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Lipophilic Antioxidants in Human Sebum and Aging

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Skin surface lipids (SSL), a very complex mixture of sebum mixed to small amounts of epidermal lipids, mantle the human epidermis, thus representing the outermost protection of the body against exogenous oxidative insults. The present work is a systematic and quantitative analysis of upper-chest SSL and their content in antioxidants in 100 healthy volunteers, divided into five age groups using TLC, HPLC, and GC–MS methods. Further, the effect of exposing SSL *in vitro* to increasing doses of UV irradiation was examined.

Straight monounsaturated and diunsaturated as well as branched monounsaturated fatty acids of triglycerides and pooled fractions were found to be higher at maturity than in childhood and in advancing age. Diunsaturated fatty acids were below 3% of the total and constituted exclusively of C18:2$_{\Delta 5,8}$, C20:2$_{\Delta 7,10}$, C18:2$_{\Delta 9,12}$. Squalene, vitamin E (vit. E) and Coenzyme Q_{10} (CoQ_{10}) were found to increase from childhood to maturity to decrease again significantly in old age. Vitamin E and CoQ_{10} were the only known lipophilic antioxidants present in SSL. In spite of their low levels they were found to synergically inhibit the UV induced depletion of squalene, cholesterol and of unsaturated fatty acids of SSL. In fact, exposure of SSL to increasing amounts of UV irradiation led preferentially to lowering of the levels of vit. E and CoQ_{10}. Four minimal erythema dose (MED) (5.6 J/cm^2) were able to deplete 84% vit. E and 70% ubiquinone, and only 13% squalene. Diunsaturated and monounsaturated fatty acids as well as cholesterol were unaffected even following 10 MED UV exposures, which produced a 26% loss of squalene. The same UV dose when applied in the absence of vit. E and CoQ_{10} produced a 90% decrease of squalene.

Keywords: Antioxidants; CoQ_{10}; Sebum; Aging

INTRODUCTION

Skin surface lipids (SSL) are a mixture of sebum and lipids originating from keratinising epidermal cells, mainly corneocytes.[1,2] The SSL in different skin areas is extremely variable, depending on the level of sebaceous lipid (sebum) secreted, and on the number of desquamating epidermal cells. In areas rich in sebaceous glands, such as scalp, face, chest and upper back, over 90% SSL are of sebaceous origin and thus represent sebum composition fairly accurately.[1–3]

The chemical composition of the different lipid fractions, the mechanism of sebum biosynthesis and the hormonal control of the sebaceous gland activity have been thoroughly studied.

Human SSL, which have been extensively studied also by our group,[1–9] mainly consist of triglycerides (about 60%) and their hydrolysis byproducts generated to a variable extent by the lipases of resident microflora.[10] Monoene fatty acids show a typical *cis* Δ6 pattern (Δ6 family), and, among the C18:2$_{\Delta 5,8}$ (sebaleic acid) is typical of sebum. Wax esters constitute about 25% of SSL, squalene is about 12% and cholesterol less than 3%.

Sebum production varies as a function of age and sex, being related to changes in gonadal and adrenal function. It is always higher in males than in females, it is low during childhood, rises with puberty, reaches a maximum at maturity, and then falls, early and more conspicuously in women, during senescence.[8,11,12]

Being closely related to the outermost barrier of the body, the stratum corneum, sebum is exposed to

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a highly pro-oxidative environment, mainly represented by ultraviolet solar radiation. Sebaceous gland secretion was shown to represent a relevant physiologic pathway for the delivery of vitamin E to upper levels of facial skin.[13] In the present paper, in order to obtain a deeper insight on the composition and on the antioxidant function of sebum, we performed a systematic and quantitative analysis of SSL and their lipophilic antioxidant content in healthy individuals; we also evaluated their variability as a function of age and sex, and checked the relative resistance of various lipid components towards UV irradiation.

