Sebaceous Gland Secretion is a Major Physiologic Route of Vitamin E Delivery to Skin

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Skin plays an important part in the protection against oxidative stressors, such as ultraviolet radiation, ozone, and chemicals. This study was based on the observation that upper facial stratum corneum contained significantly higher levels of the antioxidant α-tocopherol than corresponding layers of arm stratum corneum. We hypothesized that the underlying mechanism involves sebaceous gland secretion of vitamin E. To test this, we examined in eight human volunteers: (i) stratum corneum levels and distribution profiles of vitamin E in sites with a different sebaceous gland density (arm *versus* cheek); (ii) whether vitamin E is a significant constituent of human sebum; and (iii) if there is a correlation between levels of vitamin E and squalene, a marker of sebum secretion, in skin surface lipids. Using standardized techniques for stratum corneum tape stripping and sebum collection, followed by high-performance liquid chromatography analysis of tocopherols and squalene, we found that: (i) the ratio of cheek *versus* upper arm α-tocopherol levels was 20 : 1 for the upper stratum corneum and decreased gradually with stratum corneum depth; (ii) vitamin E (α- and γ-tocopherol forms) is a significant constituent of human sebum and is continuously secreted at cheek and forehead sites during a test period of 135 min; and (iii) vitamin E correlates well with levels of cosecreted squalene ($r^2 = 0.86$, $p < 0.001$). In conclusion, sebaceous gland secretion is a relevant physiologic pathway for the delivery of vitamin E to upper layers of facial skin. This mechanism may serve to protect skin surface lipids and the upper stratum corneum from harmful oxidation. ***Key words: lipid peroxidation/oxidative stress/sebum/squalene/α-tocopherol. J Invest Dermatol 113:1006–1010, 1999***

The skin serves as a biologic interface between body and environment and thus is exposed to multiple sources of oxidative stress, e.g., ultraviolet (UV) A/UVB, air pollutants, and chemical oxidants (Fuchs, 1992; Scharffetter-Kochanek, 1997; Cross *et al*, 1998). To counteract oxidative injury of structural lipids and proteins, human skin is equipped with a network of enzymatic and nonenzymatic antioxidant systems (Thiele *et al*, 1999). Previously, we have demonstrated that the stratum corneum (SC), which maintains the barrier function of skin, is highly susceptible to vitamin E depletion and lipid peroxidation induced by UVA, UVB (Thiele *et al*, 1998), and ozone (Thiele *et al*, 1997). α-Tocopherol, the principal naturally occurring form of vitamin E and chain breaking antioxidant (Traber and Sies, 1996), was identified as the predominant antioxidant in murine and human SC, exhibiting a characteristic gradient with lower levels towards the outer SC layers in upper arm skin (Thiele and Packer, 1999). Whereas the distribution, regulation, and relevance of naturally occurring vitamin E in the various layers and anatomic regions of human skin are still poorly understood, many studies on topically applied vitamin E point to a major protective role in oxidatively challenged skin (Thiele *et al*, 1999). It has been demonstrated by *in vivo* chemiluminescence that UVA exposure strongly increases the formation of reactive oxygen species in the skin's upper layers and that topical supplementation with α-tocopherol greatly reduces UVA-induced formation (Evelson *et al*, 1997). Topical application of α-tocopherol not only increases epidermal and dermal levels of α-tocopherol but also bolsters the levels of other enzymatic and nonenzymatic cutaneous antioxidants (Lopez-Torres *et al*, 1998). Furthermore, topical application of α-tocopherol to murine skin *in vivo* was demonstrated to inhibit epidermal lipid peroxidation, prevent UV-induced immunosuppression (Yuen and Halliday, 1997), and provide epidermal DNA photoprotection by inhibition of UVB-induced thymidine dimer formation (McVean and Liebler, 1997). Inhibition of UV-induced immunosuppression and epidermal DNA damage may account for the powerful anti-carcinogenic potential of topical α-tocopherol demonstrated in UV-irradiated C3H/HeN mice (Gensler and Magdaleno, 1991).

