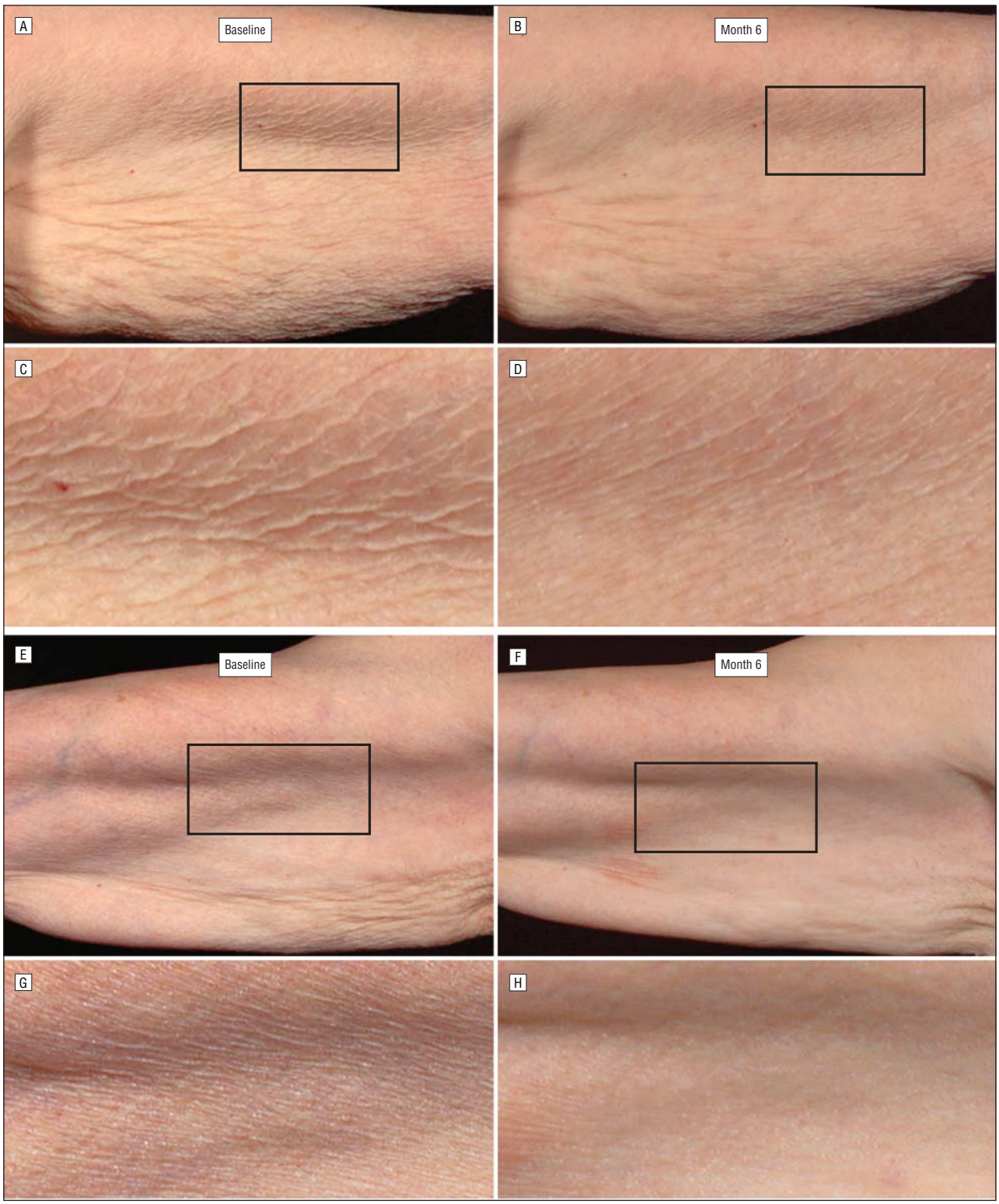

Figure 3. Clinical appearance of upper inner arms at baseline and end of study from 2 representative subjects (A and B; E and F). Magnifications (original magnification ×3.5) of the areas outlined with rectangles are also shown (C and D; G and H).

significantly reduced the fine wrinkling scores compared with vehicle treatment (–1.64 [95% CI, –2.06 to –1.22] vs –0.08 [95% CI, –0.17 to 0.01]; $P<.001$ [N=36]). In an analysis restricted to patients completing the study (n=23), the reduction in fine wrinkling was evident beginning at week 4 and continued through week 24 (**Figure 2**). Representative examples of clinical responses are seen in **Figure 3**.

Improvement in tactile roughness and overall severity in chronologically aged skin was also seen with retinol treatment. After 24 weeks, intent-to-treat analyses demonstrated significant differences between retinol-

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Table. Intent-to-Treat Analyses of 36 Patients Using the Last-Observation-Carried-Forward Method*

Variable	Baseline Score	Score at Week 24	Change in Score at Week 24	*P* Value
Tactile roughness				
Retinol	4.64 (4.09 to 5.19)	4.11 (3.57 to 4.65)	–0.53 (–0.73 to –0.33)	<.001
Vehicle	4.64 (4.09 to 5.19)	4.64 (4.09 to 5.19)	0.00 (0.00 to 0.00)	
Fine wrinkling				
Retinol	7.25 (7.03 to 7.47)	5.61 (5.14 to 6.08)	–1.64 (–2.06 to –1.22)	<.001
Vehicle	7.22 (7.01 to 7.43)	7.14 (6.91 to 7.37)	–0.08 (–0.17 to 0.01)	
Overall severity				
Retinol	6.94 (6.75 to 7.14)	5.69 (5.24 to 6.14)	–1.25 (–1.63 to –0.87)	<.001
Vehicle	6.92 (6.73 to 7.10)	6.97 (6.77 to 7.17)	0.06 (–0.02 to 0.14)	

*Data are given as mean score (95% confidence interval).

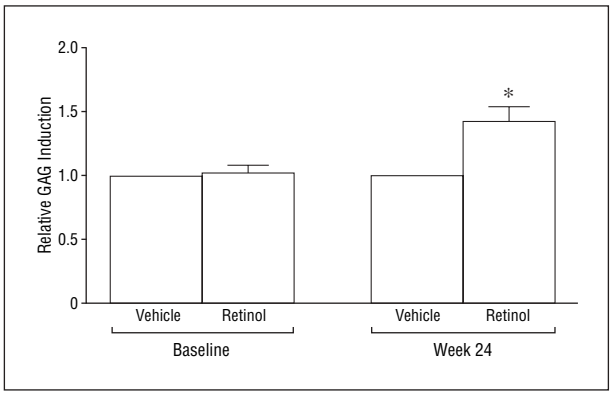

Figure 4. Topical retinol induces glycosaminoglycan (GAG) expression in chronologically aged skin (n=6). Values are ratios of levels in the retinol or vehicle at baseline and week 24. *P=.04 vs baseline. Values in retinol-treated skin are expressed as fold change from vehicle-treated skin at baseline and again at week 24. After 24 weeks, the GAG levels in retinol-treated skin were 40% higher, on average, than in vehicle-treated skin (P=.02).

treated and vehicle-treated skin for changes in tactile roughness scores (–0.53 [95% CI, –0.73 to –0.33] vs 0.00 [95% CI, 0.00 to 0.00]; P<.001 [N=36]) and in the overall severity scores (–1.25 [95% CI, –1.63 to –0.87] vs 0.06 [95% CI, –0.02 to 0.14]; P<.001 [N=36]) (**Table**).

ADVERSE REACTIONS

Overall, topical retinol was well tolerated by the subjects. By week 24, most subjects reported some degree of cutaneous irritation on the retinol-treated arm, including erythema (n=18), peeling (n=16), pruritus (n=12), dryness (n=14), and burning and/or stinging (n=3). However, most adverse reactions to the retinol were rated as mild. In 3 subjects, cutaneous reactions and/or symptoms were severe enough to withdraw consent.

BIOCHEMICAL RESULTS

Compared with vehicle, retinol treatment induced a significant increase in GAG expression (P=.02 [n=6]) (**Figure 4**). In addition, a significant increase in procollagen I immunostaining from baseline to week 24 was observed in the retinol-treated arm compared with the vehicle-treated arm (P=.049 [n=4]) (**Figure 5**).

COMMENT

The age of the population is rapidly increasing. It is estimated that by the year 2040, greater than 30% of the US population will be older than 55 years, more than doubling its current level.[23] This increase in population age will be accompanied by greater demands on health care resources. Poor wound healing and propensity to form nonhealing ulcers (eg, decubitus ulcers), in addition to development of skin cancers, are considerable dermatologic issues in the elderly population. These clinical features of increased skin fragility are believed to result from cutaneous atrophy. Therefore, a safe and effective approach to increase the dermal matrix is desirable.

As human skin naturally ages, it becomes thin, lax, and finely wrinkled. Of these changes, fine wrinkles are most easily appreciated clinically, with severity correlating strongly with age.[24] We have demonstrated through this randomized controlled clinical trial that topical 0.4% retinol improves the clinical appearance of naturally aged human skin. This clinical improvement was accompanied by an increase in 2 matrix molecules, procollagen I, and GAG.

In our study, the fine wrinkles of intrinsic aging began to efface after 4 weeks of retinol use, with continued improvement throughout the study period of 24 weeks. This is a faster response in wrinkle effacement than that observed in photoaging. Typically, at least 2 to 3 months of topical retinoic acid therapy is needed before significant improvement in wrinkles or photoaging is noted.[12,25,26] The time-course differential in wrinkle effacement may reflect differences in the nature of wrinkles between photoaging and intrinsic aging and/or severity of the matrix deficiency to be overcome by retinoids.

Occurring within the first month of retinoic acid treatment, there is an increase in GAG (hyaluronic acid) in the epidermis of photoaged skin.[3,27] As a hygroscopic material, GAG possesses the capacity to bind water roughly 1000 times its own weight.[28] Well-established tactile smoothening of photoaged skin by topical retinoic acid is believed to be mediated by induced GAG retaining water in the superficial cutaneous compartment. In our study on intrinsic aging, measurement of GAG was performed prior to and after a 6-month treatment course with retinol. As a precursor to retinoic acid, retinol is expected to induce GAG early in the treatment course like reti-

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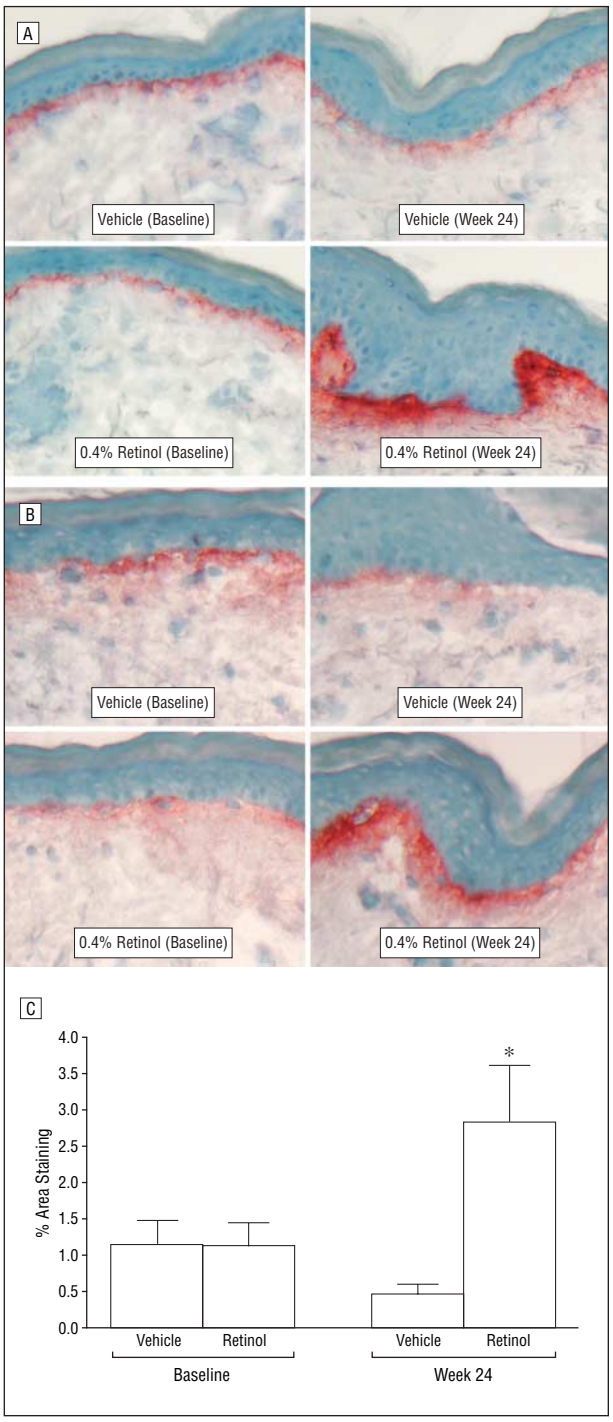

Figure 5. Topical retinol induces type I procollagen protein in chronologically aged skin. Baseline and 24 weeks after retinol- or vehicle-treated skin was analyzed for type I procollagen by immunohistologic analysis (SP1 staining [reddish-brown color represents immunostaining], original magnification ×20). Panels A and B are from 2 representative subjects. Panel C summarizes quantitation (percentage of the dermis stained by SP1) of procollagen type I immunostaining (n=4). Error bars indicate standard error. *P=.049 for retinol vs control in the difference of percentage of area staining from baseline to week 24.

noic acid. Thus, the early effacement of fine wrinkles of natural aging brought on by topical retinol is most likely due in part to GAG.

Besides GAG, procollagen I was significantly induced by retinol treatment. The paucity of procollagen I immunostaining at baseline indicates that there is minimal synthesis of collagen in intrinsically aged skin. Our study demonstrates that unoccluded treatment of retinol can stimulate procollagen synthesis in aged human skin. In photoaged human skin treated with topical retinoic acid for an extended length of time (>2 years on average), we now have evidence that mature collagen matrix is deposited in the high dermis.[14] It is expected that a similar process will occur in intrinsically aged skin treated with retinol. Such skin may withstand skin breakdown and ulcer formation to a greater degree than untreated skin.

Of the 36 subjects enrolled in our study, 11 were assessed 12 weeks after the therapy was discontinued (week 36). Although the difference in fine wrinkle severity score had lessened between the retinol- and vehicle-treated sides, it remained statistically significant (data not shown). By week 48, 24 weeks after the discontinuation of retinol treatment, no significant differences remained between the 2 sides. In photoaging, a similar relapse in effaced wrinkling caused by retinoic acid treatment has been reported.[29]

Our retinol preparation was relatively well tolerated by the elderly subjects. Despite the known relatively low irritation potential of retinol,[16] we deliberately erred on the conservative side in administering topical treatments to maximize subject retention and tolerability in this fragile population. If there was any question or concern of excessive irritation, pruritus, or erythema, our trained treatment providers were instructed to withhold further topical application until the cutaneous reaction and symptoms abated or improved. These signs and symptoms (ie, retinoid dermatitis) have always been a source of potential bias in topical retinoid trials. Our gentle treatment approach minimized cutaneous reactions evident during patient evaluations and served to reduce but not totally eliminate the possibility of bias and deblinding. Indeed, although up to 3 treatment sessions per week were planned, our patients received a mean of 1.6 applications weekly. We suspect that with a more aggressive treatment regimen, greater clinical effects and more robust biochemical changes would have been observed.

In summary, topical retinol is a promising and safe treatment to increase the dermal matrix of aged skin and improve clinical features associated with atrophic wrinkled skin. By increasing the dermal matrix of elderly skin, the substantial morbidity caused by poor wound healing and chronic ulcer formation may be reduced.

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rani, Fisher, Voorhees, and Kang. *Statistical analysis:* Hamilton and King. *Obtained funding:* Kafi, Voorhees, and Kang. *Administrative, technical, and material support:* Kafi, Kwak, Schumacher, Cho, King, Neal, Varani, Fisher, Voorhees, and Kang. *Study supervision:* Kafi, Voorhees, and Kang.

Financial Disclosure: Drs Fisher, Kang, Varani, and Voorhees are named inventors on an issued patent application concerning methods of treating skin aging. They will receive royalties under the University of Michigan's Intellectual Property Policy in the event that a commercial license is signed and a product is sold. This article describes research that was part of the basis of the approved application.

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Vitamin E Is Nature's Master Antioxidant

by Lester Packer

Oxidative destruction of subcellular membrane lipids has been implicated along with other types of intracellular oxidative damage in the normal aging process and in the pathophysiology of a number of chronic diseases. Complex antioxidant mechanisms exist to limit the effects of these reactions. Vitamin E quenches free radicals effectively in small amounts, and evidence of its usefulness as a curative and preventive agent is accumulating. Results of controlled long-term intervention trials should be available soon.

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Vitamin E was discovered at the University of California, Berkeley, in 1922 by Herbert Evans and Katherine Bishop, who observed that its deficiency caused fetal resorption in the rat. An active substance was isolated from wheat germ oil in 1936, also at Berkeley, and named "tocopherol" from the Greek words *tokos* (childbirth) and *pherein* (to carry) plus the -ol suffix designating a phenol or alcohol.

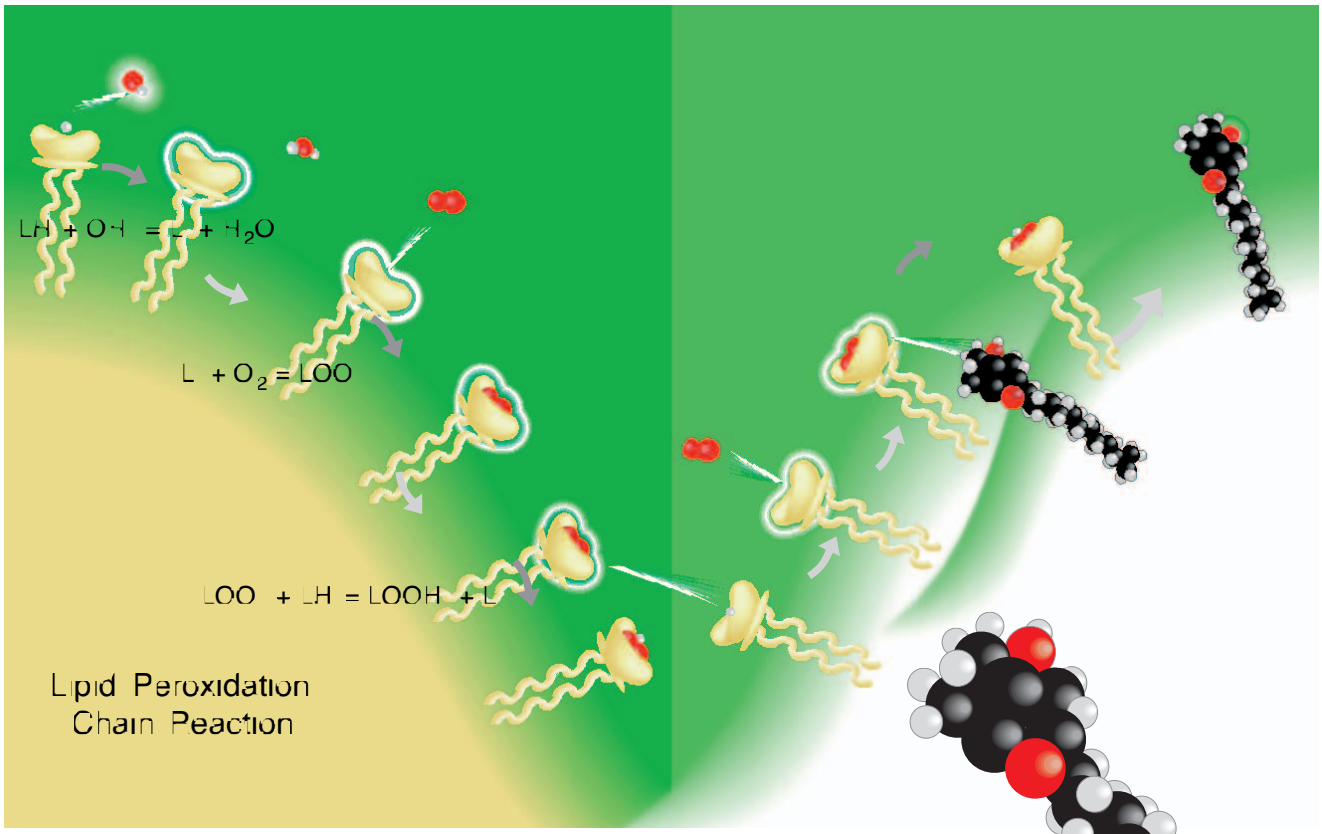
Vitamin E is a group of substances, the tocopherols and tocotrienols, found mainly in vegetable oils. Each has a chromanol head group and a phytyl side chain. The side chains of tocopherols are saturated, while those of tocotrienols contain three double bonds. Different numbers and placements of methyl groups in the aromatic ring produce α, β, γ, and δ forms of tocopherols and tocotrienols. Each form occurs in nature as a single stereoisomer. Synthetic vitamin E contains up to eight isomers, each with its own biological activity.