MATERIALS AND METHODS

Lipid composition and lipophilic antioxidants (ubiquinol, $CoQ_{10}H_2$; ubiquinone, CoQ_{10}; d-RRR; α-tocopherol, vit. E; β-carotene; lycopene; vitamin A, vit. A) were assayed in SSL from 100 healthy volunteers, with informed consensus for the study. Volunteers were divided into five age groups, A, B, C, D, E, each including 20 subjects—10 males and 10 females—for each group: 6–10 (A), 15–20 (B), 21–40 (C), 45–60 (D), and 65–80 years (E).

SSL Collection

The subjects had previously been instructed not to apply cosmetic products for the preceding week; then, one day before collection of sebum, the selected skin area was defatted with diethyl ether. To collect sebum a short open-ended glass cylinder (6.0 cm inside diameter) was firmly pressed to the upper chest of each volunteer.[9] Twenty millilitre of peroxide-free diethyl ether, containing 300 μg of pentadecane and 300 ng of CoQ_9 as internal standards, were added to the cylinder and removed by a pipette after 2 min. The extraction procedure was repeated with 10 ml of peroxide-free diethyl ether. The pooled diethyl ether solution was run through a sintered glass filter in order to remove epidermal debris and then evaporated to dryness under a nitrogen stream. The lipid extract was dissolved in 1 ml of chloroform–methanol, 2/1 v/v, and divided into 200 μl aliquots, which were placed in screw cap pyrex tubes. These, after evaporation of the solvents with nitrogen, were stored under nitrogen at −80°C and their weight and composition analysed within few days. In order to obtain larger amounts of SSL, to use for the UV irradiation, the extraction procedure was repeated on subsequent days in 10 volunteers from 20–45 years group by using glass cylinders with an internal diameter of 10 cm. CoQ_9 as internal standard was in this case added after irradiation of the samples.

Fatty Acid Composition of Triglycerides and FFA Pooled Fractions

Fatty acid composition of triglycerides and FFA pooled fractions was performed on one aliquot of SSL. Triglycerides and FFA fractions were purified by thin layer chromatography and methylated with boron trifluoride–methanol, 20% w/v. The resulting fatty acid methyl esters were analysed by capillary gas chromatography-mass spectrometry (GC-MS) according to Passi *et al.*[9]

Determination of Squalene and Cholesterol

Quantification of squalene and cholesterol was performed by capillary GC-MS on one SSL aliquot according to Passi *et al*.[9]

Analysis of Lipophilic Antioxidants

Determination of vit. E, $CoQ_{10}H_2$ and CoQ_{10} in the sebum: vit. E, $CoQ_{10}H_2$ and CoQ_{10} were quantified simultaneously by a 10 A VP Shimadzu liquid chromatograph on an analytical Supelcosil LP-18 column (24 cm × 4.6 mm, 5 μm, Supelco) plus its guard column, by using both photodiode array (SPD-M Shimadzu) and electrochemical detectors in cascade (ESA CoulArray Bedford, MA) operated with a −600 mV reduction potential and a +600 mV oxidation potential. Ubiquinone 9 (CoQ_9) was used as internal standard, once it was ascertained this molecule was not present in sebum. Mobile phase: 50 mM sodium perchlorate in methanol/isopropanol, 55/45, v/v, flow: 0.9 ml/min.

0.3 ml of 1 M KCl, 30 μl of 5 M acetic acid, and 10 μg of BHT were added to the screw cap pyrex tube containing one SSL aliquot. Three ml of ethanol–hexane (2:5) are added and the mixture vortexed vigorously for at least 2 min and then centrifuged (2000*g* × 1 min at 4°C). The hexane phase was collected and the mixture extracted again with 2 ml hexane. This procedure allowed a quantitative extraction of the lipids, as shown by the recovery of the internal standard, and this is in accordance with data from the literature.[14] The combined hexane phases were taken to dryness under nitrogen and resuspended in 200 μl of methanol/isopropanol, 30/70 (v/v). One hundred μl of this extract were injected into the HPLC system. Vit. E, $CoQ_{10}H_2$, CoQ_{10}, and CoQ_9 were quantified by comparing the areas to those of authentic standards.