This study was based on the rather unexpected observation that the upper SC layers of the environmentally exposed human facial skin contained several-fold higher levels of α-tocopherol than corresponding layers of the previously investigated, less exposed upper arm SC (**Fig 1**). It was suggested that this finding may be related to regional differences in the delivery pathway and/or regulation of vitamin E. In the environmentally highly exposed facial skin, the SC is covered by a film of skin surface lipids which consist of wax esters, triglycerides, and squalene, originating from sebum secretion by sebaceous glands (Clarys and Barel, 1995). We hypothesized that sebaceous gland secretion is a physiologic pathway of vitamin E delivery to the skin surface lipids and SC of facial skin. Therefore, in this study, we sought to examine: (i) SC

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Abbreviations: SC, stratum corneum; TEWL, transepidermal water loss.

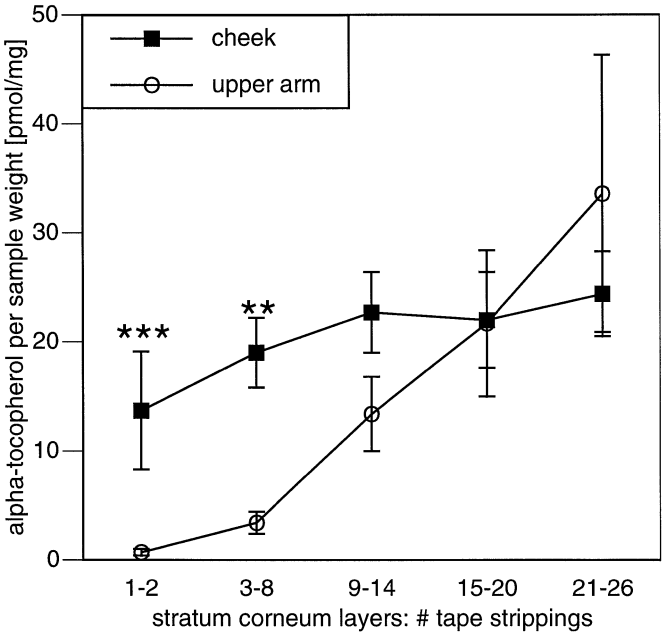

Figure 1. α-Tocopherol distribution within SC is site-dependent: arm *versus* cheek. In the upper arm α-tocopherol-levels increased 47-fold from the uppermost to the deepest SC layer. The increase in the cheek is by far less pronounced. The first two layers of cheek SC contained more α-tocopherol than the arm (***$p < 0.001$, **$p < 0.01$, $n = 8$).

levels and distribution profiles of vitamin E in sites with different sebaceous gland density; (ii) whether vitamin E is secreted by sebaceous glands; and (iii) if there is a correlation between levels of vitamin E and squalene in skin surface lipids.

MATERIALS AND METHODS

Chemicals All chemicals used were of the highest grade available. α- and γ-tocopherol standards were a kind gift from Henkel (La Grange, IL). Squalene was from Sigma (St Louis, MO).

Human subjects For tape stripping, a location within the medial portion of the proximal first third of the upper arm was chosen. This region is usually protected from UV exposure. On the face, a region of the cheek was chosen (caudal of the middle line of the eye) for tape stripping. For the procedures used to obtain SC and sebum from human volunteers, permission was granted by the UC Berkeley Committee for the Protection of Human Subjects. Nine volunteers (skin types II and III, $n = 3$ Asians, $n = 5$ Caucasians, $n = 2$ women, $n = 6$ men, median age 27 y, range of age 23–39 y) gave their written informed consent. Exclusion criteria included any history of dermatologic disorders and any current medical problem. For a total of 2 wk prior to the study, participants were not allowed to take anti-oxidant supplementation either orally or topically, nor were they allowed to apply any vitamin E-containing lotion or after-shave.

Tape stripping of human SC Before sample acquisition, the skin was cleaned with a piece of gauze soaked in 50 μl of ethanol. Samples of human SC were obtained by sequential tape stripping with D-Squame (Cuderm, Dallas, TX) using a standardized protocol as described before (Thiele *et al*, 1998). Briefly, the strips were smoothly adhered to the skin, flattened evenly three times, and removed gently using moderate and even traction. The tape strips were numbered from 1 to 26. The average weights per surface area of tape strip were 27 ± 6 μg per cm^2 for the face and 33 ± 4 μg per cm^2 for the arm, which is in accordance with previous reports (Thiele *et al*, 1998). The difference between the two groups was not significant ($n = 8$). The thickness of the removed SC was estimated based on the cumulative weight, an assumed density of 1 g per cm^3, and a uniform coverage of SC on the tape strip as described (Kalia *et al*, 1996). With a cumulative weight for 26 strips of 815 ± 157 μg per cm^2 for the cheek and 995.5 ± 37 for the upper arm, the average thickness of removed SC corresponded to 8.2 ± 1.6 μm and 10 ± 0.4 μm. Tape-stripped SC samples were stored on dry ice until extraction, which was carried out within 2 h after tape stripping.