D-α-tocopherol is the most common type of vitamin E absorbed from the human diet, except that tocotrienols predominate in areas of the world where tropical plant oils are used for cooking and as sources of food. D-α-tocopherol is about 36% more active than the synthetic, isomeric mixture.

The function of vitamin E was disputed for decades and is not yet completely understood. An antioxidant property was evident once the chemical structure had been determined, for it resides in the phenolic hydroxyl group at C-6 on the aromatic ring. Readily oxidized, the tocopherols protect less susceptible compounds. Peroxidation of polyunsaturated fatty acids causes fats and oils to become rancid when exposed to air, and an early commercial use of vitamin E was to retard food spoilage. Biochemists supporting the view that the general antioxidant property was the key biologic activity could not show that lipid peroxidation occurred in animals, while those who favored a specific metabolic function were not able to identify one.

In the meantime, while the puzzling consequences of experimental vitamin E deficiency in animals were investigated, a barrage of unsubstantiated claims arose. With a kind of reverse logic, it has been argued that if a deficiency of vitamin E in animals causes lack of potency, then an excess in humans must cure impotence and act as an aphrodisiac. If a deficiency in animals causes muscle weakness and inability to tolerate exercise, then an excess in humans must lead to enhanced physical performance. Vitamin E is still promoted as a nostrum for ailments from cancer to arthritis. To skeptics it sounds like a twentieth century version of the old "snake oil remedy," but every faith has its believers, and vitamin E is being taken in large amounts by many people.

They may be right. Of itself, the variety of disease states induced in animals by vitamin E deficiency was an argument for a general



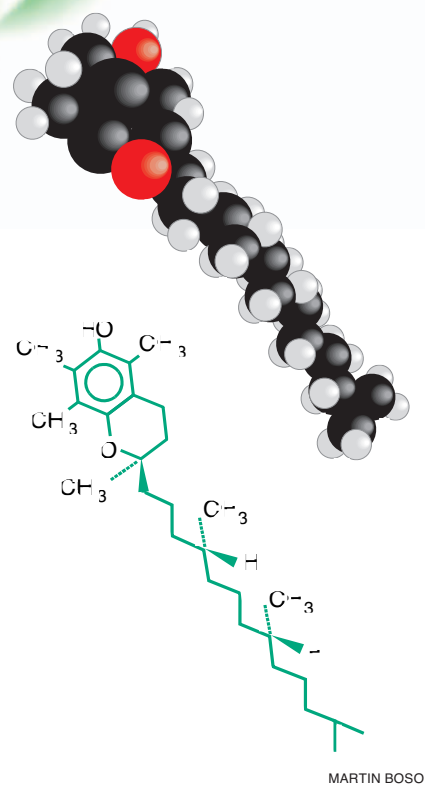
rather than a specific function of the vitamin. Experiments by Al L. Tappel and colleagues at the University of California, Davis, beginning in the late 1950's directly demonstrated in vivo lipid peroxidation and the inhibitory effect of vitamin E. By blocking oxidation of lipids in subcellular membranes, vitamin E may have a role in defending the body against diseases. Quite small amounts of vitamin E are protective; much larger amounts taken as dietary supplements are unlikely to be harmful and may be beneficial.

Vitamin E Neutralizes Free Radicals

During normal energy metabolism, electrons are passed from foodstuffs down the electron transport chain in mitochondria to oxygen molecules, which accept electrons and protons to form water. The cell harnesses the energy released as ATP. But there are "leaks" in the electron transport chain, points at which single electrons can escape to transform atoms or molecules into free radicals. For example, a superoxide radical is formed when molecular oxygen accepts a single electron.

Superoxide, like other free radicals, is highly reactive, and one reaction in which it can engage is dismutation, to form hydrogen peroxide. Hydroxide ions and reactive hydroxyl radicals are formed from hydrogen peroxide in the presence of metal ions. Superoxide radicals, hydroxyl radicals, and hydrogen peroxide are the so-called excited-oxygen or reactive oxygen species, and they cause intracellular oxidative damage in several ways. By reacting with DNA, for example, free radicals can induce mutagenic alterations.

Polyunsaturated fatty acids, susceptible to oxidative chain reactions at their double bonds, are present in plasma membranes and in mitochondrial membrane glycerophospholipids. Free radical lipid peroxidation propagates through polyunsaturated fatty acids, each completed peroxida-



MARTIN BOSO

The α-tocopherol molecule

tion producing one of a variety of products and a new fatty acid peroxyl radical. In theory, oxidation of a single membrane lipid molecule by a single hydroxyl radical could start a chain reaction that would destroy the entire membrane.

Antioxidant mechanisms have evolved to stop the oxidative processes. Some antioxidants are enzymes that destroy superoxide radicals (superoxide dismutase) and peroxides (peroxidases and catalase). Others are molecules such as ferritin that bind tightly to metal ions and prevent the breakdown of hydrogen peroxide. Vitamin E interrupts the chain of membrane lipid peroxidation and is thus a "chain-breaking" antioxidant. The reaction of a lipid peroxyl radical with a vitamin E molecule interrupts peroxidation by producing a hydroperoxide and a vitamin E radical, both of which are relatively unreactive.

The vitamin E radical, or chromanoxyl radical, can follow one of several pathways. If it reacts with another chromanoxyl radical, with an alkoxyl radical, or with a peroxyl radical the result is unreactive products with no further free radical scavenging activity. Alternatively it can be reduced back to a functional vitamin E molecule.

Though it is the major, if not the only, chain-breaking antioxidant in mitochondrial membranes, vitamin E is present at extremely low concentrations, usually less than 0.1 nmol per mg of membrane protein or in other words one molecule per 1000 to 2000 membrane phospholipid molecules. Lipid peroxyl radicals can be generated in membranes at the rate of 1 to 5 nmol per mg of membrane protein per minute, yet destructive oxidation of membrane lipids does not normally occur, nor is vitamin E rapidly depleted.

There must be an extremely efficient mechanism for regenerating or recycling vitamin E in order to sustain such minute but effective concentrations. Recycling of vitamin E by both enzymatic and nonenzymatic pathways has been demonstrated by us in artificial

Assays and Units

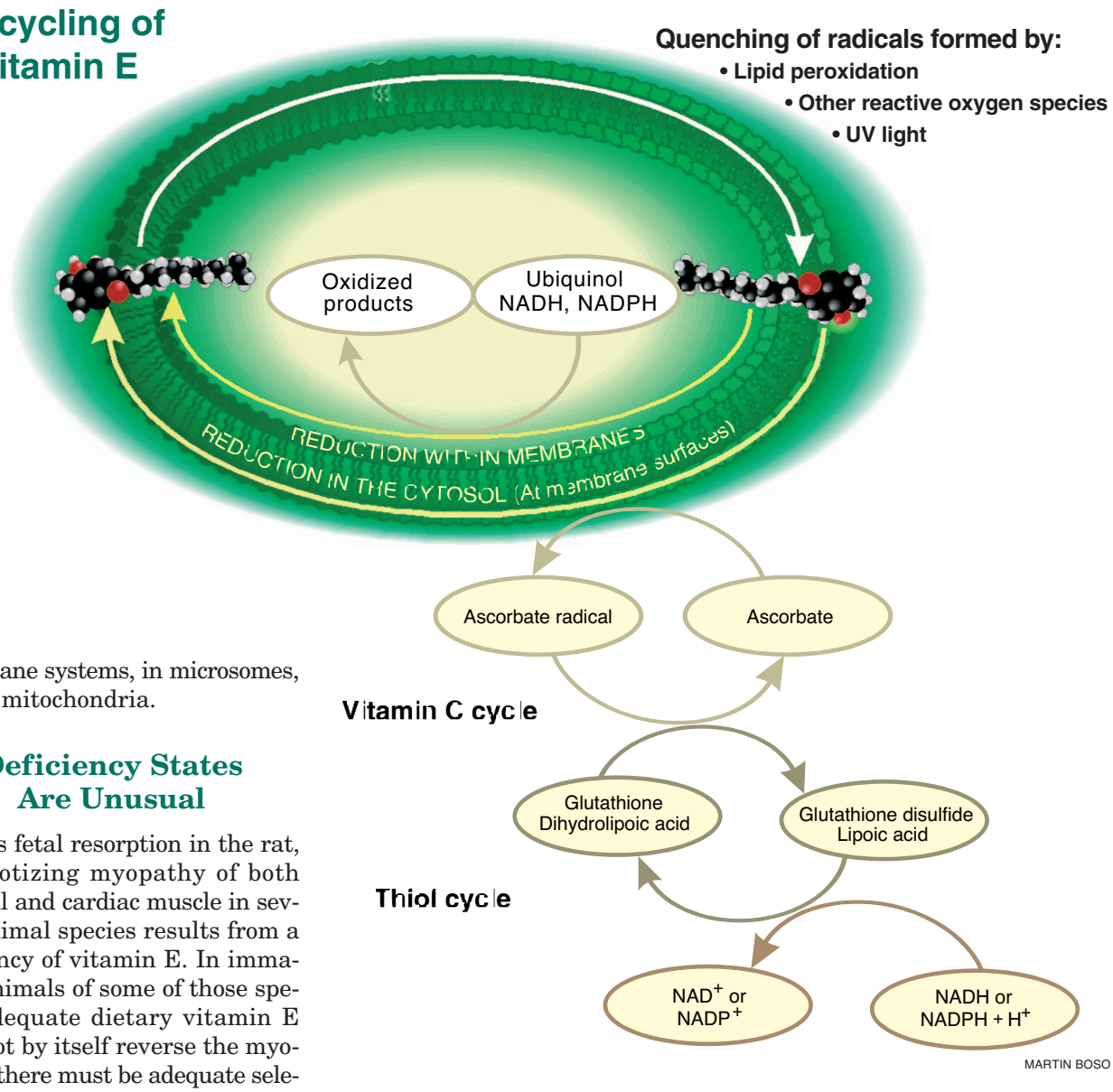
The earliest method of testing for the activity of vitamin E was a rat fetal resorption assay. Vitamin E-depleted female rats impregnated by normal males were kept on diets containing various amounts of the substance being assayed. The International Unit of vitamin E activity was based on the amount of vitamin E needed to prevent fetal resorption.

Other assay methods, equally tedious and insensitive, measured the ability of vitamin E to prevent deficiency symptoms in animals. Chromatography and sensitive electrochemical methods now allow direct measurement of the vitamin E content of a sample with ease. High performance liquid chromatography enables detection of the presence of any form of tocopherol or tocotrienol in a sample, down to the picomole level, in 15 minutes. Being able to record the exact amounts of compounds in a mixture rather than using the vague term "vitamin E activity" has clear advantages.

The contrast between the two kinds of assay procedures, one based on the appearance of deficiency symptoms and the other on chemical composition, raises a larger question about vitamin E. If its function depends only on its presence, then bioassays are useful and International Units are handy; but if its effects vary according to the specific tocopherol or tocotrienol content, then more sophisticated assays and more precise units of measurement are required.

Fairly early in the history of vitamin E research it was clear that the antioxidant effects of the four tocopherols were different ($\alpha > \beta > \gamma > \delta$). Work in our laboratory with in vitro membrane systems has shown that the most common of the tocotrienols, D-α-tocotrienol, has 40 to 60 times the antioxidant potency of D-α-tocopherol. While such differences have yet to be demonstrated in animals, they point to the necessity for careful discrimination among the various forms of vitamin E. Most research uses D-α-tocopherol, which is the most common naturally occurring form.

Each tocopherol and tocotrienol has its own conversion rate between mg and IU. As a rough rule of thumb, 1 mg of natural "vitamin E" is equivalent to 1.5 IU of D-α-tocopherol, and 1 mg of synthetic vitamin E is equivalent to 1 IU of D,L-α-tocopherol.



membrane systems, in microsomes, and in mitochondria.

Deficiency States Are Unusual

Besides fetal resorption in the rat, a necrotizing myopathy of both skeletal and cardiac muscle in several animal species results from a deficiency of vitamin E. In immature animals of some of those species adequate dietary vitamin E does not by itself reverse the myopathy; there must be adequate selenium as well. Other deficiency diseases, such as a liver necrosis in pigs, rats, and mice, are reversible by either vitamin E or selenium.

Probably because biologically active vitamin E is continually being recycled and not consumed, clinical signs of its deficiency cannot be induced in human adults. In an early experiment conducted by Max Horwitt at the Elgin State Hospital, volunteers were kept on diets containing no more than 4 mg of tocopherol per day for up to five years without developing any clinical signs of vitamin E deficiency, although their erythrocytes showed less resistance to induced peroxidation and a decrease in survival time.

There is a form of spinocerebellar degeneration produced by low levels of vitamin E due to chronic fat malabsorption. The disorder has been described in both adults and children, in some of whom plasma levels of vitamin E were not detectable at all. Recognition of vitamin E deficiency and restoration of normal vitamin E status stabilizes and may reverse the neurologic degeneration.

Children are born with relatively low tocopherol levels. Premature infants have even less vitamin E and transiently are not able to absorb it. The consequent absence of adequate protection against membrane lipid oxidation is presumably involved in the pathogenesis

Chromanoxyl radicals are reduced to "native" vitamin E molecules in a complex and highly efficient system involving ubiquinol (coenzyme Q), vitamin C, and glutathione. Elements of the hypothetical mechanism have been worked out in model systems; confirmation of the theoretical and experimental biochemistry in an intact organism still lies ahead.

Assessment of Lipid Peroxidation in Vivo

Two common methods measure secondary products, and neither is perfect. The **thiobarbituric acid** reaction usually uses plasma, easy to sample and to store. Heated with the sample at low pH, TBA is assumed to react with malondialdehyde, resulting in a pink product whose absorbance at 532 nm is taken as an indication of lipid peroxidation.

Other aldehydes, and indeed other chemicals in plasma, react with TBA, and the heating itself accelerates lipid peroxidation and triggers decomposition of lipid peroxidation products. Unless carefully controlled, the TBA assay may give values that are tens or even hundreds of times higher than the actual values of MDA in plasma. Furthermore, how well plasma reflects whole-body lipid peroxidation is not clear, nor is it known what components of plasma may be oxidized.

Hydrocarbon gases produced by lipid peroxidation are the basis for the measurement of **breath pentane**. Noninvasive and capable of being continuously monitored, the breath pentane assay is cumbersome and complicated, and its use is more or less restricted to research applications. The assumption is that increased lipid peroxidation results in increased pentane production and that the gas must ultimately be released in the lungs. Exhaled air can be collected, condensed and stored in liquid nitrogen, and assayed for hydrocarbons by HPLC.

Hydrocarbon gases are also produced by gastrointestinal bacteria and are components of air pollution. The rate of metabolism, the iron content of various tissues, the antioxidant intake, and the metabolic conversion of pentane to pentanol all complicate the assay.

of hemolytic anemia in prematures, whose risk is increased by the administration of iron because of its role in oxidative processes.

The use of oxygen to alleviate respiratory distress in prematures accelerates oxidative reactions and is associated with bronchopulmonary dysplasia, retrolental fibroplasia, and intravascular cerebral hemorrhages. In fact, retrolental fibroplasia was the first human disease to be linked to low levels of vitamin E, in 1949. Supplemental vitamin E may be helpful when oxygen must be administered to premature infants. If used, it is given intramuscularly or in very high oral doses because it is poorly absorbed by the premature infant's gut.

Lipid Peroxidation Is Associated with Human Diseases

Oxidative membrane damage has been implicated in:

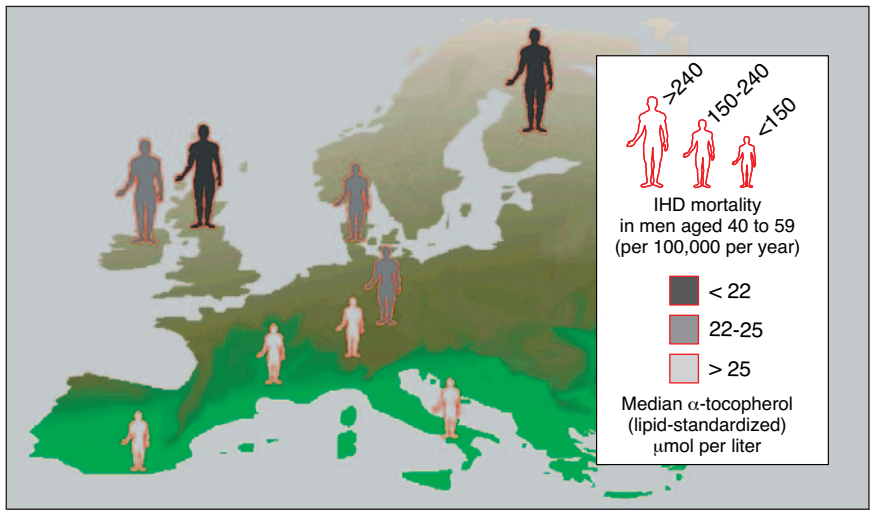
- Cardiovascular disease
- Carcinogenesis
- Neurologic disorders
- Immune system dysfunction
- Cataracts
- Arthritis

Oxidative processes are implicated in the pathophysiology of many diseases. Laboratory findings and population studies have established the associations, and interventions are being tried. The interlinked nature of antioxidant mechanisms makes it difficult to isolate the effects of vitamin E supplementation in most instances, but it should be possible to define an optimal daily requirement for vitamin E more accurately than has been done in the past.

Oxidative modification of polyunsaturated fatty acids, cholesterol, and apoprotein B in low-density lipoproteins degrades the lipoproteins, which are taken up by macrophages. The result is foam cells, and aggregations of foam cells are the fatty streaks on which atherosclerotic plaques develop. Protection of low-density lipoproteins against oxidation has been observed in cell cultures containing various concentrations of vitamin E. In animal studies as well, vitamin E has been shown to have a protective effect and possibly to reverse established atherosclerotic lesions.

As part of an epidemiologic investigation, the WHO/MONICA study, ischemic heart disease mortality was correlated with risk factors across a number of European populations. The strongest link was between decreased mortality and high plasma concentrations of vitamins E and A, attributed to greater amounts of green and yellow vegetables in southern European diets. Reports from this large survey triggered interest in the "Mediterranean" or "vegetarian-type" diet now being widely touted.