Determination of $CoQ_{10}H_2$ and CoQ_{10} in plasma: 500 μl of plasma were placed in a screw cap pyrex tube containing 0.5 ml 1 M KCl, 100 μl 5 M acetic acid, and 20 μg BHT. Twenty μg ubiquinol-dicaprilate (reference standard), 0.5 μg CoQ_9H_2 and 5 ml ethanol–hexane (2:5) were added and the mixture vortexed vigorously for at least 2 min and then

centrifuged (2000*g* × 1 min at 4°C). The hexane phase was collected and the mixture extracted again with 3 ml hexane. The combined hexane phases were taken to dryness under nitrogen and resuspended in a known volume of methanol/isopropanol, 30/70 (v/v). Half of the sample was injected into the HPLC system. CoQ_9H_2 and/or $CoQ_{10}H_2$ and $CoQ_{10}H_2$ and/or CoQ_{10} were quantified by comparing the areas to those of authentic standards, including the reference standard and taking into account the degradation of CoQ_9H_2.

Determination of vit. A, β-carotene and lycopene: vit. A, β-carotene and lycopene were determined by HPLC as described previously.[15]

UV-irradiation of SSL

Pooled SSL from C group volunteers were dissolved in chloroform:methanol, 2/1 (v/v), and divided into 200 µl aliquots, each corresponding to SSL from approximately 28 cm^2. The aliquots were placed into beakers (6 cm inside diameter), which, after evaporation of solvents under nitrogen, were exposed to different levels of UV irradiation, by using a Dermalight Vario 1 professional lamp (Dr Hohle, medizintechnick GmbH, Munchen, D) fitted with a H2 filter. The lamp stimulates the full solar UV spectrum (UV-A plus UV-B, 295–400 nm) with an uniform elliptic irradiation field of approximately 1450 cm^2 at 90 cm from the lens. Fluence of the lamp at this distance is 3.4 mW/cm^2. The uniform output was measured with a Dermalight UV radiometer (Dr Hohle, Medizintechnick GmbH, Munchen, D). Three aliquots were used for each level of UV exposure (0, 1, 2, 4, 6, 8, 10 minimal erythema dose (MED)—corresponding to 0, 1.4, 2.8, 5.6, 8.4, 14 J/cm^2 UV dose). Using phototype III human volunteers, 1 MED was estimated to result from 7 min exposure (1.4 J/cm^2). After exposure, SSL were supplemented with 300 ng of CoQ_9 as internal standard, extracted and analysed as described above.

Plasma and Sebum Total CoQ_{10} Following Oral Supplementation With Ubiquinone

Five volunteers from group C were orally treated daily with 200 mg of CoQ_{10} for 10 days. At the end of the treatment, CoQ_{10} and $CoQ_{10}H_2$ were extracted from plasma and SSL and quantified as described above.

Statistical Analysis

Statistical analysis of the antioxidant levels in the different groups were performed by Mann–Whitney *U* Test. Differences between groups were considered statistically significant at $P < 0.05$.

RESULTS

Carbon skeletal types of fatty acids of triglycerides and FFA pooled fractions in the SSL from differently aged healthy individuals are reported in Table I. Types of diunsaturated fatty acids are specified in the legend. Since there were no significant differences between males and females within the same group, the results were tabulated together. Straight monounsaturated and diunsaturated, as well as branched monounsaturated fatty acids were significantly lower in individuals from groups A and E (6–10 and 65–80 years, respectively) than in those belonging to group C (21–40 years). More specific details on the fatty acid composition and carbon skeletal types of wax esters and cholesterol esters were reported in our previous works.[8–10] In our present study CoQ_{10} and vit. E were also investigated and they resulted as the only lipophilic antioxidants of SSL. Their levels peaked in volunteers from group C, and were significantly decreased in those from the furthermost groups A and E. Such behaviour paralleled that of squalene, a peculiar and remarkable sebum

TABLE I Age variations in fatty acids of triglycerides and FFA pooled fractions in SSL of healthy volunteers