In order to characterize further the portion of SC removed by 26 D-squame tape-strips, the transepidermal water loss (TEWL; TM 210 evaporimeter, Courage & Khazaga, Cologne, Germany) was assessed in five human volunteers after 0, 2, 8, 14, 20, 26, 32, and 38 tape-strippings. Baseline TEWL levels after 0 tape-strippings were (mean ± SD) 8.17 ± 0.96 g per m^2 per h (arm) and 33.8 ± 7.59 g per m^2 per h (cheek), maximal levels after 38 tape-strippings ($TEWL_{max}$) were 65.18 ± 12.26 g per m^2 per h and 93.90 ± 0.58 g per m^2 per h, respectively. After 26 tape-strippings ($TEWL_{26}$) TEWL levels increased to 42.52 ± 23.4 g per m^2 per h (arm) and 89.7 ± 6.04 g per m^2 per h (cheek), corresponding to a relative increase ($TEWL_{26}$ to $TEWL_{max}$) of 65.2% and 95.5%, respectively. These results indicate that most of the facial SC was removed, whereas approximately the upper two-thirds of the arm SC were removed by our sampling method.

Collection of sebum Prior to sample acquisition, the skin was cleaned with a piece of gauze soaked in 50 μl of ethanol. Sebum was obtained by application of standardized adhesive patches designed to collect sebum (Sebutape, Cuderm, Dallas, TX). The surface area of one patch is 5.1 cm^2. The patches were sequentially applied to one cheek (caudal of the middle line of the eye), the unilateral side of the forehead (cranial of the middle line of the eye), and the unilateral inner side of the upper arm (upper third). The first patch was removed after 15 min. Subsequently, the second and third patches were applied one after the other, and left on the same spot for 60 min each. The average weights of the samples obtained by the first patch were 0.3 ± 0.1 mg (arm), 1.1 ± 0.2 mg (cheek), and 0.8 ± 0.1 (forehead). The average weight of the samples obtained by the second and third patches were 0.3 ± 0.1 mg (arm), 1.0 ± 0.2 mg (cheek), and 0.8 ± 0.1 (forehead) ($n = 8$). The amount of sebum was (semi)quantitated by scoring on a scale from 0 to 5 according to the pore size on the patch, as described by the supplier. A score of 0 means no sebum visible on the patch, and a score of 5 means the maximum amount of sebum. Average scores of three independent observers were determined (0.5 ± 0.4 for the arm samples, 3.8 ± 0.9 for the cheek, and 3.0 ± 1.3 for the forehead). The patches were stored on dry ice until extraction.

Simultaneous HPLC – detection of α- and γ-tocopherol, and squalene For SC experiments, tapes 1 and 2 were pooled and hereafter referred to as "layer 1", tapes 3 through 8 as "layer 2", tapes 9 through 14 as "layer 3", tapes 15 through 20 as "layer 4", and tapes 21 through 26 as "layer 5". SC lipids were extracted as described earlier (Thiele and Packer, 1999). Briefly, pooled tapes and single adhesive patches were transferred into 50 ml centrifuge tubes containing 2 ml phosphate-buffered saline with 1 mM ethylenediamine tetraacetic acid and 10 μM butylated hydroxytoluene, 1 ml of 0.1 M sodium dodecyl sulfate, and 4 ml ethanol. The samples were mixed vigorously and extracted with 4 ml of hexane. The hexane layer was taken to dryness under nitrogen, and the residue was resuspended in ethanol/methanol 1 : 1 for injection. The HPLC system (Shimadzu, Kyoto, Japan) consisted of an SCL-10A system controller, an LC-10AD pump, an SIL-10A autoinjector with sample cooler, an Ultrasphere ODS C-18, 5 μm particle size column, and two detectors: a Waters 490 multiwavelength detector (Millipore, Milford, MA), set at 208 nm for squalene detection, and, set-up in-line, an LC-4B electrochemical detector (BAS, West Lafayette, IN), operating at a 500 mV oxidizing potential for vitamin E detection. The mobile phase was composed of methanol/ethanol 1:3 (vol/vol) with 20 mM lithium perchlorate.