An added benefit obtained from supplemental vitamin E has been



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Blood levels of vitamin E were inversely correlated with mortality from ischemic heart disease in a cross-cultural survey of European populations. The data were reported by Fred Gey of the University of Bern.

suggested by reported findings from the Nurses' Health Study, in which 87,000 healthy women were followed for eight years. Those who took 100 mg or more of vitamin E per day had 36% less chance of developing heart diseases than those who did not, and if supplements were taken for more than two years the risk dropped by more than 40%.

To consider a clinical situation, the major tissue damage seen in ischemia-reperfusion injury occurs not during ischemia but during reperfusion. When deoxygenated blood is used for reperfusion, there is less tissue damage. Although the mechanism is debated, there is agreement that reactive oxygen species generated during reperfusion cause most of the damage. Hence vitamin E and other antioxidants should reduce ischemia-reperfusion injury.

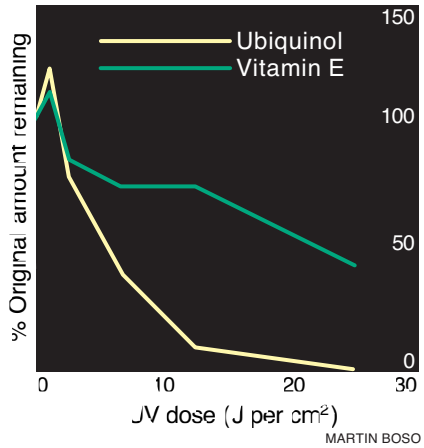
In our laboratory, hearts from rats fed a diet supplemented with vitamin E experienced 70% better recovery on reperfusion as measured by enzyme release from damaged cardiac muscle after mechanical recovery. Others have reported similar results, and in some hospitals it is now routine to add antioxidants to the bathing medium during open heart surgery.

Populations exposed to high levels of ultraviolet radiation have the highest incidence of cutaneous melanoma, and the incidence of this disease has doubled in the United States over the past decade. Recent work from our laboratory and others indicates that free radicals are involved in UV-induced skin cancer and that antioxidants can play a role in its prevention.

The hypothetical steps in UV-induced skin cancer are (1) ultraviolet light induces the formation of free radicals in skin; (2) antioxidant defenses nullify free radicals, but if the dose of UV light is too great the defenses will be overwhelmed; and (3) the resulting free radical load causes damage to proteins, lipids, and DNA.

Using the hairless mouse as a model, we found decreased concentrations of all major antioxidants in the skin, and a simultaneous increase in lipid hydroperoxides, when the animals were irradiated with doses of ultraviolet light in the range commonly encountered by humans, equivalent to a few minutes to a few hours in the sun. Administering vitamin E before irradiation, as a dietary supplement or topically, reduced the level of UV-induced lipid hydroperoxide formation by two thirds.

Studies by others, notably Homer Black, have shown that dietary supplementation with a combination of antioxidants including vitamin E reduced the incidence of skin tumors in irradiated mice and also that mice fed a diet high in polyunsaturated fats expe-



Defense of the skin against UV-induced cancer by antioxidants is suggested by animal experiments that showed steadily decreasing antioxidant concentrations and increasing amounts of lipid hydroperoxides with increasing exposure to UV light. Ubiquinol diminishes first, presumably because it is consumed by the reduction of vitamin E radicals.

rienced more tumors than those fed diets lower in polyunsaturated fats. Mammary, colon, esophageal, and oral cancers have also been studied in laboratory animals, and vitamin E has been shown to be effective in reducing the incidence of tumors in the majority of studies.

As with atherosclerosis, epidemiologic studies suggest a possible role for antioxidants in cancer prevention. A case-control study of cancer patients in Finland found that those with low plasma vitamin E and selenium levels were at higher risk of dying than those with high antioxidant levels. American women with cervical dysplasia or cancer had lower levels of β-carotene and vitamin E than controls. Both Japanese and U.S. lung cancer patients had lower plasma vitamin E levels than matched controls.

No case-control study, however, can distinguish cause from effect. Among prospective studies in the United States, blood vitamin E levels were lower in subjects who subsequently developed lung cancer than in controls. A high intake of fiber, carotene, and vitamins C and E was associated with a lower risk of oral and pharyngeal cancer in black American men. People who took vitamin E supplements were found to have a lower risk of developing oral and pharyngeal cancer than people who did not.

Overall, Gladys Block of the University of California, Berkeley, analyzed 130 studies of the relationship between dietary intake or blood levels of antioxidants, or both, and the subsequent development of a variety of different cancers. She found that in 120, increased levels of antioxidants were associated with decreased cancer risk.

Problems plague this area of investigation, besides that of separating out the influences of the various antioxidants. Measurements must be made soon after blood samples are drawn, because vitamin E is unstable. The vitamin E content at the time the samples were taken cannot be reliably determined after years in cold storage, a difficulty that vitiates the results of numerous early studies and some more recent ones.

Evidence for a preventive or curative role may be scant, but supplemental vitamin E may be useful during cancer chemotherapy. Anti-cancer agents such as doxorubicin that generate reactive oxygen species damage normal tissue as well as cancerous tissue. Vitamin E and other antioxidants seem to protect healthy tissue from free radical attack while allowing chemotherapy to destroy cancer cells. Patients whose antioxidant status has been bolstered during chemotherapy seem to tolerate it better, increasing the likelihood that they will complete the course of treatment.

Antioxidants May Improve Performance and Extend Lifespan

Oxygen consumption by working muscles can increase by 100 times or more during exercise. Wherever there is more oxygen, there are more free radicals, so that damage to cells induced by reactive oxygen species should also increase during strenuous exercise. Kelvin Davies, formerly at Berkeley and now at Albany Medical College, demonstrated free radical formation and lipid peroxidation in the muscles of strenuously exercised animals.

With training, there is a concomitant increase in antioxidant defenses. However, Eric Witt, of our group at Berkeley, has shown that even highly trained college oarsmen have increased plasma lipid peroxidation after a strenuous exercise bout. Untrained rats develop not only signs of lipid peroxidation but also oxidative protein damage in their muscles when they are exercised strenuously. Vitamin E can significantly reduce the damage.

Carefully controlled studies by Witt and others failed to support exaggerated claims that antioxidants improve physical performance, with

one exception. Mountain climbers given 400 IU of vitamin E per day showed improved physical performance and decreased breath pentane output during prolonged exposure to high altitudes, in a study done by Irene Simon-Schnass.

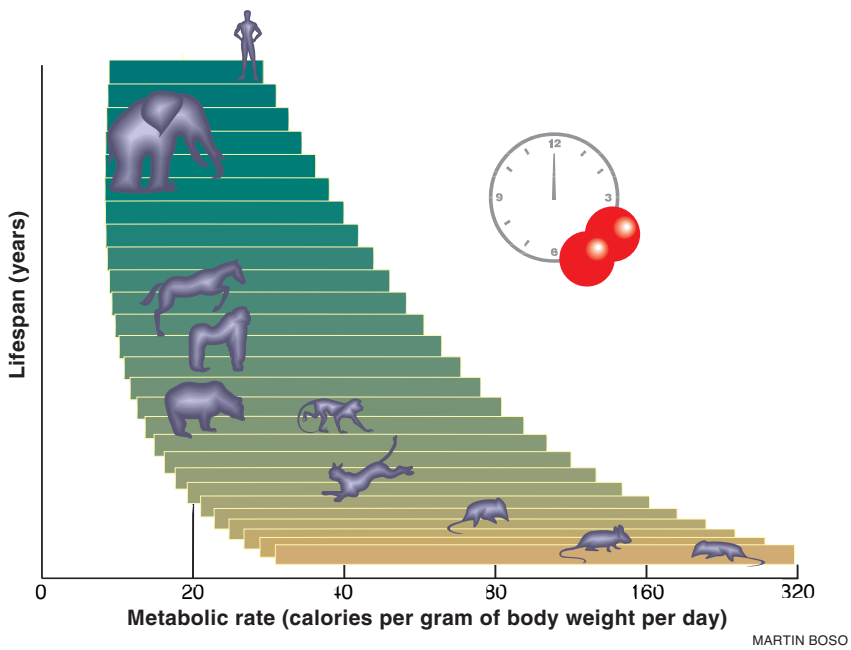
There is a remarkably consistent inverse correlation between metabolic rate and lifespan: the faster an organism uses oxygen, the more quickly it seems to age. Thus the constant appearance of free radicals during normal energy metabolism may be an important and even overriding factor in aging. If this is so, then antioxidant supplementation should slow the aging process. In animal experiments, average lifespan has been increased by supplementing diets with a variety of antioxidants, increases of 10% to 30% being reported. But such supplementation does not generally extend maximum lifespan.

Studies of aging human subjects show that vitamin E and other antioxidants can reverse some of the events of aging to some extent. For example, cell-mediated immunity tends to decline with age. In a study of subjects over 60, supplementation with vitamin E improved delayed-type hypersensitivity. In Poland, blood lipid peroxide levels were evaluated in people aged 60 to 100 who were then asked to take antioxidant supplements for a year. Those receiving vitamin E experienced a 26% decrease in lipid peroxides over the course of the study.

Daily Requirements Vary with Diet

The National Research Council first established Recommended Daily Allowances for vitamins in 1948, based on the prevention of observable deficiency syndromes. Vitamin E was omitted at first because of the difficulty of inducing clinical deficiency states. A Recommended Daily Allowance of 30 IU was established more or less arbitrarily in 1968. It was reduced in 1974 to 15 IU for men and 12 for women, since redefined as 10 tocopherol equivalents (10 mg of D-α-tocopherol) for men and 8 tocopherol equivalents for women.

Taking the antioxidant property of vitamin E as paramount, it is clear that the daily requirement depends on the individual diet. Someone who consumes a diet high in polyunsaturated fat, more susceptible to peroxidation, would benefit more from a high vitamin E in-



Possible aging effects of energy metabolism emerge when energy consumption is plotted against lifespan. The diagram is adapted from one originally prepared by Richard G. Cutler of the Gerontology Research Center, National Institute on Aging.

FOODS HIGH IN VITAMIN E
(Amount needed to get 30 IU)

Wheat germ oil	1 tablespoon
Sunflower seeds	1½ ounces
Sunflower oil	3 tablespoons
Safflower oil	3½ tablespoons
Almonds	3 ounces
Peanut oil	8 tablespoons
Mayonnaise	11 tablespoons
Margarine (soft)	6 ounces
Wheat germ	6 ounces
Margarine (stick)	7 ounces
Peanuts (dry roasted)	10 ounces
Peanut butter	12 ounces
Soybean oil	13 tablespoons
Butter	2 pounds
Brown rice (boiled)	2¼ pounds
Asparagus	2½ pounds
Spinach	2½ pounds
Broiled liver	7 pounds
Baked shrimp	7½ pounds
Whole wheat bread	124 slices
Peas	8 pounds
Broccoli	9½ pounds
Eggs	8 dozen
Bacon	10 pounds

SOURCES OF VITAMIN E IN THE AMERICAN DIET
(USDA Nationwide Food Consumption Survey, 1987-88)

Margarine	13%
Mayonnaise	10%
Fortified breakfast cereal	6%
Shortening	5%
Salad dressing	3%
Peanut butter	3%
Eggs	2%
Soybean oil	2%
Potato chips	2%
Milk	2%
Tomato sauce	1%
Apples	1%
All other sources	51%

take. Dietary habits being what they are, such a person's sources of vitamin E are likely to be vegetable oils used in food processing.

At the high end of the scale, vitamin E causes few side effects in animals even at massive dosage levels, and those effects are minor and reversible. Mice have been given single doses roughly the equivalent of a human adult taking 3,500,000 IU without adverse effects. Doses up to the equivalent of 5000 IU per day for 10 to 60 days caused only a possible increase in coagulation time due to interference with vitamin K metabolism. Amounts equivalent to about 200,000 IU per day for two to three months resulted in increased liver weight, decreased hematocrit and hemoglobin, interstitial inflammation, and adenomatous pulmonary hyperplasia; these effects were not seen at doses up to an equivalent of 50,000 IU per day.

Unsubstantiated claims for the therapeutic effectiveness of vitamin E some years ago were accompanied by equally exaggerated notions of its side effects, notably dizziness, giddiness, intestinal cramps, and emotional disturbances. The supposed side effects disappeared along with many of the putative benefits when placebo-controlled double-blind studies were done.

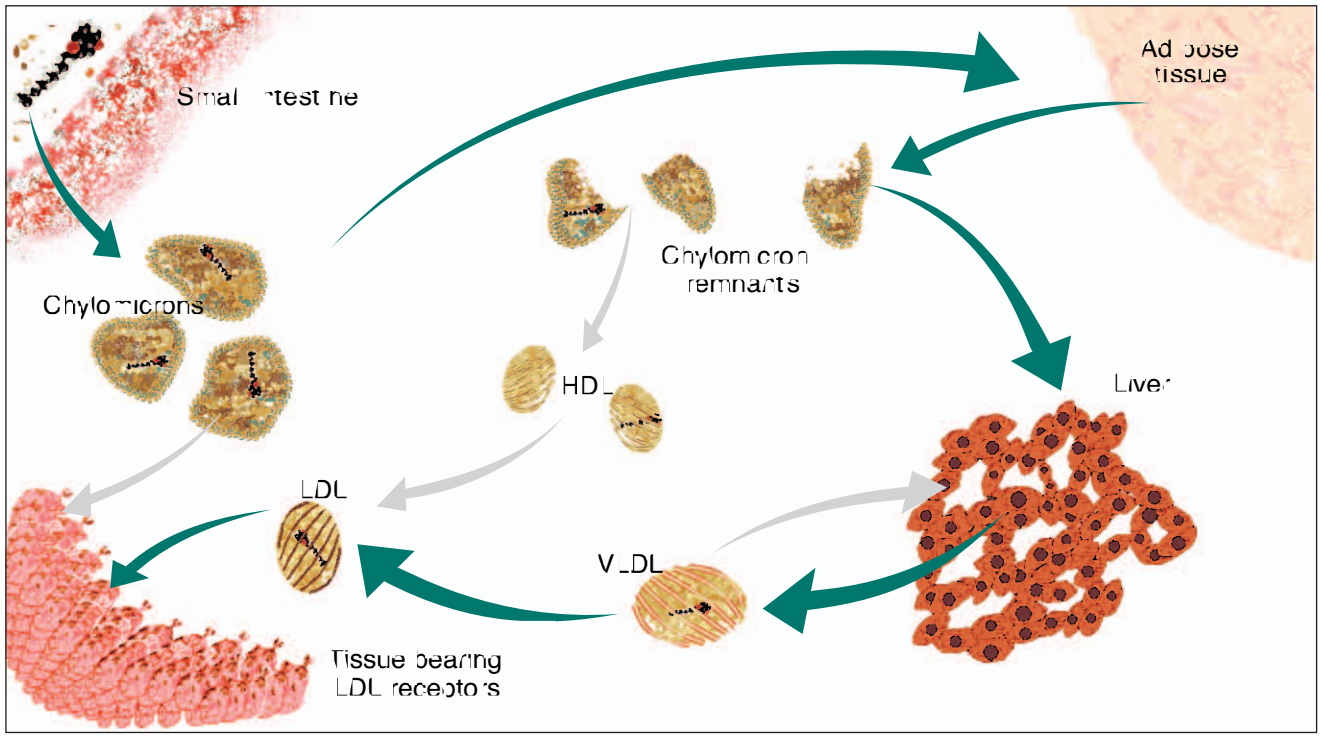
The World Health Organization considers daily doses of vitamin E up to 150 mg to be "absolutely safe" and doses between 150 and 720 mg to be a "range without side effects." From 720 mg to 3000 mg, transient gastrointestinal complaints begin to appear along with increased coagulation time, which is a problem in patients with vitamin K deficiency or who are taking anticoagulants. Studies of doses above 3000 mg per day have not been done in humans. Topical preparations containing synthetic α-tocopherol may cause skin irritation, while those containing α-tocopherol acetate do not.

The average American consumes 11 to 13 IU of α-tocopherol per day and much smaller amounts of the less common tocopherols and tocotrienols. Depending on the individual diet, the range is probably from 5 IU or less up to 30 IU or more. On the average intake, the average blood level is about 23 μmol per liter. A widely promoted multivitamin supplement provides 30 IU per day, which is almost certainly safe and which may be beneficial.

Biokinetics and Regulatory Functions Are Being Studied

The biokinetics and tissue absorption of vitamin E have not been well studied in humans. Dietary vitamin E is absorbed with fat and transported in chylomicrons to the liver and other tissues. There is an α-tocopherol–binding protein in the liver; there may be others for the other tocopherols and the tocotrienols. Tocopherols in plasma tend to be associated with phospholipid-rich lipoproteins, but tocotrienols are primarily found in triglyceride-rich lipoproteins. Therefore, different pathways must exist for the incorporation of these compounds into lipoproteins. Specificity is also expressed at the interfaces between plasma lipoproteins, probably including chylomicrons, and various tissues. Adipose tissue becomes enriched in tocotrienols, while in our research we have observed that most other tissues contain equivalent amounts of α-tocopherol and α-tocotrienol when equivalent amounts of both compounds are fed.

Antioxidants may exert regulatory effects on cellular signaling mechanisms, including gene expression. A nuclear transcription factor, NFκB, present in many cells that activates and down-regulates genetic systems including viral proliferation has been shown to be activated by reactive oxygen species. Tumor necrosis factor and phorbol esters activate NFκB in human T cells. Thiol antioxidants such as N-acetylcysteine and α-



MARTIN BOSO

lipoic acid are potent inhibitors of NFκB activation. Recent studies in our laboratory have demonstrated that α-tocopherol succinate and α-tocopherol acetate may inhibit NFκB activation by tumor necrosis factor and phorbol esters.

Influences on the cyclooxygenase and lipooxygenase pathways of arachidonic acid metabolism have also been suggested.

Classically, a vitamin is defined as a substance necessary in small amounts in the diet to assist normal metabolism and whose lack causes one or several clearly defined deficiency states. Vitamin E has not appeared to fit the classical definition because the conditions that it prevents take years to develop. This view has been supported by epidemiological and laboratory studies. Clinical trials now in progress will redefine vitamin E requirements in terms of a dosage necessary for optimal functioning. Continuing laboratory efforts are certain to enlarge understanding of the intricately related antioxidant mechanisms that function in living cells, and may at last establish with certainty the biologic functions of vitamin E.

Transport of α-tocopherol from intestines to tissues may involve three pathways: hepatic secretion of VLDL that is delipidated to LDL; transfer via HDL and LDL to tissues with LDL receptors; and direct release upon catabolism of chylomicrons by circulating lipoprotein lipase.

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Cosmetic dermatology of the aging face

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Abstract Aging is a complex process involving both genetically determined and environmental factors that result in functional and aesthetic changes in the skin, soft tissue, and skeletal support structures. These age-related changes are particularly apparent on the human face. In recent years, there has been increasing interest in reversing the effects of these age-related changes to restore a youthful appearance and improve patients' self-perception. Although many nonsurgical treatments for aesthetic correction of facial aging focus on skin restoration and removal of the effects of photoaging on the skin, other treatments, such as dermal fillers, address the soft-tissue volume loss that underlies many of the effects of aging. Advances in cosmetic dermatology, particularly in the area of soft-tissue augmentation, have expanded the options for older patients seeking to improve their facial appearance.
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Effects of aging on the human face

Age-related dermal changes

The aging process causes fundamental changes in the skin, soft tissue, and skeletal support structures of the human face. Dermal changes are due to intrinsic and extrinsic factors. Intrinsic factors refer to genetically determined hormonal and biochemical processes that cause irreversible degeneration of skin tissue, whereas extrinsic factors refer to environmental influences, particularly ultraviolet (UV) radiation, that damage the skin and compromise skin integrity.

Intrinsic aging of the skin leads to various histologic changes throughout the skin layers, including flattening of the epidermal–dermal interface, loss of dermal papillae, reduction in the number of melanocytes and Langerhans cells in the epidermis, dermal atrophy, reduction in the number of dermal fibroblasts, mast cells, and blood vessels, loss of elastic tissue in the fine subepidermal elaunin network, and an unusual thickening and fragmentation of elastic tissue in the reticular dermis.[1,2] Intrinsic aging also leads to functional changes in the human skin, including reduced type I and type III collagen production, a lower epidermal turnover rate, and reduced melanocyte activity.[1]

Extrinsic aging, caused primarily by UV radiation, results in histologic changes that are distinct from those caused by intrinsic aging. Whereas intrinsically aged skin is characterized by loss of elastic tissue and a reduction in cellularity, photodamaged skin is characterized by elastosis, the overgrowth of abnormal elastic fibers, and increased populations of mast cells, histiocytes, and fibroblasts.[1] Photodamaged skin tissue is generally inflamed, with dilated, tortuous blood vessels and a thickened basement membrane. Although histologic changes in intrinsically and extrinsically aged skin

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differ, many of the functional changes are similar in both types of skin aging. Collagen content and melanocyte activity are reduced and wound healing is impaired in both photoaged and intrinsically aged skin; however, these changes may be more pronounced in sun-exposed skin.[1,2]

Age-related structural changes

Beginning as early as the third decade of life, the soft-tissue structures of the face gradually weaken. In the upper third of the face, the brow begins to droop as a result of reduced skin elasticity, the force of gravity, and repeated contraction of muscles in the periorbital area. Skin of the upper eyelids loses its elasticity, resulting in excess lid folds that contribute to an aged and tired appearance. Fine and deep horizontal and vertical rhytides develop on the forehead and glabella as a result of repeated contraction of the frontalis, procerus, and corrugator supercilii muscles.[3]

In the midface region, the soft-tissue prominences near the cheeks recede, exposing the bony orbital rims.[4] The loss of soft tissue over the orbital rims appears as excess fat in the lower lid. The area between the lower lid and the cheek develops a hollow, with a deepening of the nasojugal fold and development of a palpebronasal groove.

In the lower third of the face, the aging process leads to thinning of the subcutaneous fat. The malar fat pad descends because of weakening of the malar and orbital ligaments and overlaps with the more firmly attached ligaments of the cheek–lip groove, creating a prominent nasal fold.[5]

In the perioral region, vertical rhytides develop above the vermilion border as the skin in this area thins.[5,6] Lips become elongated and lose their fullness.[7] Excess skin and soft tissue accumulate near the jaws and chin as a result of volume loss and laxity of ligaments and skin in the malar and perioral areas, obscuring the well-defined jawline of youth and giving rise to jowls.[8]

Loss of soft-tissue volume is a hallmark of the aging face. Volume loss occurs in the periorbital, forehead, malar, temporal, mandibular, mental, glabellar, and perioral sites. At the same time, redistribution of subcutaneous fat occurs, whereby other areas of the face, such as the submental area, lateral nasolabial fold and labiomental crease, jowls, infra-orbital fat pouches, and malar fat pad, are characterized by fat hypertrophy.[8,9] This loss of fat in certain facial areas and accumulation in others results in an unbalanced appearance that has lost the defining arcs and lines of the youthful face.

Aesthetic treatment of the aging face

Facial aging is associated with changes in skin quality and appearance (eg, thinning, reduced elasticity, and wrinkling due to extrinsic and intrinsic factors) as well as structural changes (eg, loss of soft-tissue volume, redistribution of subcutaneous fat) that change the contour of the face. Accordingly, treatments designed to reverse the effects of aging on the face may address dermal or structural changes.

Correcting age-related changes in skin quality and appearance

Skin protection and skin care

Photoprotection through the use of protective sunscreens and reduced exposure to sunlight can improve and even reverse the gross alterations in skin appearance and quality due to cumulative sun exposure.[10,11] Daily sunscreen applications can reduce skin roughness and the appearance of fine wrinkles, correct dyspigmentation, and improve the overall appearance of the facial skin.[1] Broad-spectrum sunscreens that offer effective protection against both UV-B and UV-A radiation are widely available. A skin protection factor rating scale is currently used to indicate the level of UV-B protection. A rating scale for UV-A protection is being developed.

Proper daily skin care is an essential step in improving the appearance and quality of aging skin. The skin barrier tends to become compromised with age, photodamage, and long-term exposure to irritants. The result is transepidermal water loss and a reduced capacity of the skin to serve as a mechanical barrier to toxins and infectious agents.[1] Gentle daily skin care can not only improve the appearance of the skin but can also heal, restore, and maintain the integrity of the skin barrier.

Retinoid therapy is the mainstay of topical treatment for photoaging. All-trans-retinoic acid and the second-generation retinoid tazarotene are indicated for the improvement of fine wrinkling, mottled hyperpigmentation and hypopigmentation, and facial lentigines.[1,12,13] The effects of retinoids may be partly due to their ability to upregulate collagen synthesis, which results in greater skin strength and resiliency.[11] Retinoids may also help increase water retention in the epidermis.[10]

Topical antioxidants help reduce UV-induced oxygen free radicals, reducing skin damage from UV radiation.[1] Anti-oxidants used in the treatment of photoaging include vitamin C (L-ascorbic acid), ferulic acid, α-lipoic acid, coenzyme Q10, idebenone, kinetin, coffee berry, and green tea.[10]

Long-term topical application of α-hydroxy acids (eg, ascorbic acid, glycolic acid, lactic acid, citric acid, and malic acid) has been reported to improve wrinkling, roughness, and skin discoloration in photodamaged skin. These agents may also increase epidermal thickness, induce collagen production in the dermis, improve perfusion of the dermis, and increase moisture retention in the epidermis, thereby improving skin elasticity and appearance.[10] The efficacy of these agents increases with higher concentrations and greater acidity (lower pH) of the agents. Over-the-counter products contain only moderate concentrations of α-hydroxy acids with pH values exceeding 3.5, thus limiting their activity and efficacy.[1] Higher-concentration products are available for use by cosmetic dermatologists.

Chemical peels

Chemical peels that use α-hydroxy acids, salicylic acid, trichloroacetic acid, or phenol can improve the appearance of aged skin. The chemicals, which are applied directly to the skin, damage layers of the epidermis and dermis and cause the treated skin layers to blister and slough, enabling new, younger skin to regenerate. Chemical peels are classified as superficial, medium, or deep, corresponding to the depth of dermal penetration and damage.[10,11]

- Superficial chemical peels (eg, glycolic acid) penetrate the epidermis and can improve fine lines and wrinkling, as well as roughened skin texture, mottling, lentigines, and actinic keratoses.
- Medium-depth peels (eg, trichloroacetic acid) penetrate to the level of the papillary dermis and may be appropriate for deeper wrinkles and severe photodamage.
- Deep chemical peels (eg, phenol) penetrate to the reticular dermis and can be used to remove deep wrinkles and precancerous lesions; however, these deep peels are associated with significant organ toxicity and must be used with caution.[10]

The recovery time generally is correlated with the depth of the peel as well as individual patient characteristics. Chemical peels are not the best choice if the patient has deep folds and creases or visible volume loss.

Topical treatments such as imiquimod (Aldara Cream, 5%; Graceway Pharmaceuticals, LLC, Bristol, Tennessee) and 5-fluorouracil cream (Efudex Cream, 5%; Valeant Pharmaceuticals North America, Costa Mesa, California) can be applied daily or weekly as part of a skin care regimen. A pulse peel, consisting of a light Jessner peel, followed by 5-fluorouracil solution, can also improve overall skin appearance. The 5-fluorouracil solution remains on the skin for 6 to 8 hours and is then washed off. Pulse peels are recommended weekly for eight treatments. Pulse peels are a more conservative and more cosmetically elegant approach toward skin rejuvenation; they require less downtime than do daily topical treatment with Aldara and 5-fluorouracil, and have fewer side effects such as redness and swelling.

Nonablative therapies

Nonablative skin rejuvenation—a noninvasive alternative to traditional laser resurfacing or surgery—provides a variety of cosmetic benefits with a minimum of downtime. Nonablative therapies can improve skin texture, discoloration, and scarring by using devices that deliver light or thermal energy, or both, from various sources, including visible and infrared lasers, intense pulsed light, light-emitting diodes (LEDs), and radiofrequency (RF) energy sources.[14,15]

Visible light lasers, such as the pulsed dye laser (585 nm), the long-pulsed dye laser (595 nm), and the pulsed potassium titanyl phosphate laser (532 nm) are vascular lasers that target dyspigmentation and vascular lesions; they have essentially no effect on dermal remodeling.[14] Nonablative lasers with a longer wavelength, such as the neodymium: yttrium-aluminum-garnet laser (1064 and 1320 nm), and diode laser (1450 nm), that emit light in the infrared portion of the electromagnetic spectrum (ie, 1000 to 1500 nm) induce thermal injury selectively to the papillary and upper reticular dermis while leaving the epidermis undamaged.[14,16] Thermal injury of the dermis induces a wound-healing response involving fibroblast activation, regeneration of subsurface collagen, and neocollagenesis, thereby improving skin texture and appearance.[17] Infrared laser treatment is helpful in removing mild to moderate rhytides and is associated with little or no morbidity. Although most patients experience minimal or no downtime after treatments, the cosmetic improvements tend to be modest.[16,17]

A number of nonlaser, nonablative rejuvenation technologies are currently available that also stimulate collagen production and tighten the skin. Similar to lasers, the RF devices generate heat, whereas the low-intensity LED photomodulation systems do not generate heat, but both can stimulate mitochondrial activity, increase the production of collagen and fibroblasts, and decrease the production of collagenase.[17] Several of the nonlaser devices most commonly used to treat fine wrinkles and improve skin texture are ThermaCool (Thermage Inc, Hayward, California), Titan (Cutera Inc, Brisbane, California), and ReFirme ST (Syneron Inc, Irvine, California).

The Thermage ThermaCool system uses monopolar RF energy to selectively heat collagen-based structures in the deep dermis while sparing the epidermis. Dermal remodeling occurs gradually over 6 months.[18] Only one treatment is recommended; a second treatment can be performed 6 months after the first treatment. Patient discomfort is a concern with Thermage ThermaCool, although with oral sedatives about 30 minutes before and narcotic analgesics can improve patient comfort.[18] The nonlaser Titan device uses controlled infrared light (1100 to 1800 nm) to selectively heat the dermis. The infrared light is filtered around the peak of water absorption to produce gradual, even heating in the dermis without thermal damage to the epidermis.[19] Absorption by melanin and hemoglobin is minimal at these wavelengths; thus, there is little risk of pigmentary changes.[19]

Nonlaser device improvements for facial rejuvenation and skin tightening include the use of bipolar RF and newer dual-technology devices that combine bipolar RF and various types of optical energy, such as the eLight systems based on electroptical synergy (elos) technology (Syneron Medical Ltd, Yokneam, Israel). The use of bipolar RF—rather than monopolar RF—provides an additional measure of patient comfort. The ReFirme ST procedure combines nonlaser pulsed infrared light (700 to 2000 nm) simultaneously with bipolar RF current to create controlled thermal energy in the dermis with minimal epidermal absorption.[15,20] Both Titan and ReFirme ST are used for the

lower one-third of the face (primarily jowls and submentum) and neck. Patients experience a mild sensation of heat during procedures with Titan and ReFirme ST, but do not usually require sedation or topical anesthesia. Both procedures require multiple treatments; treatments are recommended once a month for up to four or five treatments. Results can be seen after each treatment. Another nonlaser combination device for facial rejuvenation is the elos SRA Applicator (Syneron Medical Ltd), which uses a combination of focused light and bipolar RF for treatment of dyschromia and improvement of skin texture.

LED photomodulation and intense pulsed-light photodynamic therapy with blue light illumination are also effective nonthermal, nonablative skin treatments. Intense pulsed-light devices emit a spectrum of visible light (515 to 1200 nm) controlled by various cutoff filters.[14,15] Intense pulsed light is used primarily to target pigment and vascular abnormalities such as diffuse erythema, discrete vessels, mottling, and hyperpigmentation. Intense pulsed light has only modest effects on fine wrinkles.

A proprietary, two-step photodynamic therapy process is available that uses a topical application of aminolevulinic acid, a photosensitizing agent, followed by blue light illumination (Levulan Photodynamic Therapy system; DUSA Pharmaceuticals Inc, Wilmington, Massachusetts). Aminolevulinic acid/photodynamic therapy can improve the appearance of moderate to severe sun-damaged skin and reduce the number of visible and underlying precancerous lesions, such as actinic keratoses.

Low-intensity LED photomodulation (eg, GentleWaves LED Photomodulation device, Light BioScience, Virginia Beach, Virginia) also uses a nonthermal mechanism to reverse signs of photoaging.[21,22] LED photomodulation has been theorized to have anti-inflammatory and cell regulatory components that may improve the outcome of thermal-based rejuvenation treatments used with LED.[21]

Another nonlaser, nonablative procedure combines ultra–high-frequency electrical energy with nitrogen gas, which is converted to highly energized charged plasma (ie, ionized nitrogen gas). The non–chromophore-dependent, nitrogen-based plasma (Portrait Plasma Skin Regeneration, Rhytec Inc, Waltham, Massachusetts) energy is absorbed by all skin layers to induce remodeling of the skin's architecture, generating new collagen, and improving wrinkles, tone, texture, and discoloration of the skin.

Ablative therapies

Ablative laser resurfacing is the gold standard against which the nonablative techniques are measured. The two principal lasers used for ablative resurfacing are the carbon dioxide (10,600 nm) and erbium:yttrium-aluminum-garnet (Er:YAG, 2930 to 2940 nm). These lasers have an affinity for water, which allows for ablation of water-containing tissue (ie, epidermal tissue) without excessive penetration of the dermis.[16,23] Epidermal ablation occurs after the first pass of the carbon dioxide laser, and collagen remodeling of the dermis occurs after additional passes. The Er:YAG laser has an even higher affinity for water and is more efficiently absorbed by the epidermis, allowing for fine-tissue ablation and less collateral thermal damage.[16] The morbidity and recovery time after ablative laser resurfacing with the carbon dioxide laser, in particular, and, to a lesser extent, the newer Er:YAG devices are greater than what is seen after nonablative procedures; also greater is the risk of complications, such as infection, postoperative edema, erythema, and dyspigmentation.[10,16,17] Ablative laser therapy is effective, however, for long-lasting improvements in moderate to severe signs of aging and photodamage, including facial rhytides and scars.[16]

Fractional photothermolysis technology is now a popular treatment option. Fractional photothermolysis allows for corrective ablative laser treatment with less downtime and greater patient comfort; however, multiple treatments are necessary. Whereas full ablation can be achieved in one treatment, fractional photothermolysis requires up to four treatments a month apart. Fractional photothermolysis systems are now available that use the carbon dioxide laser (eg, SmartXide DOT; Eclipsemed Ltd, Dallas, Texas), the 1540-nm laser (eg, Palomar Lux 1540 Fractional Laser; Palomar Medical Technologies Inc, Burlington, Massachusetts), and the 1550-nm laser (eg, Fraxel SR; Reliant Technologies Inc, Mountain View, California). Topical anesthesia is usually required.

Addressing structural changes in the aging face

Although surgical techniques such as blepharoplasty, brow-lifts, and face-lifts (cervicofacial rhytidectomy) are commonly used to reverse the structural effects of aging on the face, surgery is associated with complications and significant recovery time. These surgical techniques, moreover, focus only on the problems of ptosis and skin laxity, without addressing age-related soft-tissue volume loss in the face. Nonsurgical techniques used to correct the structural effects of aging include botulinum toxin injections, autologous fat transfer, and dermal fillers to restore soft-tissue volume loss and improve facial contour. These techniques may be used alone or with other techniques, such as chemical peels or lasers, to rejuvenate facial appearance.

Botulinum toxin type A injection

Local injection of botulinum toxin causes temporary muscle denervation (described by some as paralysis), resulting in relaxation of hyperfunctional facial muscles and smoothing of the skin overlying these muscles. Botulinum toxin type A, in a formulation intended for cosmetic application (Botox Cosmetic; Allergan, Inc, Irvine, California), is approved by the United States Food and Drug Administration (FDA) for moderate to severe glabellar lines

associated with corrugator or procerus muscle activity, or both, in individuals aged 65 years or younger.[24] In addition to its approved indication, this agent is used for improving other facial lines and wrinkles, including periorbital wrinkles (crow's-feet and the brow),[25,26] horizontal forehead lines,[26] nasolabial folds,[27] perioral rhytides,[27] and melomental folds (marionette lines).[27] Poor injection technique, however, may result in suboptimal outcomes, particularly when botulinum toxin is used for off-label indications. For example, botulinum toxin-induced denervation of the frontalis muscle may exacerbate brow ptosis, which may already be severe in the aging face.[26]

Botulinum toxin injections are particularly useful for improving dynamic lines and wrinkles in the upper third of the face, whereas other techniques are more appropriate for addressing static wrinkles. Our preference is to reconstitute botulinum toxin type A with bacteriostatic water that is preservative-free. We currently use a 2-mL dilution with bacteriostatic water; 1- and 4-mL dilutions are also used.

Nonserious adverse events associated with botulinum toxin injections include lack of cosmetic effect, injection-site reactions, ptosis, muscle weakness, and headache. Serious adverse events are rare with cosmetic use of botulinum toxin.[28]

Dermal fillers

Aging of the human face is characterized by soft-tissue volume loss in the periorbital, forehead, malar, temporal, mandibular, mental, glabellar, and perioral sites as well as a

Table 1 Injectable dermal fillers used in aesthetic rejuvenation of the aging face

Category/product	Description of effect	Use	Duration
Bovine collagen			
Zyderm 1	3.5% purified bovine dermal collagen + 0.3% lidocaine	Superficial wrinkles and lines	3-4 mon
Zyderm 2	6.5% purified bovine dermal collagen + 0.3% lidocaine	Moderately deep defects, lip augmentation	3-6 mon
Zyplast	3.5% purified bovine dermal collagen cross-linked with glutaraldehyde + 0.3% lidocaine	Deep defects, lip augmentation	3-6 mon
ArteFill	20% volume percent PMMA microspheres + 80% volume percent bovine collagen (3.5%) + 0.3% lidocaine	Deep defects, lip augmentation	Permanent
Human collagen			
CosmoDerm 1	3.5% human fibroblast culture–derived collagen + 0.3% lidocaine	Superficial wrinkles and fine lines, lip augmentation	3-5 mon
CosmoDerm 2	6.5% human fibroblast culture–derived collagen + 0.3% lidocaine	Superficial wrinkles and fine lines, lip augmentation	3-5 mon
CosmoPlast	3.5% human fibroblast culture–derived collagen cross-linked with glutaraldehyde + 0.3% lidocaine	Deep lines and folds, scars, lip augmentation	3-5 mon
Porcine collagen			
Evolence	3.5% ribose cross-linked porcine collagen without lidocaine	Moderate to deep facial wrinkles and folds	9-12 mon
Fat			
Autologous fat	…	Deep defects	Variable, up to 7 y
HA			
Restylane	Cross-linked bacterial HA (20 mg/mL)	Superficial and moderate defects, lip augmentation	6-12 mon
Perlane	Cross-linked bacterial HA (20 mg/mL)	Moderate to severe facial folds and wrinkles	6-12 mon
Juvéderm	Cross-linked bacterial HA (24 mg/mL)	Moderate to severe facial wrinkles and folds	Up to 12 mon
Elevess	Cross-linked bacterial HA (28 mg/mL) + 0.3% lidocaine	Moderate to severe facial wrinkles and folds	6 mon
Hylaform	Cross-linked avian HA (5 mg/mL)	Superficial and moderate defects, lip augmentation	3-6 mon
CaHA			
Radiesse	CaHA microspheres in aqueous gel	Moderate to severe facial wrinkles and folds	2-5 y
PLLA			
Sculptra	Injectable PLLA microspheres suspended in sodium carboxymethylcellulose gel	Deep lines, creases, and folds, and for volumization in lower two thirds of face	18-24 mon

CaHA, calcium hydroxylapatite; HA, hyaluronic acid; PLLA, poly-L-lactic acid; PMMA, polymethylmethacrylate.

concomitant redistribution of subcutaneous fat in the submental areas, lateral nasolabial fold and labiomental crease, jowls, infraorbital fat pouches, and malar fat pad. Dermal fillers serve to restore soft-tissue volume in these areas and can also be used to address the problem of static wrinkles, furrows, and folds. Dermal fillers can be injected in the forehead, glabella, nasal tip, eyelids, cheeks, nasolabial folds, melolabial folds, and lips. Although these agents are especially useful in the lower third of the face, the use of dermal fillers in the eyelid area can improve age-related volume loss in the lower eyelid tear trough and improve the superior sulcus deformity of the upper eyelid. In the perioral area, injection of dermal fillers can help reduce perioral lines, reduce nasolabial folds and marionette lines, and enhance lip volume and shaping.[29]

Many of the commonly used dermal fillers replace or replenish volumizing components of the skin and surrounding tissue and may be considered passive or replacement fillers (Table 1). These include bovine and human collagen, autologous fat, and hyaluronic acid (HA). The newer-generation dermal fillers are biostimulatory and actually stimulate the production of endogenous collagen and dermal fibroblasts. Biostimulatory dermal fillers include injectable poly-L-lactic acid (PLLA) and calcium hydroxylapatite (CaHA). These fillers are discussed in detail elsewhere in this supplement.