Gas chromatography–mass spectrometry analysis of squalene For further characterization of SC and sebum squalene, the fraction containing the squalene peak was collected from the HPLC-system in a salt-free mobile phase and taken to dryness under liquid nitrogen. The residue was resuspended in acetone for analysis in a gas chromatography–mass spectrometry system consisting of a HP6890 gas chromatograph (Hewlett Packard, Palo Alto, CA), with a 30 m HP5 column (Hewlett Packard), and an electron impact mass spectrometry unit HP 5974 (Hewlett Packard) operated at 70 eV with a mass range from 35 to 500. The spectra obtained were characterized by a base ion at 69 m/z and a weak parent ion at 410, and found to be identical to squalene standards.

Statistics Statistical analyses were carried out using InStat for the Macintosh (Graphpad, San Diego, CA). A one-factor, repeated measure ANOVA was performed for successive SC layers and subsequent sebum samples. To determine differences between groups, the Tukey post-test was used. For the comparison of upper SC layers, the data were log transformed. $p < 0.05$ was considered statistically significant. Linear regression was used to correlate the different parameters derived from sebum samples. All data are expressed as mean ± standard error of the mean, n is given for each experiment.

RESULTS

Vitamin E levels in upper facial SC are 20-fold higher than in corresponding layers of upper arm SC Vitamin E levels in the face and the upper arm were not significantly different, when the SC thickness removed by 26 consecutive tape-strippings was compared. 102.0 ± 18.3 pmol of α-tocopherol per mg (n = 8) were found in facial SC as compared with 72.7 ± 21.9 pmol per mg (n = 8) in the SC of the upper arm. A dramatic difference in α-tocopherol levels, however, became apparent when analyzing the uppermost SC layer obtained by two consecutive tape strips (corresponding to layer 1). Layer 1 of the upper arm contained 0.7 ± 0.3 pmol α-tocopherol per mg (n = 8), whereas the corresponding layer in the cheek contained 20-fold higher levels (13.7 ± 5.1 pmol per mg) (n = 8, $p < 0.001$). These findings indicate a different distribution of α-tocopherol within the SC of the arm than the face.

SC vitamin E gradients differ in the upper arm and the cheek The distribution of α-tocopherol in a total of five SC layers was analyzed for each subject individually. Consistent with our earlier reports, in the upper arm site, the α-tocopherol level steadily increased from the outside towards the inside ($p < 0.005$) 47-fold. Layer 1 contained 0.7 ± 0.3 pmol per mg, layer 2 3.4 ± 1.0 pmol per mg, layer 3 13.4 ± 3.4 pmol per mg, layer 4 21.7 ± 6.7 pmol per mg, and layer 5 33.5 ± 12.7 pmol per mg. Significant differences were observed between layers 1 and 5 ($p < 0.01$) and 2 and 5 ($p < 0.01$) (n = 8 for all data). Notably, a strikingly different pattern was recorded for facial SC. Even though the vitamin E levels slightly increased from the outside towards the inside ($p < 0.05$, layer 1 *versus* layer 5), the increase from layer 1 to layer 5 was only 1.8-fold. Layer 1 contained 13.7 ± 5.1 pmol per mg, layer 2 19.1 ± 3.2 pmol per mg, layer 3 22.7 ± 3.7 pmol per mg, layer 4 22.1 ± 4.4 pmol per mg, and layer 5 24.4 ± 3.9 pmol per mg (n = 8 for all data). The first two layers of facial SC contained significantly more vitamin E than the corresponding layers of the upper arm ($p < 0.001$ layer 1, $p < 0.01$ layer 2, **Fig 1**).

Squalene forms gradients in SC of the upper arm and the cheek In both arm and cheek, the first SC layer contained more squalene than the subsequent layers ($p < 0.001$ for arm and $p < 0.0001$ for face). Facial SC, however, contained significantly more squalene ($p < 0.001$) than each corresponding layer of the arm (**Fig 2**). In the cheek, layer 1 contained 85.1 ± 13.9 pmol per mg, layer 2 35.5 ± 9.3 pmol per mg, layer 3 36.3 ± 13.0 pmol per mg, layer 4 27.2 ± 8.7 pmol per mg, and layer 5 30.1 ± 10.5 pmol per mg. In the upper arm, layer 1 contained 14.1 ± 4.4 pmol per mg, layer 2 4.1 ± 0.9 pmol per mg, layer 3 2.4 ± 0.5 pmol per mg, layer 4 1.9 ± 0.6 pmol per mg, and layer 5 1.8 ± 0.5 pmol per mg (n = 8 for all data points).