Dermal fillers may also be classified by the duration of their effects. The effects of bovine collagen, for example, are short-lasting, on the order of a few months, whereas the effects of ArteFill (Artes Medical Inc, San Diego, California), a product composed of polymethylmethacrylate (PMMA) microspheres suspended in a 3.5% bovine collagen solution containing lidocaine, are permanent. Other volume-restoring dermal fillers have long-lasting but nonpermanent effects that are maintained for 18 to 24 months.

Collagen

Aging causes a reduction in collagen content of the skin, with the overall collagen content per unit area of skin surface decreasing by 1% per year.[30] Injection of collagen fillers into the dermis can replace collagen that is lost because of the aging process. Currently available collagen dermal fillers are derived from bovine or human sources (ie, tissue from deceased donors or human fibroblast cell culture). Collagen fillers are very short-acting, usually a maximum of 3 months; therefore, they can be used as a starting filler, especially for first-time filler patients. Collagen is also recommended for correction of superficial rhytides, particularly periorbital and perioral lines, and can occasionally be recommended for lip augmentation when a quick plump is needed for a special occasion.

Bovine collagen fillers

Bovine collagen has been used as a cosmetic filler for more than 30 years. The currently available bovine collagen fillers, Zyderm 1, Zyderm 2, and Zyplast (Allergan Inc), are purified fibrillar suspensions of bovine collagen in a saline solution containing lidocaine.[31] Depending on the formulation, these products can be used to correct deep nasolabial folds, restore lip fullness, and eliminate facial scars, lines, and wrinkles such as frown lines, smile lines, and crow's-feet.

After bovine collagen saline suspensions are injected, the saline is gradually lost and the filler forms a fiber network over which the patient's own connective tissue cells grow. After several weeks to months, a foreign-body reaction occurs, and collagenases and inflammatory cells clear the injected material.[31] Thus, the treatment effects last for only 2 to 6 months, and patients may require frequent retreatment to maintain the aesthetic benefits.[31] With Zyplast, the bovine collagen is cross-linked with glutaraldehyde to inhibit degradation by collagenases, thereby increasing the duration of effects.[31] Approximately 3% to 5% of patients experience a hypersensitivity reaction to bovine collagen, so a skin challenge test is required before treatment.[29,32] Patients who have a positive reaction on testing should not be treated with these products. Some patients who do not experience hypersensitivity on initial injections may present with such reactions after subsequent treatments, underscoring the importance of repeat testing and monitoring.[29,32]

Human collagen fillers

The commonly used human collagen fillers, CosmoDerm 1, CosmoDerm 2, and CosmoPlast (Allergan, Inc), contain purified collagen derived from human fibroblast cell-culture lines dispersed in a saline solution with lidocaine. These products are indicated for the restoration of the lip border and correction of facial wrinkles, acne scars, and soft-tissue contour deformities.[29] CosmoPlast, analogous to the bovine collagen Zyplast, is cross-linked with glutaraldehyde to inhibit degradation and increase duration of effect and is indicated for deeper folds and wrinkles.[30] The mechanism of human collagen fillers is the same as that of bovine collagen; however, the duration of action may be reduced compared with that of bovine collagens.[29] Unlike bovine collagen, the human-derived products are not associated with hypersensitivity reactions, so no allergy testing is required before treatment.[30]

Porcine collagen filler

Injectable porcine-derived collagen gel (Evolence Collagen Filler; ColBar LifeScience Ltd, Rehovot, Israel, and OrthoNeutrogena, Los Angeles, California) is now approved in the United States for treatment of moderate to deep facial wrinkles and folds. Skin testing is not required, but the product is contraindicated in patients with a history of anaphylactic or recurrent allergic reactions. Unlike other collagens, Evolence does not contain lidocaine, so topical anesthesia may be needed. Cosmetic effects reportedly last longer than those obtained with human or bovine collagens, up to 1 year in a recently published study.[33]

Polymethylmethacrylate

ArteFill, an injectable formulation consisting of 20% PMMA microspheres and 80% bovine collagen (3.5% denatured bovine collagen plus 0.3% lidocaine), was developed recently as a permanent collagen-based filler.[34] The collagen in this formulation is chemically modified to reduce antigenicity and serves as a delivery system for the PMMA microspheres.[29,31] The collagen degrades over several weeks, but the PMMA microspheres remain in place and induce tissue fibroblasts to encapsulate them into the network. Although this product offers the benefit of permanent results—effects can last for 7 years—it leaves little room for injection errors. PMMA is useful for structural improvements, deep rhytides and fold correction, deep acne scar correction, and bulk volume correction in the lower face, jowls, and midcheeks. PMMA is an excellent option for individuals who have been treated with other dermal fillers such as HA, CaHA, and PLLA. Bovine-derived PMMA requires a skin test 1 month before injection, but the product manufacturer is currently working on a formulation that will eliminate the skin test.

Autologous tissue

Autologous fat was the first filler ever to be used for cosmetic correction of the face. In this procedure, the patient's fat cells from the abdomen, thigh, or buttocks are harvested, processed, and then injected subdermally into the face. Fat can be used to fill larger and deeper defects and is nonimmunogenic.[29] Adipocytes, however, are fragile and may be damaged during harvesting and preparation. The duration of effect is variable, but with newer techniques, the effects have been reported to last up to 7 years. Localized resorption of fat also may occur in certain treated areas, with unpredictable effects on facial appearance. The best results are seen when fat transfer is used to fill nondynamic facial areas, such as nasolabial folds.[31]

The use of autologous fat as a volume corrector is technically more involved than the use of a prepared volumizing filler. The fat is harvested through an appropriate harvesting cannula after the donor site receives tumescent anesthesia. The collected fat and tumescent fluid remain in the syringe and are allowed to separate into their respective layers, consisting of a supranatant layer of fat and infranatant layer of serosanguineous tumescent fluid. After complete separation, the fat is transferred to an appropriate syringe and injected using a retrograde technique or placement of small aliquots (0.25 to 0.5 mL) into the muscle or subcutaneous layer, depending on the desired result and anatomic site. Injections of autologous fat can be used to fill in the infraorbital, malar cheek, zygomatic arch, jowl, and chin areas. Autologous fat can be stored for future injections if a state-approved storage facility is available.

The use of autologous human fibroblasts as another treatment option is being investigated. In this procedure, skin is obtained from the patient, and the fibroblasts are cultured, harvested, and suspended for injection into the papillary dermis. Fibroblast injections are potentially useful for treatment of wrinkles, fine lines, and scarring.[35]

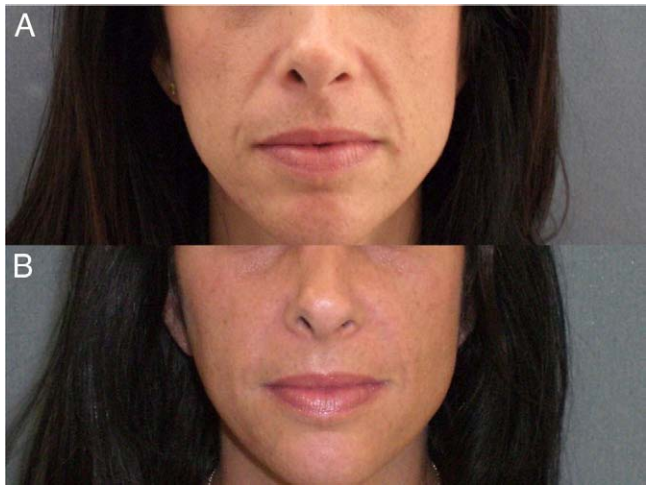

Fig. 1 Injectable hyaluronic acids (Restylane and Perlane) were injected in the bilateral nasolabial folds of a 37-year-old woman. (A) Photograph taken August 28, 2007, before treatment. (B) Photograph taken September 5, 2007, after treatment with 1 mL of Restylane (0.5 mL each injected into right and left nasolabial fold) and 1 mL of Perlane (0.5 mL each injected into the right and left nasolabial fold). No other filler was injected into the nasolabial folds during the interim. (Photographs courtesy of Neil S. Sadick, MD.)

Hyaluronic acid

HA is the fluid portion of the connective tissue matrix in which collagen and elastin fibers are embedded in the dermis. HA is highly hydrophilic and therefore helps maintain water in the extracellular space, which not only increases tissue hydration but also enhances dermal volume. Dermal HA content is reduced in older skin, which may explain the decrease in tissue hydration that is characteristic of aging skin. In its natural form, HA has a very short half-life and therefore must be chemically stabilized through cross-linking to serve as a dermal filler.[29] HA as a dermal filler does not affect the structure of the collagen network or correct the tissue HA deficiency characteristic of aging, but rather works by augmenting volume.[36] In this sense, it is a passive filler.

HA gels have the unique property of dynamic viscosity—the viscosity of the gel decreases under increasing shear force. Thus, the HA gel is more fluid when injected under the pressure of a syringe needle, but once the injection pressure is removed, the material becomes viscous and does not migrate from the site of implantation. HA gels also maintain their volume even as the individual gel particles degrade, which translates into a longer-lasting space-filling effect.[37]

HA gels are indicated for dermal implantation for the correction of moderate to severe facial wrinkles and folds, such as deep nasolabial and glabellar folds. HA is also

indicated for correction of most types of scar, correction of facial contours, restoration of the lip border, and lip enhancement.[29]

The HA used in dermal fillers is primarily derived from bacterial culture (eg, Restylane and Perlane, Medicis Aesthetics Inc, Scottsdale, Arizona; Juvéderm Ultra and Juvéderm Ultra Plus, Allergan, Inc, Santa Barbara, California; Elevess, Artes Medical Inc) or avian culture (eg, Hylaform, Biomatrix Inc, Ridgefield, New Jersey). Unlike collagen, the chemical structure of HA is similar across species, and it is therefore less immunogenic than, for example, bovine collagen; allergy testing is not required before treatment.[36] The duration of effect of HA products is 6 to 12 months, depending on the product and location of the defect.[37] HA products generally have more durable effects than do bovine collagen products.[32]

The currently available HA formulations (Figure 1) are highly viscous and, with the exception of Elevess, do not contain an anesthetic; they are thus likely to be associated with more discomfort on injection than, for example, human and bovine collagen injections. A local anesthetic is usually necessary. Bruising may also occur.[29] We currently use 0.2 mL of 1% lidocaine without epinephrine mixed with 1 mL of Restylane or Juvéderm to maintain patient comfort. Elevess injectable gel comes prefilled in a 1.0-mL syringe containing 0.3% lidocaine and has a higher concentration of HA (28 mg/mL) than does either Restylane or Perlane (20 mg/mL) or Juvéderm (24 mg/mL), which may offer more injection comfort and more durable cosmetic effects in some patients.

Calcium hydroxylapatite

CaHA is the major mineral component of bone. Radiesse (BioForm Medical, Inc, San Mateo, California) is a biostimulatory dermal filler consisting of CaHA microspheres suspended in an aqueous gel matrix that also contains glycerin and sodium hydroxycellulose.[29] The bone mineral microspheres do not cause hypersensitivity reactions, so allergy testing is not necessary.[29]

The gel matrix is absorbed at 6 to 8 weeks after injection; during that same time, the host's fibroblast response is stimulated, and a bony matrix is formed that serves as a scaffold for the new collagen. The bone mineral microspheres degrade into calcium and phosphate ions over time, and the augmentation effect gradually diminishes.[29] CaHA is considered a semipermanent filler, with clinical effects reported to last for 2 to 5 years.[29,37]

Injection into the subdermal or intramuscular tissue is recommended because of its high viscosity.[31] Radiesse is approved by the FDA for use in correcting moderate to severe facial folds, such as nasolabial folds, and wrinkles around the nose and mouth. It is also effective for glabellar folds and tear trough deformities. CaHA filler should not be used on the lip because of the increased incidence of submucosal nodule formation (20%).[31]

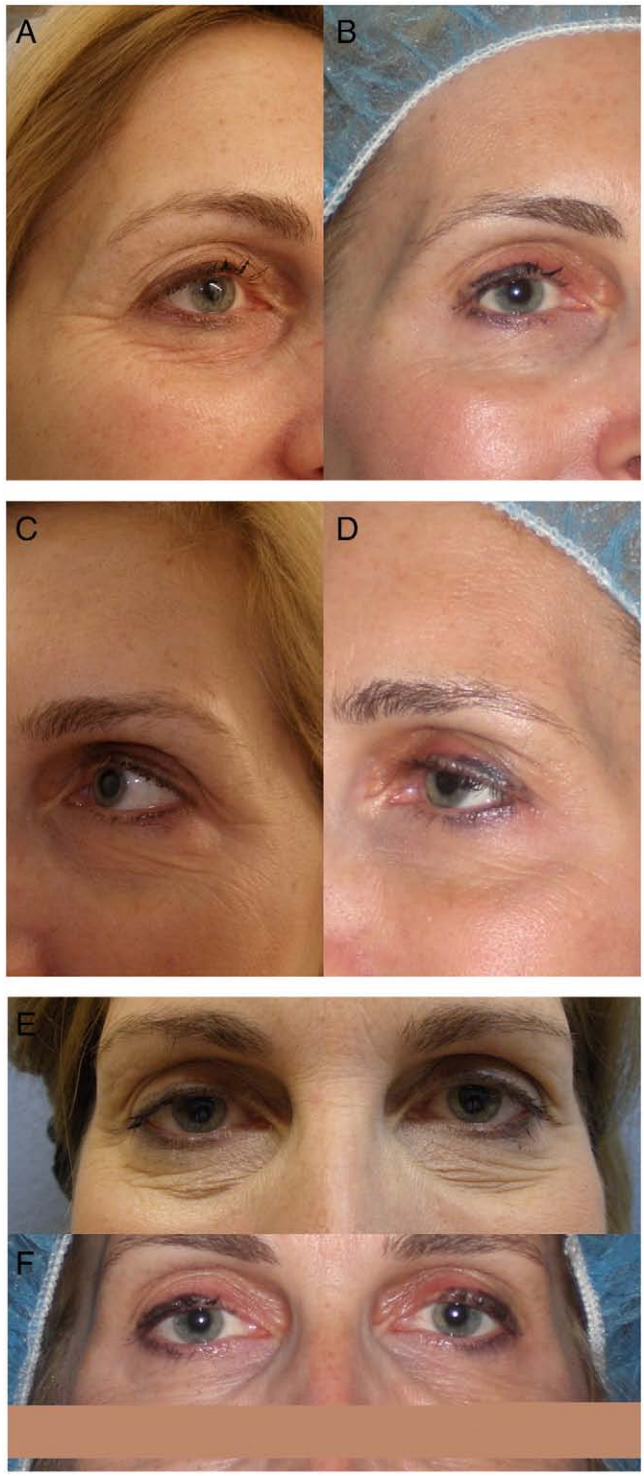

Fig. 2 Injectable poly-L-lactic acid was injected in the bilateral infraorbital regions of a 52-year-old woman. (A, C, E) Photographs were taken September 8, 2006, before treatment. (B, D, F) Photographs were taken June 23, 2008, after treatment with an 8-mL dilution (7 mL of bacteriostatic water and 1 mL of lidocaine 1%) injection of 4 mL each in the right and left infraorbital region. No other filler was injected into the infraorbital regions during the interim (Photographs courtesy of Neil S. Sadick, MD).

CaHA is also used to correct volume loss in the hands, especially when there are prominent vessels, and to improve the appearance of the aging hand. Injection in the hand with this product is technique-dependent. The recommended injection is 0.7 to 1.0 mL into the dorsum of each hand according to the amount of volume depletion. A bolus should be placed subcutaneously and then massaged thoroughly so that the product may be evenly distributed. We generally use 1.0 mL of 1% lidocaine added to 1.0 mL of CaHA.

Poly-L-lactic acid

Sculptra (Dermik Laboratories, Bridgewater, New Jersey) is an injectable implant that contains microparticles of PLLA, a biocompatible, biodegradable, synthetic polymer from the α-hydroxy acid family.[38] An injectable formulation of PLLA was first approved by the FDA in 2004 for the treatment of facial lipoatrophy associated with HIV infection.[39] Although not yet approved for the following uses, injectable PLLA is also widely used for the correction of deep lines, wrinkles, folds, and creases, such as crow's-feet, nasolabial folds, marionette lines, and oral rhytides, as well as for volume augmentation in the buccal and temple areas. It is often used for diffuse volume enhancement but is not appropriate for very superficial fine lines or for lip augmentation.[29]

Injectable PLLA works in two phases. The PLLA microspheres initially act as a transient space-filling volumizer and are gradually degraded and metabolized to carbon dioxide and water. The PLLA injection also stimulates fibroblastic activity, resulting in the formation of collagen and other connective fibers during a period of several months.[40] Soft-tissue volume is gradually corrected over time, with effects lasting for 18 to 24 months.[41]

We reconstitute injectable PLLA with 5 mL of bacteriostatic water and 1 mL of 1% lidocaine, with or without epinephrine. The 6-mL dilution is commonly used when we treat the middle and lower face. PLLA can be further diluted depending on the treatment site. For example, an 8-mL dilution can be used for the infraorbital area, and a 12- to 20-mL dilution can be used for the forehead, neck, décolleté, and hands, although the product is not yet approved for these uses.[38] Layered injections can also be used; that is, depot injections placed first in the deep dermis or just above the periosteum can be used to rebuild structure, followed by more superficial injections to provide additional volume correction, especially for deep rhytides and folds. Even distribution of product, followed by aggressive massage, will optimize results (Figure 2).

Conclusions

The aging process, combined with environmental factors such as UV radiation exposure, leads to profound aesthetic and functional changes in the skin, soft tissue, and support structures of the face. Aging also leads to changes in the quality and appearance of skin (eg, loss of elasticity and collagen) as well as structural changes due to soft-tissue volume loss, redistribution of subcutaneous fat, and skin laxity. Treatments that address age-related changes in the quality and appearance of the skin include retinoid therapy, antioxidants, chemical peels, and ablative and nonablative laser therapy. Structural changes may be addressed with botulinum toxin therapy or dermal fillers, including autologous fat. The use of dermal fillers is a good nonsurgical option to restore age-related volume loss. Newer-generation dermal fillers not only restore soft-tissue volume loss but also stimulate host fibroblast activity for a long-lasting response. Augmentation of soft-tissue volume in the aging face helps remove deep facial folds and wrinkles, particularly in the middle and lower third of the face, and partially restores the facial arcs and lines characteristic of the youthful face.

Drug names

5-fluorouracil cream/solution: Efudex Cream/Solution
Botulinum toxin type A: Botox Cosmetic
Calcium hydroxylapatite: Radiesse
Collagen (purified bovine dermal): Zyderm 1, Zyderm 2, Zyplast
Collagen (human fibroblast culture–derived): CosmoDerm 1, CosmoDerm 2, CosmoPlast
Collagen gel (porcine-derived): Evolence
Hyaluronic acid (cross-linked avian): Hylaform
Hyaluronic acid (cross-linked bacterial): Restylane, Perlane, Juvéderm Ultra, Juvéderm Ultra Plus, Elevess
Imiquimod: Aldara Cream
Poly-L-lactic acid: Sculptra
Polymethylmethacrylate: ArteFill

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REVIEW

How mitochondria record the effects of UV exposure and oxidative stress using human skin as a model tissue

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The accumulation of mitochondrial DNA (mtDNA) mutations has been proposed as an underlying cause of the ageing process and mutations have been associated with cancer in many tissues, including human skin. This involvement is linked to the key roles of mitochondrial function and mtDNA in oxidative stress production and as a mediator of apoptosis. We and others have pioneered the use of mtDNA damage as a highly sensitive biomarker of ultraviolet exposure in human skin and have also shown that the accumulation of an ageing-dependent mtDNA mutation is accelerated by exposure to sunlight, which is known to induce oxidative stress in skin. This is important as ultraviolet radiation (UVR)-induced gene mutations play a key role in the development of skin cancer and ageing in human skin. Novel applications of mtDNA as a biomarker of UVR-induced oxidative stress will also be highlighted in this review.

Mitochondria

Mitochondria are thought to have originated when an α-proteobacterium was engulfed by and established residency in the cytoplasm of a primitive eukaryotic cell. A symbiotic relationship existed, whereby the aerobic bacterium supplied energy to the host cell in exchange for a stable protected environment and a constant supply of nutrients. Through evolution, most of the genes present in the bacterium were gradually transferred to the nucleus of the host (1). Mitochondria now exist as sub-cellular organelles present within the cytoplasm of all nucleated eukaryotic cells.

Textbook descriptions usually portray mitochondria as oval discrete organelles of 1–2 μm in length and 0.5–1 μm in diameter; however, in reality mitochondria are not isolated static organelles but constantly change their shape, forming reticular networks, which are maintained by continual cycles of fission and fusion (2).

Mitochondria have several functions within the cell, including their key involvement in apoptotic events, such as the release of the caspase activator cytochrome *c*. However, their primary function is to carry out oxidative phosphorylation (OXPHOS), which generates ∼90% of cellular energy in the form of adenosine triphosphate (ATP) from products of cellular metabolism (3). OXPHOS is carried out by five enzyme complexes that are located on the inner mitochondrial membrane. Complexes I, II, III and IV and two non-enzyme electron carriers, coenzyme Q and cytochrome *c*, transfer electrons along the mitochondrial respiratory chain, which is coupled to the formation of a proton gradient across the inner mitochondrial membrane. Complex V, or ATP synthetase, uses the free energy released when the protons flow back across the membrane to produce ATP from ADP and inorganic phosphate.

Mitochondrial genetics

Despite loosing the majority of genes through evolution, mitochondria have retained some of their own genetic material, which remains within the mitochondrial matrix. This mitochondrial DNA (mtDNA; Figure 1) is the only extranuclear source of functional DNA in mammalian cells and is a 16 569 bp, double-stranded, circular DNA molecule that encodes 37 genes, 13 proteins of the mitochondrial respiratory chain plus the 22 tRNAs (mt-tRNAs) and 2 rRNAs (mt-rRNAs) required for their translation (4,5).

The remaining respiratory chain protein subunits plus all the proteins required for mtDNA replication, transcription, translation and mitochondrial maintenance are encoded for by the nuclear genome and are subsequently imported into mitochondria. Due to the dual genetic control of the respiratory chain, it is essential that the two genomes are coordinated, and thus crosstalk between the two must occur (6,7).

mtDNA is highly compact. It does not contain introns, with coding sequences being continuous or having very few bases between them; 95% of mtDNA is encoding in comparison to 3% of nuclear DNA, and so any mutagenesis to mtDNA is likely to affect a coding region. The only non-coding region is the ∼1-kb D-loop (displacement loop), which is formed by the displacement of the two genomic strands of mtDNA by a third strand of 500–700 bp that gives this region a triple-stranded structure. It is the least conserved region of mtDNA and functions as the major regulatory region for both replication and transcription of the genome (8).

In contrast to nuclear genes where there are two copies of each gene, a maternal allele and a paternal allele, mtDNA is present in multiple copies (polyploidy) of up to thousands of copies of mtDNA per cell (9). As a result, mtDNA has the capacity to form a mixture of both wild-type and mutant mtDNA genotypes within a cell, a phenomenon known as heteroplasmy (Figure 2). mtDNA mutations are functionally recessive, that is the remaining wild-type molecules compensate for any deleterious effects of mutant genomes (complementation), and cellular dysfunction only occurs when the ratio of mutated to wild-type mtDNA exceeds a threshold level (10). This level is often between 50 and 60% for deletions (11–13), but is generally higher for point mutations at 60–90% mutant mtDNA (14–16). A growing collection of reported

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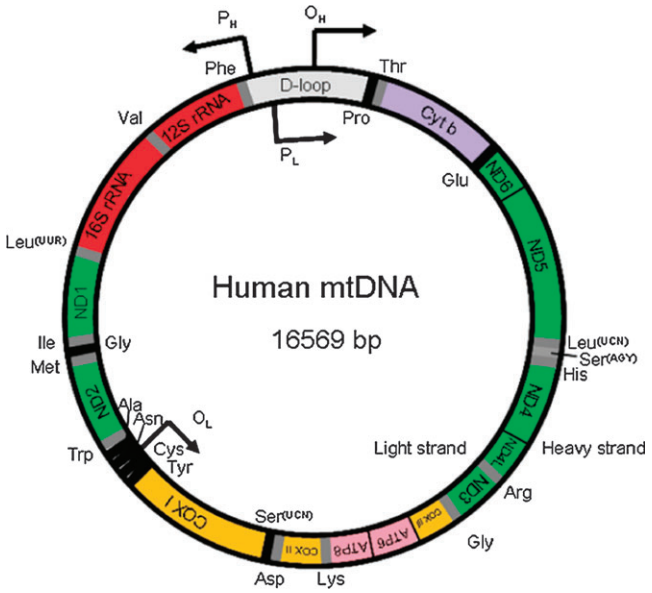

Fig. 1. Human mtDNA. mtDNA encodes for 13 subunits of the mitochondrial respiratory chain: seven subunits of Complex I (ND1-6 and ND4L, green), one subunit of Complex III (cyt *b*,purple), three subunits of Complex IV (COXI–III, yellow) and two subunits of Complex V (ATP6 and 8, pink). In addition mtDNA encodes for two rRNAs (12S and 16S, red), and 22 tRNAs required for the translation of these subunits. The tRNAs are abbreviated to indicate the corresponding amino acid and are indicated by black or grey stripes (black, encoded on light stand; grey, encoded on heavy strand). O_H, origin of heavy strand replication; O_L, origin of light strand replication; P_H, promoter of heavy strand transcription; P_L, promoter of light strand transcription.

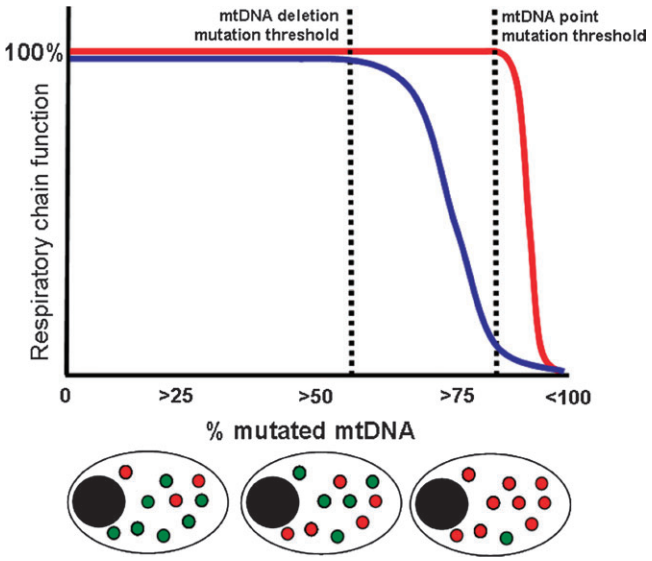

Fig. 2. mtDNA heteroplasmy. Individual cells can harbour a mixture of wild-type (green) and mutant mtDNA (red). If the ratio of mutant to wild-type genome exceeds a threshold, respiratory chain dysfunction occurs.

mtDNA point mutations and rearrangements has been associated with muscle and neurodegenerative diseases, a proportion of which exhibit skin manifestations (10). These are reviewed elsewhere [for example ref. (17)].

mtDNA is not distributed homogenously throughout the mitochondrial matrix but is organized into structures termed 'nucleoids', each containing around two to seven mtDNA molecules (18,19). Nucleoids may be considered as the units of mtDNA inheritance (20), which incidentally is inherited exclusively through the maternal linage (21).

mtDNA mutagenesis

Although the nucleoid formation may offer some protection in insulating mtDNA molecules from their environment (22), unlike nuclear DNA, mtDNA has a lack of association with protective histones (23). mtDNA also has a scarcity of the efficient DNA repair mechanisms that are present in the nucleus. The literature agrees that mitochondria are deficient in nuclear excision repair pathways and cannot repair ultraviolet (UV)-induced photoproducts such as pyrimidine dimers. However, mitochondria do show repair of a variety of other types of DNA damage confirming that mitochondria possess base excision repair pathways, although evidence for repair involving both recombination and mismatch repair is not fully resolved in human (10,24).

Approximately 90% of the oxygen consumed within a eukaryote is used in mitochondrial respiration. Incomplete oxygen reduction within the mitochondrial respiratory chain can lead to the formation of the superoxide radical, the first molecule in the pathway responsible for the production of reactive oxygen species (ROS) (24), as electrons 'leak' from the respiratory chain and react with oxygen. Mitochondrial ROS formation is mainly due to the natural leakage of electrons, which occurs at Complexes I and III of the respiratory chain (25). Recent evidence in human skin cells postulated an additional contribution by Complex II (26). Under normal physiological conditions, without any additional stressor on the respiratory chain, up to 5% of oxygen consumed by mitochondria is converted to ROS (27); however, this level predominantly comes from experiments done in isolated mitochondria with air-saturated aqueous buffers. Mitochondria *in vivo* are exposed to a considerably lower concentration of oxygen, and thus this conversion is likely to be lower (28).

ROS are known to damage proteins, membranes and DNA by oxidation and as mtDNA is in close proximity to the site of ROS generation, it is therefore highly susceptible to damage by these reactive species (29–32), especially superoxide ($O_2\cdot$), hydrogen peroxide (H_2O_2), hydroxy radicals ($OH\cdot$) and peroxynitrite ($ONOO\cdot$).

The consequence of a lack of histone protection coupled to limited repair and vulnerability to attack by reactive species is that mtDNA has a rate of mutation 10–17 times higher than that of nuclear DNA, and these mutations persist for a longer period of time in the cell (33–37). Individual mitochondrial genomes are constantly subject to undergo random mutation (38), and unless damaged mtDNA is repaired or eliminated, a mitochondrial genome harbouring a sequence variation may be replicated allowing its level in cells to increase by intracellular drift (39). In this way, mtDNA mutations can accumulate during ageing (40).

A vicious cycle hypothesis

The 'vicious cycle' mechanism, first proposed by Harman (41), summarizes how the characteristics of mitochondria can contribute to disease and ageing.

The hypothesis remains the most vigorous although a somewhat controversial contender to explain the basis of intrinsic ageing in a wide range of species by postulating that

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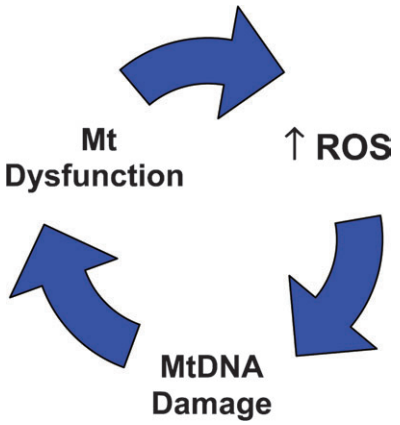

Fig. 3. The vicious cycle of ageing hypothesis. Accumulation of mitochondria DNA mutations as a consequence of ROS production results in cell disruption and ageing.

the production of intracellular ROS is the major determinant of life span.

Intracellular ROS are primarily generated by the mitochondrial respiratory chain and as such it is a prime target for oxidative damage. mtDNA is located in the mitochondrial matrix close to the site of ROS production (the respiratory chain) making it highly vulnerable to oxidative damage. As the integrity of mtDNA is essential for mitochondrial function (as it encodes respiratory chain subunits), the accumulation of mutations is considered a possible contributor to ageing and cell death (via apoptosis). Dysfunctional mitochondria will produce more ROS, and so a feed-forward loop is set up whereby ROS-mediated oxidative damage to mitochondria favours more ROS generation, resulting in a so-called 'vicious cycle' (Figure 3) (42). The postulated role of this in ageing is discussed later.

Effects of ultraviolet radiation on the skin

Human skin is continuously exposed to external stressors, such as ultraviolet radiation (UVR). As a general rule, the shorter the UV wavelength the greater is the biological effect (Figure 4). UVB therefore is considered to be more harmful to skin than UVA, which is highlighted in erythema (skin reddening), more commonly known as sunburn. In this respect, UVB is known to be a potent carcinogen and mutagen and has been suggested as the major component of sunlight causing DNA mutations leading to human skin cancer (43). However, not only is UVA able to penetrate deeper into the skin when compared to UVB (Figure 4), Agar *et al*. (44) demonstrated a greater frequency of UVA compared to UVB fingerprint mutations in human non-melanoma skin cancer (NMSC) samples thereby suggesting an important role for UVA in human skin carcinogenesis.

Studies suggest that skin cancer is the most commonly diagnosed human cancer throughout the world (45), accounting for 40% of all cancers diagnosed in the USA (46). There are broadly two categories of skin cancer, malignant melanoma and NMSC, with the latter representing 85–90% of the incidence of skin cancer (47). According to Cancer Research UK figures, 75 000 new cases of NMSC were diagnosed in the UK in 2005 and studies suggest a trebling of skin cancer incidence by 2025 (48).

Human skin ageing is the combined effect of intrinsic (time-dependent) and extrinsic (environmental) processes. UV irradiation from sunlight is the major extrinsic factor to impact upon skin ageing producing cellular changes in the skin and it is generally thought that UVA is the main determinant for these changes (49).

Oxidative stress

The exposure of skin to UVR is known to stimulate the intracellular production of ROS (e.g. superoxide and hydrogen peroxide) and reactive nitrogen species (RNS; e.g. nitric oxide). It is this imbalance in the production of ROS/RNS and the antioxidant defence systems that leads to oxidative stress. UVB can be directly absorbed by DNA bases, therefore, inducing damage within cells. The most common UVB-induced DNA modulations are dimeric photoproducts between adjacent pyrimidines on the same strand of DNA. However, UVB has also been shown to cause oxidation of guanine residues in a process mediated by ROS (50), as well as altering the levels of intracellular antioxidant enzymes (e.g. superoxide dismutase, glutathione peroxidase and catalase). The harmful effects of UVA are thought to be mainly mediated through the generation of ROS and RNS rather than direct DNA damage (51), which has shown a correlation with UVA-induced DNA damage (52). These latter studies have particularly focussed on the role of mitochondria in UV-induced oxidative stress where the vast majority of cellular ROS is produced as free electrons 'leak' from the respiratory chain (32,53).

mtDNA as a biomarker of sun exposure in human skin

A major limitation of studies relating genotype to phenotype of human skin cancer is the absence of reliable markers of exposure to UVR and this is compounded by inter-individual differences in the ability to repair photoproducts in nuclear DNA. To determine a reliable marker of cumulative UVR exposure in human skin, we and others have pioneered the novel idea of using mtDNA, rather than nuclear DNA, as a biomarker of UV-induced DNA damage (54,55) or commonly termed 'sunburnt DNA' in the public domain. Compared to mutation screening of candidate nuclear DNA genes such as p53, there are certain advantages of studying mtDNA damage in sun-exposed skin (10,55) particularly the absence of nucleotide excision repair of photoproducts, higher mutation rate and mtDNA complementation. Therefore, cells are able to accumulate photodamage in mtDNA without compromising cell function, a necessary requirement for a reliable and sensitive biosensor of UV exposure. There has been a spectrum of mtDNA deletions associated with UV exposure (56). Of the spectrum of mtDNA deletions identified in sun-exposed human skin, the major species have been the 4977 bp common deletion [reviewed in refs (55,57)] and a 3895 bp deletion (58,59). These mtDNA deletions can be also be induced in human skin and cultured skin cells by sub-lethal repetitive doses of UVR (58,60). Apart from deletions, a higher frequency of tandem mtDNA duplications has been observed in sun-exposed human skin (59). These deletions and tandem duplications occur more frequently in usually sun-exposed skin as opposed to occasionally sun-exposed skin (58,61). This is important because the relative density of NMSC is highest on body sites 'usually' exposed to the sun when outdoors (such as scalp, face, neck and ears) compared to occasionally sun-exposed sites (such as shoulders, back and chest). These data show that mtDNA damage in human skin provides

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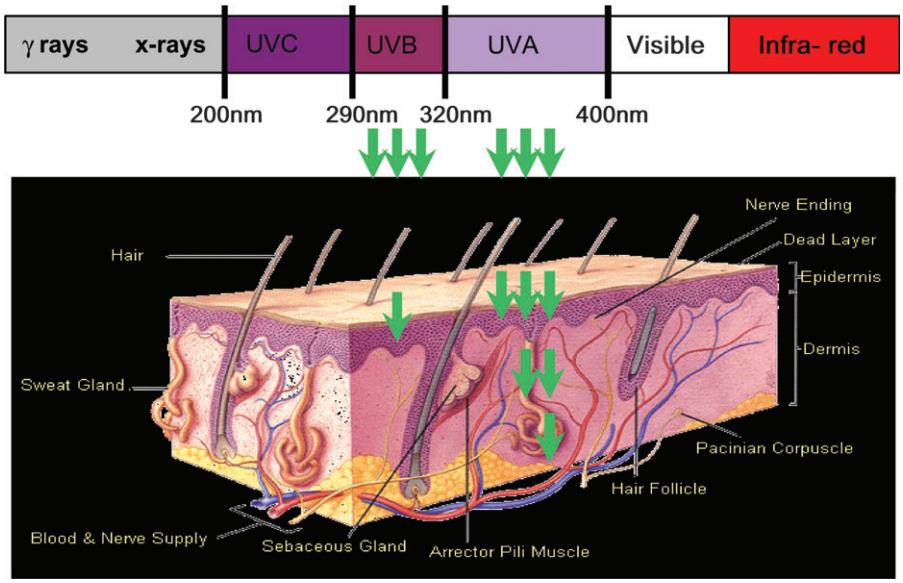

Fig. 4. Differential penetration of UVA and UVB into human skin.
Extensively modified from Gawkrodger, D. (ed.) (2002) *Dermatology—An Illustrated Colour Text*, Churchill Livingstone, ISBN-0-4443-07140-3.

a potential biomarker for cumulative UV exposure in human skin. In addition, it may provide a method of monitoring long-term safety of clinical UV phototherapy regimes and possibly an early warning system for development of skin cancer.