Vitamin E is excreted in sebum The α- and γ-form of tocopherol were analyzed in sebum obtained from adhesive patches that were applied to defined skin sites and removed at scheduled time points. After 60 min a total of 54.4 ± 11.1 pmol α-tocopherol (5.4 ± 1.5 pmol γ-tocopherol) was collected on the patch from the forehead and 76.5 ± 13.4 pmol α-tocopherol (8.7 ± 1.8 pmol γ-tocopherol) from the cheek (no significant difference). The arm patch contained significantly less vitamin E ($p < 0.001$, n = 8, α-tocopherol 5.1 ± 1.2 pmol, γ-tocopherol 0.7 ± 0.5 pmol, **Fig 3**). Both forms of vitamin E were intermittently detected in sebum samples obtained during a time course of 135 min in both the forehead and the cheek (no significant difference). When data were normalized per weight of the collected sebum, no significant differences were found between forehead, cheek, and arm. As the vitamin E gradients were analyzed in the cheek, the corresponding sebum tocopherol levels obtained from this location are shown. After 15 min 66.5 ± 8.3 pmol of α-tocopherol per mg were found (11.0 ± 3.1 pmol of γ-tocopherol per mg) in the cheek. After another 60 min (75 min total time), 72.0 ± 10.6 pmol of α-tocopherol per mg (8.2 ± 1.8 pmol of γ-tocopherol per mg) were detected, and after a further 60 min collection period (135 min total elapsed time), 66.2 ± 8.9 pmol of α-tocopherol per mg were found (6.5 ± 1.8 pmol of γ-tocopherol per mg) (n = 8 for all values). α-Tocopherol and γ-tocopherol levels correlated closely ($r^2 = 0.86$, $p < 0.0001$) with an average ratio of 9:1.

Furthermore, squalene was present in all samples. Within a sebum collection period of 60 min, 135.3 ± 26 nmol of squalene were detected per forehead patch, 182.9 ± 29.2 nmol in the cheek and significantly less in the arm (19.3 ± 3.2 nmol, $p < 0.001$, n = 8, **Fig 3**). Regression was performed for vitamin E and squalene contents in sebum samples. Both α- and γ-tocopherol correlated closely with squalene ($r^2 = 0.86$, $p < 0.001$ for α-tocopherol and

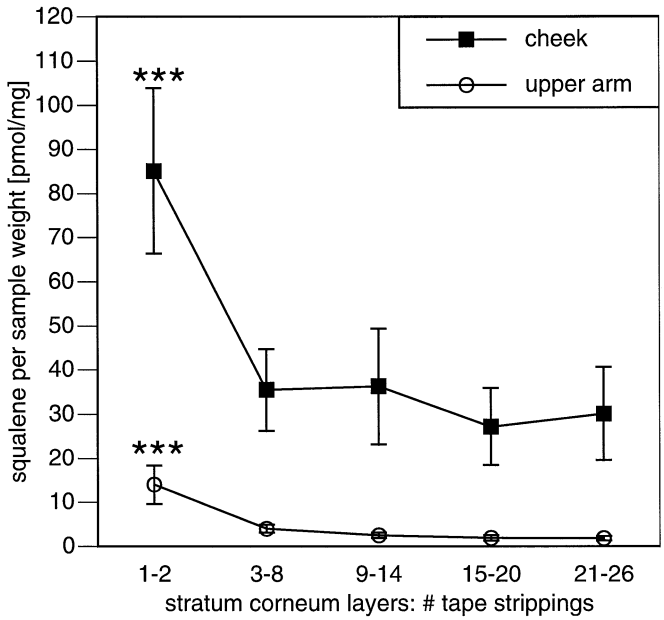

Figure 2. Cheek SC contains more squalene than SC of the upper arm. SC samples were obtained from defined cheek and upper arm sites by tape stripping. In both locations, the first SC layer contained more squalene than the subsequent ones (***$p < 0.001$, n = 8). Squalene levels in each cheek SC layer were higher than the corresponding arm layers ($p < 0.001$, n = 8).