Mitochondria and ageing

The mitochondrial theory of ageing predicts that the vicious cycle contributes to the ageing process (Figure 3). Therefore, mtDNA damage and mitochondrial dysfunction not only serve as important downstream markers of mitochondrial ROS production but are also themselves involved in exacerbating the excessive ROS scenario (38,62–65). Although there are recent data supporting a direct functional role of mtDNA in ageing and photoageing (66), there is still considerable debate as to the type of mtDNA species associated with ageing. For example, the most frequently reported species is the 4977 bp common deletion but its significance is still under debate (67). In addition, there are single somatic mtDNA control region mutations associated with ageing tissues, including skin, but their functional significance is still unclear (68). This process of chronological ageing can of course be accelerated in skin by chronic exposure to UVR, which is associated with a further increase in mtDNA damage (as described above).

One interesting area of human ageing biology is the large age-dependent accumulation of mtDNA point mutations in the non-coding control region observed in skin, muscle and brain (69–71). The control region harbours genetic elements important for transcription and replication of mtDNA, giving potential functional relevance to these mutations and making them good candidates as contributors to ageing. In ageing skin, a T414G mutation has been shown to be one of the most common control region mutations (70) and we have recently shown in human skin that its accumulation is accelerated by UV exposure (72) although there is not a clear segregation with the process of senescence (6,73–75).

Mitochondria and cancer

Growing evidence suggests that cancer cells exhibit increased intrinsic ROS stress, due in part to oncogenic stimulation, increased metabolic activity and mitochondrial malfunction. Since the mitochondrial respiratory chain is a major source of ROS generation in the cells, the vulnerability of the mtDNA to ROS-mediated damage appears to be a mechanism to amplify ROS stress in cancer cells. Mitochondria have therefore been implicated in the carcinogenic process (76) not only because of their role in apoptosis and other aspects of tumour biology but also due to their role as a generator of ROS (77). Many types of human malignancy such as colorectal, liver, breast, pancreatic, lung, prostate and bladder as well as skin cancer have been shown to harbour somatic mtDNA mutations (57,77–79). Moreover, sequence variations of mtDNA have been observed in pre-neoplastic lesions, which suggest mutations occur early in tumour progression (80). Durham *et al*. (81) provided the first detailed study of multiple forms of mtDNA damage (including deletions, tandem duplications and point mutations) in NMSC. Somatic heteroplasmic point mutations were identified in addition to clear differences in the distribution of deletions in the tumours compared to perilesional skin. There are three recent studies that have identified somatic mtDNA mutations in cutaneous malignant melanoma (82–84). It is currently unknown whether the observed mtDNA damage has a primary and causative link to the process of cancer development or it may simply represent a secondary 'bystander effect', which reflects an underlying nuclear DNA instability. The interplay between nuclear and mitochondrial genes requires careful investigation and may hold the final understanding of the mitochondrial role in tumorigenesis.

Antioxidants

There is now considerable interest in antioxidants as they may provide one way (in addition to the intrinsic antioxidant defence mechanism) of reducing oxidative damage caused as a result of increased ROS levels (85–92). There is also some evidence that dietary antioxidants directly protect mtDNA damage and function (93,94). The Rizwan *in vivo* study showed that lycopene (the bright red pigment found in a number of red fruit and vegetables including tomatoes) administered as tomato paste reduced the UVR-induced

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damage to mtDNA in human skin. Compared to the control group, the group who had eaten the tomato paste were also found to have 33% more protection against sunburn, which can lead to skin cancer. Furthermore, the tomato paste supplementation was associated with a reduction in the UV induction of matrix metalloproteinase-1 (MMP-1) expression, abolished the UV-induced reduction in fibrillin-1 expression and increased the procollagen I deposition in the treated subjects. This pilot study indicates that tomato paste containing lycopene provides protection against acute and potentially long-term aspects of photodamage and suggests that acute UVR-induced mtDNA damage is a promising tool for assessing evidence of photoprotection.

Summary

In summary, the accumulation of mtDNA mutations appears to play a predominant role in the ageing process and mutations have been associated with cancer in many tissues. This is associated with the key roles of mitochondrial function and mtDNA in oxidative stress production and as a key mediator of apoptosis. We and others have pioneered the use of mtDNA damage as a highly sensitive biomarker of UV exposure in human skin, which is known to induce direct DNA damage and oxidative stress and is strongly associated with increased skin ageing and cancer. This review has highlighted the several applications of mtDNA as a biomarker of UVR-induced oxidative stress.

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Successful Aging

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Objective: Until now, prospective studies of aging have begun with 50–60-year-olds, not adolescents. Premature death, childhood variables, and alcohol abuse have been often ignored, as has successful aging.

Method: The authors reviewed the existing literature on health in late life in order to highlight that, increasingly, successful aging is not an oxymoron. The present study followed two cohorts of adolescent boys (237 *college* students and 332 *core-city* youth) for 60 years or until death. Complete physical examinations were obtained every 5 years and psychosocial data every 2 years. Predictor variables assessed before age 50 included six variables reflecting uncontrollable factors: parental social class, family cohesion, major depression, ancestral longevity, childhood temperament, and physical health at age 50 and seven variables reflecting (at least some) personal control: alcohol abuse, smoking, marital stability, exercise, body mass index, coping mechanisms, and education. The six outcome variables chosen to assess successful aging at age 70–80 included four objectively assessed variables (physical health, death and disability before age 80, social supports, and mental health) and two self-rated variables (instrumental activities of daily living and life enjoyment).

Results: Multivariate analysis suggested that "good" and "bad" aging from age 70–80 could be predicted by variables assessed before age 50. More hopeful still, if the seven variables under some personal control were controlled, depression was the only uncontrollable predictor variable that affected the quality of subjective and objective aging.

Conclusions: One may have greater personal control over one's biopsychosocial health after retirement than previously recognized.

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This review of successful aging will be presented in two parts. First, we shall review selected findings from the past 15 years. This research dispels many myths and helps us attend to what is most salient about aging, both in terms of decline and development. Second, we will report heuristic findings from the Study of Adult Development, arguably the first prospective interdisciplinary study in the world to study physical and mental health from adolescence until old age.

What Is Known About Successful Aging

In the future, geriatric psychiatry must learn to pay as much attention to health as to disease. With his concept of "compression of morbidity," James Fries (1) was among the first to assert that the 1955 motto of the American Gerontological Society was coming true; modern medicine was "adding life to years; not just more years to life." In 1990 there were 4 million people age 85 and older (the old-old) in the United States. There will be 10 times that many in 2040. It is surprising that this is not so much the result of greater longevity among the elderly but of more people living to age 65. In the past century, the lifespan of 85-year-old men has increased only 1.2 years (from 4.0 years in 1900 to 5.2 years in 1987) (2); but because of medical advances and changes in lifestyle, however, these surviving octogenarians are more likely to be active. For example, by the end of his life, the average 85-year-old man will have spent only 0.56 years in an institution, and the average 85-year-old woman will have spent 1.5 years (2). Between ages 75 and 84, 73% of elderly people report no disability; and after age 85, 40% of the population remain fully functional (3). Over the last century, the number of years an individual spends in active retirement has increased 10-fold. Thus, psychiatry in the 21st century must pay attention not only to the ills of the old-old but also to the determinants of successful biopsychosocial aging.

In order that successful aging not seem an oxymoron, the concept of aging must be viewed from three dimensions: decline, change, and development. The term "aging" can connote decline, and decline is not successful. After age 20 our senses slowly fail us. By age 70 we can identify only 50% of the smells that we could recognize at 40 (4). Our vision in dim light declines steadily, until by age 80, few of us can drive at night (5); by age 90, 50% of us can no longer use public transportation (6).

But the term "aging" also conveys change, a relatively neutral meaning. Analogous to the transformation of trees from spring to winter, our hair changes from chestnut to white, our waistline becomes portly, our eyes acquire crow's feet, and our frequency of making love shifts from three times a week to twice a month. But equally impor-

tant, our ability to love and be loved does not diminish with age. At the beach we pick up grandkids instead of sweethearts, but our capacity for joy is undiminished.

Finally, the term "aging" also conveys development and maturation. Analogous to a grand cru wine evolving from bitterness to perfection, at 70 we are often more patient, more tolerant, and more accepting of affect in ourselves and others (7). We are more likely to tolerate paradox, to appreciate relativity, and to understand that every present has both a past and a future (8). Adults, like toddlers, can lose millions of neurons even as their cognitive skills evolve, and the midline laminar bundle linking the limbic brain to the frontal lobes evolves until age 50 (9). Finally, like age itself, experience can only increase with time.

The Berlin Aging Study recently concluded, "Old age is not foremost a negative and problem-ridden phase of life" (10, p. 506), and the MacArthur Study of Aging (3) also provided excellent support that our greater longevity is resulting in less, not more, years of disability. By making a careful cross-sectional study of a representative sample of 516 urban Berliners aged 70–100, the Berlin Aging Study has shattered many common beliefs about old age. The Berlin Aging Study included 43 men and 43 women in each of six 5-year age cohorts between age 70 and 100. On one hand, after age 70, almost all of the subjects suffered at least one serious illness; and many suffered up to five. Fifty percent had painful arthritis; after age 95, 50% experienced significant dementia. On the other hand, before age 95, less than 10% manifested dementia; nine out of 10 still retained life goals. In another study of still-active octogenarian men (11), 76% still had sexual partners, 17% had intercourse at least once a week, and 35% more still masturbated.

When we look at mental functioning first, the bad news is that the Berlin Aging Study's old-old subjects (aged 85–100 years) resembled humans coping with severe stress. They experienced fewer positive emotions, more emotional loneliness, and a feeling that others controlled their lives. After age 90, only 50% felt they had a confidante. Such grim statistics, however, would be less bleak if individuals in "terminal decline" (i.e., in the 1–3-year period before death) had been excluded. The good news was that with the exception of dementia, there was not more mental illness among the elderly—even among the old-old. Like arthritis and hip fracture, even dementia should be viewed as a common, but not inevitable, consequence of longevity. If after age 85, Alzheimer's disease is both a common disease and an enormous burden on nursing homes and relatives; nevertheless, Alzheimer's disease affects only one-half of centenarians (12). Social networks had declined only from an average of 13 individuals at age 70 to seven at age 90.

These findings are confirmed by other studies. Most mental deterioration before age 80 reflects disease and not the normal aging process. Schaie (13) observed that "virtually none of the individuals contained in our data set showed universal decline in all of the [five] abilities monitored—even by the eighties" (p. 114). Our IQs at age 75 are roughly what they were at 20 (14). True, after 30, our ability to recall proper names steadily declines, but such anomia does not predict dementia.

Careful epidemiological study of the aged from multiple centers (10, 15) has revealed that there is no increase in depression among elderly individuals. Similarly, literature reviews have shown that happiness and life satisfaction are stable over the last half of the lifespan (16, 17). The best predictors of happiness in a septuagenarian were those high on trait extroversion and low on trait neuroticism—traits that are stable over the adult lifespan (10). After age 40, the fear of death declines steadily (18), and belief in the afterlife increases.

After age 85, however, 60% of the Berlin Aging Study women and 30% of the men needed help bathing or showering; only one in five needed nursing-home care. These figures are inflated by the fact that participants over age 95 were oversampled. Even after age 85, only one-quarter of waking life was spent "resting."

The MacArthur Study (3) and the national survey on which the SF-36 norms are based (19) confirm the findings of the Berlin Aging Study. Although elderly people may be taking an average of three to eight different medicines and may be chronically ill in the eyes of their physicians, they often do not regard themselves as sick (10)—an important distinction. Subjectively, two out of three elderly individuals perceive their own health as superior to that of their peers, whereas only one in seven feels less healthy than their peers.

Of great interest, multivariate analysis of the Berlin Aging Study data revealed that successful aging was relatively free from the effects of social class. Certainly, because of more disease due to lessened self-care and access to medical care, the socially disadvantaged are less likely to survive until age 75. But having reached 75, the cohort-to-cohort decline in mental and physical functioning was no more rapid for the disadvantaged than for more privileged individuals. Between ages 70 and 100, there was a 30% decline in "fluid" intelligence (e.g., memory and digit symbol substitution), but the predictors of this decline were impaired visual and hearing acuity and not past social disadvantage.

When examiners used a global definition of successful aging at age 75, 80% of the Berlin Aging Study cohort were still considered in "good health" (cognitively fit, active, and involved in life) or in "average health" (relatively healthy, still independent, and satisfied with life). At age 95, this level of health was still maintained by 30% of the subjects. These figures would have been significantly better had those with "terminal decline" been excluded. For example, the average centenarian lives without major disability until age 97 (12)!

Among the healthy old-old, it is increasing fatigability and reduced vision and hearing that lead most commonly

to impairment—not dementia or the often cited but largely irrelevant 10% increase in reaction time (20). Perhaps the most inevitable consequence of aging is an inexorable decline in vital capacity and in the efficiency of oxygen utilization. This decline begins at age 20 and steadily declines, reaching the 50% mark by age 75 (21).

The shifts in coping strategies of elderly people in the Berlin Aging Study between ages 70 and 95 were fascinating (10). With increasing age, spirituality and serenity increase. By "serenity" we mean faith, acceptance, and allowing someone else to take over. The strategies of "giving up" or of information seeking were more common among the young-old, whereas the more "Buddhist" strategy of perceiving life as "being without meaning" was preferred by the old-old. Coping strategies that did not change between ages 70 and 90 were humor and comparing oneself with others more severely afflicted.

The two most important psychosocial predictors of successful aging were high level of education (which probably reflects traits of self-care and planfulness as much as social class) and having an extended family network. In a hierarchical regression model, the important correlates of poor aging (defined as dependence, dissatisfaction with living, and being bedridden) were trouble walking, poor vision, age per se, depression, and dementia (10). In contrast, variables commonly construed as important to poor aging from ages 50–70 (e.g., emphysema, arthritis, loss of a partner, and diabetes) were no longer salient. Indeed, because of selective survival after age 85, only one person in two suffered from significant arteriosclerotic heart disease.

A Prospective Study of Successful Aging

In 1948 the World Health Organization defined health not as the absence of illness but as the presence of well-being—physical, mental, and social (22). In order to offer a heuristic model of successful aging for the 21st century, we shall draw on the Study of Adult Development at Harvard University, which prospectively followed two socially diverse cohorts of adolescents (a *college* cohort and a *core-city* cohort) until they became great-grandfathers. In this study the complexity of aging will be simplified by holding gender (male), nationality (United States), and skin color (white) constant. Birth cohort was confined to the period 1918–1932; nevertheless, the *college* cohort has enjoyed the same longevity as white males born in 2000. Contrast will be achieved by looking at similarities between, and differences within, these two demographically different cohorts. Physically and mentally healthy *college* septuagenarians and *core-city* sexagenarians, the happy-well, will be contrasted with the sad-sick and the prematurely dead.

To define well-being in old age, we chose six domains of function that permitted classifying the old along a continuum stretching from the happy-well to the sad-sick. Between the two poles, of course, were individuals whose outcome was mixed. The first domain was physician-assessed objective physical health and absence of irreversible physical disability. The second domain was subjective physical health. Do octogenarians allege that they can still carry out most instrumental tasks of daily living (e.g., yard work, climbing stairs, walking 2 miles) as before, albeit more slowly?

The third, more longitudinal, domain was length of active life. This was defined as the number of years before age 80 or age 70 that an individual had survived without either objective or subjective physical disability. The fourth domain was objective mental health. This domain reflected objective evidence of competence in four areas: work, relationships, play, and the absence of need for psychiatric care or medication. The fifth domain was subjective life satisfaction. Over the last 20 years, had the person reported subjective satisfaction in multiple facets of his or her life (e.g., marriage, job, children, and friendship)? The sixth domain was social supports. Was there objective evidence of friends? Was the individual's satisfaction with spouse and children mutual?

Individuals who did well in all six areas until age 80 were be classified as happy-well; those who were both psychosocially unhappy and physically disabled were classified as sad-sick. Those who fell in between were classified as intermediate.

Until now we have not known how to predict successful aging. True, there have been several prospective studies of aging. Well-known examples include the Duke Longitudinal Studies of Normal Aging (23), the Baltimore Longitudinal Study of Aging (24), the Bonn Longitudinal Study (25), the Seattle Longitudinal Study of Intellectual Aging (26), and the Veterans Affairs Normative Aging Study (27). All have contributed valuable understanding about the course of old age, but each of these studies was flawed by "selective mortality" (28). By beginning late in life, these studies failed to include those who died before age 60 or 70. As Baltes and Baltes (8) pointed out, "In the field of psychological gerontology, research has not yet reached a stage where there is good causal evidence about predictor variables including knowledge about the role of risk and protective factors" (p. 16). The Study of Adult Development provides a way around some of these difficulties.

Method

The *college* cohort in the study included 268 Harvard sophomores selected for physical and mental health circa 1940 (29, 30). The socially disadvantaged *core-city* cohort included 456 nondelinquent schoolboys with a mean IQ of 95 and a mean education of 10 years (31, 32). The details of the study have been well described in previous reports (33–37).

Independent predictor variables (assessed before age 50) were the following:

1. Smoking: In pack-years from age 15–50.

2. Alcohol abuse: DSM-III criteria were used; 0=no abuse, 1=alcohol abuse or dependence.

3. Body mass index (kg/m$^{2)}$: At age 50, men with a body mass index >28.00 (overweight) or <22.00 (underweight)=2; men with body mass index <28.01 and >21.99 (optimal weight)=1.

4. Years of education: Used only for *core-city* men because the range of education for the *college* men was truncated.

5. Some regular exercise (*college* men only): Exercise that burned more than 500 kilocalories/week (38); 1=yes, 2=no.

6. Stable marriage: 1=married without divorce, separation, or serious problems until age 50; otherwise=2.

7. Maturity of defenses: Defenses are involuntary mental mechanisms that adaptively alter inner or outer reality in order to minimize distress. Mature (adaptive) defenses were defined as suppression, humor, altruism, anticipation, and sublimation; immature (maladaptive) defenses were defined as projection, schizoid fantasy, acting out, passive aggression, dissociation, and hypochondriasis. For each man, the mean maturity of defensive behaviors, largely identified from the age of 47 (SD=2) by interview, were scored on a 9-point scale consistent with the DSM-IV Defensive Functioning Scale on which 1=most adaptive and 9=most maladaptive. The method is described elsewhere (39); 1=mature defenses present scored 1–3; 2=mature defenses absent scored 4–9.

8. Depression: A clinician reviewing men's entire records before age 50, including interviews, felt there was reasonable evidence of a major depressive disorder (40); 0=no, 1=yes.

9. Parental social class: Scaled from upper class=1 to unskilled laborer or on welfare=5 (41).

10. Warmth of childhood (until age 18 for *college* men; until age 14 for *core-city* men): Two research assistants who were blind to all later data rated five facets of the men's childhood environmental strengths (global impression, family cohesion, and relations with mother, father, and siblings) on scales from 1–5; 5=warmest environment and 25=bleakest environment (42).

11. Ancestral longevity: For the *college* men, this was the mean age at death of the oldest parent or grandparent on both the mother's and father's sides; for the *core-city* men, this was the mean mother's and father's age at death (43).