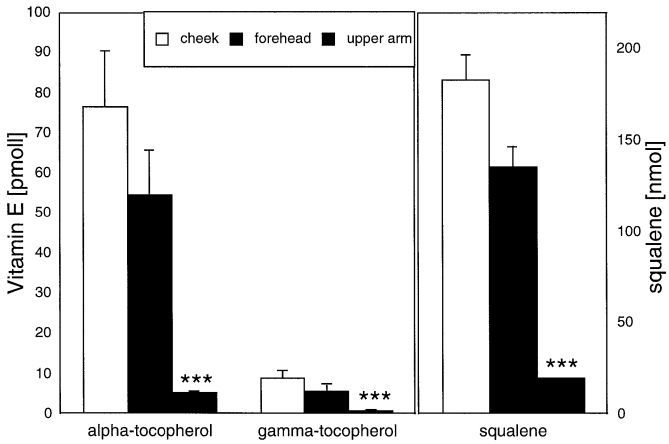

Figure 3. Facial skin surface contains more vitamin E and squalene than the upper arm. Sebum from the forehead, the cheek, and the upper arm was collected as described in *Materials and Methods*. The total amount collected in 60 min is shown. Whereas no significant difference was found between the two facial locations, both were higher in both α- and γ-tocopherol than the surface of the upper arm (both ***$p < 0.001$, n = 8). The same pattern was observed for squalene (***$p < 0.001$, n = 8).

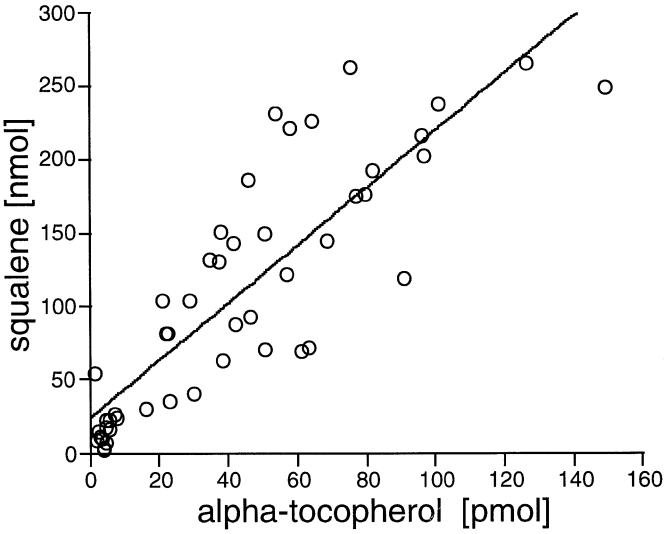

Figure 4. Levels of α-tocopherol and squalene in sebum are closely correlated. Sebum samples were collected from upper arm, forehead, and cheek sites as described in *Materials and Methods* and thereafter analyzed for tocopherol and squalene levels by HPLC using electrochemical detection. All data pairs from all locations were pooled for regression analysis ($r^2 = 0.86$, $p < 0.0001$).

$r^2 = 0.71$, $p < 0.0001$ for γ-tocopherol) (**Fig 4**), with sebum weight ($r^2 = 0.68$, $p < 0.001$ for α-tocopherol and $r^2 = 0.60$, $p < 0.0001$ for γ-tocopherol) and with sebum quantitation ($r^2 = 0.52$, $p < 0.0001$ for α-tocopherol and $r^2 = 0.49$, $p < 0.0001$ for γ-tocopherol). In addition, the amount of squalene correlated well with sebum weight ($r^2 = 0.75$, $p < 0.0001$), as well as with visual sebum quantitation ($r^2 = 0.56$, $p < 0.0001$). These correlations indicate, that vitamin E indeed constitutes a part of the sebum.

DISCUSSION

This study demonstrates: (i) increased levels of α-tocopherol in the upper SC of facial skin *versus* upper arm skin (**Fig 1**); (ii) sebaceous gland secretion of α-tocopherol as the responsible mechanism of α-tocopherol delivery to facial skin surface lipids and upper SC (**Fig 3**); and (iii) a close correlation between squalene and α-tocopherol levels in sebum (**Fig 4**).

Intriguingly, whereas a large body of evidence points to photoprotective effects of topically applied vitamin E against immunosuppression, DNA damage, and carcinogenesis, little is known about the role of physiologic vitamin E regulation in cutaneous tissues. Remarkably, the herein reported human sebum levels of α-tocopherol (moles per wet weight) are more than 3-fold higher than levels found in human blood plasma (Lang *et al*, 1986), human dermis, epidermis (Shindo *et al*, 1994), and SC (Thiele *et al*, 1998). As was demonstrated for other sebum lipids (Blanc *et al*, 1989), and for exogenously applied α-tocopherol (Traber *et al*, 1998), sebaceous α-tocopherol is likely to penetrate into subjacent SC layers. Thus, sebaceous α-tocopherol could account for the increased levels of α-tocopherol detected in the upper SC of the sebaceous gland regions of facial skin as compared with upper arm skin (**Fig 1**). These findings suggest that sebaceous gland secretion is a relevant physiologic delivery pathway of α-tocopherol to sebaceous gland-rich skin regions, such as the environmentally exposed facial skin. Similar to α-tocopherol, which is a vitamin and hence must be provided by oral uptake, orally administered drugs have been reported to be transported to the skin surface and the SC by the sebaceous gland secretion route (Faergemann *et al*, 1995). It should be pointed out, however, that the sebaceous gland secretion route appears to be mostly responsible for the α-tocopherol levels in the upper SC layers of sebaceous gland rich regions. The steep vitamin E gradient with the highest levels in the lower SC found in arm skin (**Fig 1**) indicates that α-tocopherol is delivered from differentiating epidermal cells and thus is based, at least in part, on the epidermal turnover rate.

As the levels of α-tocopherol in sebum are substantially higher than those reported for skin and most other body tissues and fluids (Fuchs, 1992), the question arises whether this serves a physiologic defense strategy to protect specific sebum lipids. A prime candidate appears to be squalene, a major sebum constituent of predominantly sebaceous origin (Stewart and Downing, 1985). Remarkably, a very close correlation was found between sebum levels of α-tocopherol and squalene (**Fig 4**). Considering the chain-breaking anti-oxidant properties of vitamin E in the protection against lipid peroxidation, this finding might of be great relevance for maintaining a physiologic balance of squalene and its UVA/UVB-induced peroxides in the uppermost epidermal layers and surface lipids. Upon oxidative challenges with singlet oxygen, squalene is readily oxidized to mono-, di-, and tri-hydroperoxides, whereas unsaturated phospholipids are more stable (Nakano *et al*, 1998). In skin, singlet oxygen is produced upon UVA exposures of endogenous and exogenous photosensitizers, such as psoralens and chlorpromazine, and leads to the decomposition of squalene and the formation of squalene peroxides (Fujita *et al*, 1986; Hayakawa and Matsuo, 1986). Among the various human skin surface lipids, squalene was shown to be the most susceptible to UVB-induced photooxidation (Picardo *et al*, 1991b). Furthermore, it has been demonstrated that both UVB and UVA irradiation of squalene yield squalene peroxides and malondialdehyde, a stable, cytotoxic lipid peroxidation product (Matsuo *et al*, 1983; Dennis and Shibamoto, 1989; Yeo and Shibamoto, 1992). Notably, there is evidence that squalene oxidation products induce a number of harmful effects in skin cell cultures and *in vivo*, including keratinocyte cytotoxicity (Picardo *et al*, 1991b), histologic changes, immunosuppression (Picardo *et al*, 1991a), and comedogenicity (Mills *et al*, 1978; Motoyoshi, 1983).

Whereas the presence of high levels of vitamin E in human sebum should be sufficient to protect skin surface lipids such as squalene, at least in part, from harmful photooxidation, a number of intriguing questions arise that should be addressed in follow-up studies. These involve possible changes in the physiologic vitamin E balance by sebostatic treatment (e.g., isotretinoin and anti-androgens), physiologic hormonal changes affecting sebaceous gland secretion (pre- and postpuberty; aging skin), and the consequences of frequent face cleansing procedures.

In summary, this study provides conclusive evidence that sebaceous gland secretion is a relevant physiologic delivery route for vitamin E to sebaceous gland rich skin. In view of the adverse effects of squalene peroxides on skin and the powerful anti-oxidative potential of vitamin E, the herein presented correlation between α-tocopherol and squalene levels in human sebum may reflect a physiologic anti-oxidant strategy to maintain low levels of squalene oxidation products in skin surface lipids and their penetration into subjacent skin layers.

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