12. Stable childhood temperament: Parental report of childhood temperament; 1=easy baby and toddler, 3=minor problems, 5=phobias, shyness, tantrums, enuresis (42).

13. Objective disability (age 50): Rated using the same 4-point scale for physician-rated objective physical health that is defined in the next section; 1=no irreversible disability, physical health rated 1–3; 2=physical health rated 4 (irreversible disability).

The six outcome domains (assessed at ages 75–80 for the *college* cohort and ages 65–70 for the *core-city* cohort, i.e., the dependent variables) were as follows:

1. Objective physical health: Every 5 years, the study sought from each man a complete physical examination, including chest X-rays, routine blood chemistries, urinalysis, and an ECG (44). A study internist who was blind to the patients' psychosocial adjustment rated these examinations on a 4-point scale on which 1=without any irreversible illness, 2=minor irreversible illness that was neither life shortening nor disabling (e.g., treatable glaucoma, reversible hypertension, or noncrippling arthritis), 3=irreversible life-threatening illness (e.g., coronary thrombosis or diabetes), and 4=irreversible illness with significant disability (e.g., multiple sclerosis, chronic congestive heart failure). A score of 4=objective irreversible disability present.

2. Subjective physical health: A 14-point questionnaire (scored 1–14), similar to question 6 on the SF-36 (19) was administered every 2 years; it inquired whether the men could still climb two flights of stairs or walk 2 miles without resting, drive, care for the yard, and travel and shop without assistance, etc. (40). A score of 5–14=subjective disability present.

3. Years of active life: The age that a man first became irreversibly disabled, either objectively or subjectively.

4. Objective mental health (range=9 [best] to 21 [worst]): Independent raters assessed the men's lives from age 50 until age 65 on nine items: 1) quality of marriage, 2) job success, 3) job satisfaction, 4) no early retirement, 5) vacation of 3 weeks or more, 6) social activities, 7) no use of psychiatrists, 8) no use of tranquilizers, 9) overall rater impression (36). A score of 15–21=mental health rating in worst quartile.

5. Subjective life satisfaction (range 10 [best] to 40 [worst]): At age 75 (SD=2) the *college* men and at age 65 (SD=2) the *core-city* men were asked to rate their degree of satisfaction on a 5-point scale in four life areas over the past 20 years (marriage, children, job or retirement, and friends). To this was added their best score from one out of four additional areas (hobbies, sports, community activities, or religious participation) (37). A score of 18–32=life satisfaction rating in worst third.

6. Objective social supports (range=2.5 [best] to 14.0 [worst]): Independent raters reviewed information from seven biennial questionnaires and also those from wives and children and usually one 2-hour interview. The *college* men were assessed on seven facets of social support: closeness to wife, children, siblings, "playmates" (e.g., bridge and golf), religious affiliation, social networks (e.g., country clubs, civic organizations), and confidantes (37). This variable was not available for the *core-city* group. A score of 10.5–14.0=social supports rating in worst quartile.

In addition, successful aging was assessed with a global measure at circa age 80 for *college* men and at circa age 70 for *core-city* men; 1=happy-well: survival to age 75 (*college* men) or 65 (*core-city* men), no objective or subjective physical disability, objective psychosocial adjustment in top three-quarters, subjective life satisfaction in top two-thirds, and (*college* men only) social supports in top three-quarters; (Cutoff points adjusted to capture one-quarter of each cohort.) 2=intermediate: survival with either subjective or objective physical disability but without psychosocial disability (i.e., bottom third in life satisfaction or bottom quarter in mental health or in social supports) or survival with psychosocial disability but without physical disability; 3=sad-sick: survival with 5 or more years of objective or subjective physical disability *and* psychosocial adjustment in bottom quarter or life satisfaction in bottom third or (*college* men only) social supports in bottom quartile; 4=prematurely dead: dead before age 75 (*college* men) or before 65 (*core-city* men). (Among the happy-well, one *college* man died between ages 75 and 79, and no happy-well *core-city* men died between ages 65 and 69. In contrast, among the sad-sick, seven *college* men and six *core-city* men died between ages 75 and 79.)

Results

In order to assess the predictors of successful aging, we needed relatively complete data sets at age 50. By that point, the *college* cohort had shrunk from 268 to 237 because of 12 deaths (six killed in action in World War II) before age 45 and 19 withdrawals. The *core-city* cohort had shrunk from 456 to 332 because of 33 deaths before age 45 and 91 men who had withdrawn or had incomplete data sets. The withdrawn men did not differ from the active members except that among the *core-city* cohort, they were more likely to come from a multiproblem childhood, have a lower IQ, and have more limited education. It was not surprising that war, homicides, suicides, and accidents accounted for the majority of deaths among the men excluded because of early mortality.

Length-of-active-life data for the youngest men in the study were missing. When this article was written (2000),

TABLE 1. Quality of Aging for *College* Men and *Core-City* Men Studied Since Adolescence[a]

	College Men at Age 75–80 (N=237)				*Core-City* Men at Age 65–70 (N=332)			
			Age at Death or Disability (years)				Age at Death or Disability (years)	
Quality of Aging	N	%	Mean	SD	N	%	Mean	SD
Happy-well	62	26	>80		95	29	>70	
Intermediate	75	32	77.6	4	114	34	65.6	5
Sad-sick	40	17	71.4	7	48	14	62.3	5
Prematurely dead	60	25	62.3	9	75	23	55.0	6

[a] The happy-well, intermediate, and sad-sick all survived until age 75 if *college* men and until age 65 if *core-city* men.

6% of the *college* men (N=14) were still aged 76–77, 34% (N=81) were aged 78–79, and 14% of the *core-city* men (N=46) were aged 68 or 69. When age was statistically controlled, the relationships presented in this article were not affected.

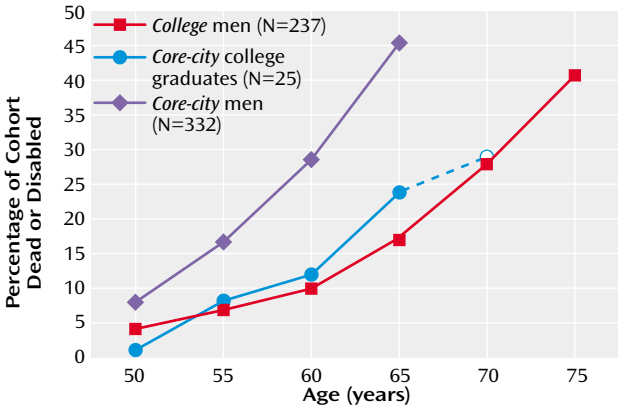
Table 1 illustrates that the distribution of the four categories of aging (happy-well, intermediate, sad-sick, and prematurely dead) were proportionately the same for *college* men at age 75 and for *core-city* men at age 65. Figure 1 illustrates the increase in physical disability and death for the two groups over time. The slopes are quite similar, but the *college* cohort reached every stage of death and disability about 10 years later than the *core-city* cohort. For example, at age 65, 25% of the *core-city* men were dead, and 20% were disabled; at age 75, 27% of the *college* men were dead, and 14% were disabled (Figure 1).

However, despite great differences in parental social class, prestige of college, intelligence test scores, current income, and job status, the health decline of the 25 *core-city* men (7.5% of all *core-city* men) who completed 16 or more years of education was no more rapid than that of the *college* men. In other words, education may have been a more robust cause of the differences between the rate of health decline in the two cohorts than other differences in socioeconomic status.

Table 2 illustrates the ability of 13 variables measured before age 50 to predict successful aging and four of its principle domains. Table 2 arranges the protective factors in terms of self-care variables over which an individual has some personal control, and those variables, such as heredity and parents, over which an individual is relatively powerless. For both the *college* and the *core-city* men, absence of alcohol and cigarette abuse (less than 30 pack-years) before age 50 were arguably the most important protective factors for successful aging. Subjective life satisfaction was the only outcome domain not significantly affected.

Although the educational range was too truncated to be relevant in the *college* men, and exercise was not assessed for the *core-city* men, both variables (which apart from their face value are each indirect measures of self-care and perseverance) appeared to be important predictors of multiple domains of successful aging. As an illustration of

FIGURE 1. Rates of Permanent Disability[a] or Death After Age 50 for *College* Men, *Core-City* Men, and *Core-City* College Graduates[b] Studied Since Adolescence



[a] Irreversible physical illness with significant disability.
[b] The 25 *core-city* men who graduated from college are shown both as an individual cohort and as part of the larger *core-city* cohort. The data point at age 70, marked with an open circle, includes only the oldest 21 college-educated *core-city* men, with a mean age of 69.5. Four men born after 1930 were excluded.

the predictive power of perseverance (and the adaptive defense mechanism of suppression), at age 19, 61% (38 of 62) of the *college* men who were classified as happy-well six decades later, but only 13% (5 of 40) of the sad-sick men were able to complete the full 5 minutes of a treadmill test (χ^2=10.7, df=2, p=0.005). The findings could not be explained by physical fitness (45) at the time.

Mature (adaptive) defenses from age 20–50 were an important predictor of successful psychosocial aging, but the maturity of defenses was quite irrelevant to objective, although not to subjective, physical disability. Before age 50, almost two-thirds of the happy-well men in both groups, and only one-10th of the sad-sick men had used mature defenses, especially humor, suppression, and anticipation, rather than immature defenses, especially projection and dissociation.

As Table 2 shows, three other protective variables assessed before the age of 50 were independent predictors of successful aging. These three variables were a warm marriage, absence of major depressive disorder, and, predictably, good physical health at age 50. Body mass index, ancestral longevity, and the two childhood variables (environmental warmth and temperament) were only marginally significant in univariate analyses.

Next, an effort was made to assess the ability of the protective factors—factors over which the individual has some control—to predict the future. To control for the deleterious effect that poor health might have exerted on the six protective factors, we excluded all men who by age 50 were disabled or even chronically ill. The absence of these protective factors (with the exception of a stable marriage and mature defenses) was just as important in predicting which men would be among the prematurely dead as among the sad-sick. For example, among *college* men still

TABLE 2. Correlation of Predictor Variables Before Age 50 With Five Aging Outcomes 15–25 Years Later for 237 *College* Men and 332 *Core-City* Men Studied Since Adolescence

	Correlation (Spearman's r_s, two-tailed)									
							Mental Health			
			Physical Health (Range=40–80)				Subjective Life Satisfaction (Range=10–40)		Objective Mental Health (Range=9–21)	
	Quality of Aging (Range=1–4)		Length of Active Life		Age at Death					
Variable[a]	*College* Men	*Core-City* Men	*College* Men	*Core-City* Men	*College* Men (<75 years)	*Core-City* Men (<65 years)	*College* Men	*Core-City* Men	*College* Men	*Core-City* Men
Controllable factors at ages 10–49										
Pack-years of smoking (range=0–90)	0.35**	0.31**	–0.30**	–0.31**	0.30**	0.23**	n.s.	n.s.	0.26**	0.14*
Alcohol abuse (DSM-III) (range=0–1)	0.42**	0.19**	–0.38**	–0.18**	0.40**	0.15*	0.21*	n.s.	0.32**	0.21**
Exercise (range=1–2) or education (range=6–19)[b]	0.22**	0.20**	–0.18*	–0.20**	n.s.	n.s.	n.s.	n.s.	0.24**	0.25**
Body mass index (range=1–2)	0.14*	0.11*	–0.14*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Stable marriage (range=1–2)	0.27**	0.22**	–0.15*	–0.13*	n.s.	0.17*	0.33**	0.27**	0.39**	0.33**
Maturity of defenses (range=1–9)	0.32**	0.23**	–0.27**	–0.17*	n.s.	n.s.	0.34**	0.28**	0.41**	0.46**
Uncontrollable factors at ages 10–49										
Depression (range=0–1)	0.22**	0.12*	–0.23**	n.s.	n.s.	n.s.	0.17*	0.16*	0.39**	0.32**
Parental social class (range=1–5)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Warmth of childhood (range=5–25)	0.18*	n.s.	–0.14*	n.s.	n.s.	n.s.	n.s.	n.s.	0.19*	0.14*
Ancestral longevity (range=40–100)	–0.15*	n.s.	0.16*	0.15*	–0.19*	–0.11*	n.s.	n.s.	n.s.	n.s.
Childhood temperament (range=1–5)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.18*	n.s.	0.20*
Objective disability at age 50 (range=1–2)	0.39**	0.40**	–0.42**	–0.68**	0.30**	0.48**	n.s.	n.s.	0.24**	0.23**

[a] See text for detailed explanation of variables. For all variables except ancestral longevity, a low score indicates better health.
[b] Exercise was rated only for *college* men, and education was rated only for *core-city* men.
*p<0.05. **p<0.001.

in good health at age 50 were 66 men with fewer than four protective factors. At age 80, 50 of these 66 men were among the sad-sick or prematurely dead, and not even one man was among the happy-well. In contrast, 44 *college* men had all six factors present; 25 were among the happy-well, and only one was among the sad-sick. There were 69 *core-city* men with five or six factors present; 35 were among the happy-well, and only one was among the sad-sick.

Table 3 demonstrates that each of the six protective factors over which an individual has some control predicted successful aging when the other predictive factors were statistically controlled. The confounder of poor prior physical health was controlled by excluding from the analysis reported in Table 3 all men who by age 50 were irreversibly chronically ill or disabled. (Including these men did not significantly alter the results.) The importance of alcohol abuse for the *core-city* cohort and the importance of smoking for the *college* cohort were masked by collinearity with the other variable. Among the uncontrollable factors, parental social class, unhappy childhood, and ancestral longevity were not significant. Only depressive disorder before age 50 appeared to be an independent predictor.

Years of education are more often perceived as a reflection of socioeconomic status and intelligence than as a reflection of self-care. Therefore, we used the same logistic regression procedure as in Table 3 to model the effects of parental social class (range=II–V), IQ (assessed by an individually administered WAIS in junior high school; range=61–130), and years of education to predict whether *core-city* men aged 65–70 were among the happy-well or the sad-sick/dead. Only education was significant (odds ratio=0.79, 95% confidence interval=0.71–0.88, p<0.0001). A surprising finding was that former cigarette and alcohol abuse explained most of the strong association between physical health and social supports.

Discussion

Although the narrowness of our cohorts limits generalization, the data are offered in a spirit of a heuristic schema of successful aging for the new millennium. The intent is not to test a specific hypothesis as much as to offer a testable model for successful aging and to highlight solutions to problems that have compromised prior studies.

First, a major problem in understanding the relevance of protective or risk factors for successful aging is selective

TABLE 3. Multivariate Model Contrasting Most and Least Successful Aging in *College* Men and *Core-City* Men Studied Since Adolescence

	Odds for Happy-Well Men Relative to Men Who Were Sad-Sick or Prematurely Dead[b]			
	College Men at Age 75–80 (N=162)		*Core-City* Men at Age 65–70 (N=217)	
Variable[a]	Odds Ratios[c]	95% CI	Odds Ratios[c]	95% CI
Controllable factors				
Smoking <30 pack-years	4.81[d]	0.84–27.7	4.56***	2.29–9.11
No alcohol abuse	—[e]		1.11[f]	0.53–2.35
Mature defenses (1–3 on 9-point scale)	2.65*	1.22–6.80	2.98**	1.40–6.10
Stable marriage	1.94	0.70–5.35	2.75*	1.24–6.81
Body mass index >21 and <29	3.05[d]	0.99–9.40	1.71	0.85–3.43
Some regular exercise	3.09*	1.30–9.75		
Education (in years)			0.86*[g]	0.77–0.96
Uncontrollable factors				
Without depressive diagnosis	10.4*	4.75–23.2	3.51*	1.20–9.99
Parental social class (range=I–V)	1.46	0.91–2.36	1.12	0.63–1.96
Childhood temperament (range=1–5)	0.92	0.68–1.24	1.10	0.85–1.42
Warmth of childhood (range=5–25)	0.98	0.89–1.12	0.99	0.92–1.11
Ancestral longevity (in years)	1.00	0.97–1.04	1.00	0.97–1.00

[a] See text for detailed explanation of variables.
[b] Men having an intermediate quality of aging (rating=2) were excluded: 75 *college* men and 114 *core-city* men. One *core-city* man was excluded owing to missing data.
[c] With control for the other 10 variables (education was not applicable for *college* men; exercise was not applicable for *core-city* men).
[d] Odds approached but did not achieve significance for smoking (p=0.08) and body mass index (p=0.051) among *college* men.
[e] Since no *college* men who abused alcohol were among the happy-well, the odds ratio could not be calculated, but alcohol abuse was left in the model.
[f] Alcohol abuse appeared to make no independent contribution owing to its collinearity with smoking.
[g] For each additional year of education, the likelihood of being sad-sick or dead at age 65 was reduced by 0.85.
*p<0.05. **p<0.01. ***p<0.001.

advocacy of one factor at the expense of others. All of the 13 putative predictors in Table 2 except social class were significant, but many predictors were affected by the others. For example, the putative risk factors of low education, depression, and alcohol abuse each increased the likelihood of sustained smoking (46). Similarly, alcohol abuse not only damages the liver, heart, and immune system (47), but it increases the risk of depression and divorce. The problem becomes where to put causal emphasis. In various prior studies, each of the following factors has been treated as critical, whereas other equally important factors were ignored: cholesterol level and smoking (48), depression (49), exercise (50), social supports (51), job status (52), body mass index (3), and alcohol abuse (53). In order to separate cause from association, however, all the confounding variables must be simultaneously studied.

A second problem in aging studies is confusion about causal direction. Social supports (3) are often assigned a causal role in successful aging. However, in their classic review of the evidence that social supports cause good health, House et al. (54) acknowledged that almost no attention had been paid to social supports as a dependent variable. In our prolonged prospective study, social supports at age 70 were powerfully affected by the pre-age-50 protective factors identified in Table 3. Good social supports in old age may be in large part a result of the same earlier good habits that preserve physical health.

A third problem inherent in prior studies is that the relative importance of predictor variables changes over time (55). Unhappy childhood predicted poor health in the *college* cohort at age 50 but not at age 80 (44). Serum cholesterol levels are an important predictor of heart disease in young adults (48), but in our two elderly cohorts, cholesterol levels at age 50 were identical for the happy-well and for the sad-sick/dead. More representative samples also suggest that after age 70 elevated cholesterol levels are not a general risk factor (56). Shortened ancestral longevity is a risk factor for men dying between ages 40 and 60, but most people die after age 60. Thus, Rowe and Kahn (3) have reported, "Our MacArthur twin studies leave very substantial room for factors other than genetics in determining life expectancy" (p. 28). Investigators using the Swedish Twin Registry noted "that most of the variance in longevity was explained by environmental factors" (57).

A fourth problem is that until recently most major longitudinal studies of health, for example, the Framingham Study (48) and the Alameda County Study (58), have only controlled for reported alcohol consumption. Unfortunately, because of "dieting" and underreporting, alleged alcohol consumption is almost as poor an index of alcohol abuse as reported food consumption is for obesity.

As a caveat, we must acknowledge that we, too, have ignored important risk variables (e.g., minority status, nutrition [59], insurance status [60], and occupational status [52]). In addition, the cohort is very narrow: white American men born principally in the 1920s. Our findings require confirmation in other populations, especially women and men of color.

However, unlike sociological science, biological science is built on diverse studies of homogeneous subspecies, not random surveys of Noah's ark. Thus, Table 3 may still

contain a hopeful message for young adults destined by actuarial tables to live past age 80. The seven protective factors that distinguish the happy-well from the sad-sick are under at least some personal control. We have considerable control over our weight, our exercise, our education, and our abuse of cigarettes and alcohol. With hard work and/or therapy, our relationship with our spouse and our coping styles can be modified. A successful old age, Horatio, may lie not so much in our stars and genes as in ourselves.

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