		Fatty acids				
		Straight (%)			Branched (%)	
Age cohorts (years)	Total SSL (µg/cm^2)	Saturated	Monounsaturated	Diunsaturated	Saturated	Monounsaturated
6–10 (A)	84 ± 18*†	52.4 ± 5.3**	31.6 ± 5.2**	1.8 ± 0.4**	12.1 ± 2.2	2.1 ± 0.4**
15–20 (B)	134 ± 10	45.2 ± 3.2	37.2 ± 4.0	2.4 ± 0.3	12.4 ± 1.6	2.8 ± 0.5
21–40 (C)	140 ± 11	44.3 ± 4.0	38.3 ± 3.7	2.7 ± 0.3	11.7 ± 0.8	3.0 ± 0.4
45–60 (D)	122 ± 14	45.8 ± 5.3	35.8 ± 4.9	2.2 ± 0.5	13.6 ± 2.5	2.6 ± 0.3
65–80 (E)	99 ± 15*†	53.8 ± 6.4**	30.6 ± 4.1**	2.1 ± 0.3**	11.5 ± 3.0	2.0 ± 0.5**

There were no significant difference between males and females within the same group and therefore the results are tabulated together. Statistical analysis is by Student's *t*-test. Straight saturated: saturated FA with both even and odd number of C atoms from C12:0 to C26:0. Straight monounsaturated: monounsaturated Delta 6 family FA with both even and odd number of C atoms from C14:1 to C24:1. Straight diunsaturated: mainly $C18:2_{\Delta5,8}$ and $C20:2_{\Delta7,10}$ (plus traces of $C18:2_{\Delta9,12}$). Branched saturated and monounsaturated: iso, antiso and other methyl branched saturated and monounsaturated FA with even an odd number of C atoms. $*p < 0.001$ and $**p < 0.01$ vs. the corresponding values of group C. $^{\dagger}p < 0.01$ vs. the corresponding values of group B and D.

TABLE II Age and sex variations of vitamin E, CoQ_{10}, squalene, and cholesterol in SSL from healthy volunteers

Age range (years) and sex	Vitamin E ($ng/10\,cm^2$)	Coenzyme Q_{10} ($ng/10\,cm^2$)	Squalene ($\mu g/10\,cm^2$)	Cholesterol ($\mu g/10\,cm^2$)
6–10(A)				
M	133 ± 21*†	120 ± 24*†	112 ± 14*‡	11.4 ± 2*†¶
F	127 ± 27*†	116 ± 27*†	110 ± 13*‡	11.6 ± 2*†¶
15–20 (B)				
M	186 ± 28	161 ± 22	135 ± 14	16.6 ± 2
F	169 ± 29	160 ± 17	130 ± 14	16.3 ± 2
22–42 (C)				
M	195 ± 32	176 ± 23	147 ± 10	17.9 ± 1
F	178 ± 25	173 ± 26	141 ± 10	16.8 ± 2
46–60 (D)				
M	183 ± 27	156 ± 24	134 ± 13	17.6 ± 3
F	166 ± 31	153 ± 23	127 ± 15	17.1 ± 3
70–85 (E)				
M	154 ± 27***	128 ± 24**	123 ± 8*	18.2 ± 2
F	141 ± 18***	124 ± 32**	118 ± 10*	17.8 ± 1

M: males, F: females. Each results represents the mean ± SEM of 20 experiments. $*p < 0.001$, $**p < 0.01$, and $***p < 0.05$ vs. the corresponding (sex) values of group C. $^{\dagger}p < 0.01$ vs. the corresponding (sex) values of group B and D. $^{\ddagger}p < 0.05$ vs. the corresponding (sex) values of group B and D. $^{\P}p < 0.01$ vs. the corresponding (sex) values of group E.

fraction, while the percentage concentrations of cholesterol were significantly lower in group A (Table II), probably because of the diminished levels of SSL in young people as compared to the other groups. $CoQ_{10}H_2$, vit. A, lycopene, and β-carotene were not detected.

Within the same group squalene, CoQ_{10}, and vit. E were constantly, but not significantly, higher in males than females.

The administration of CoQ_{10} to humans resulted in 185–215% plasma increase of total CoQ_{10} ($CoQ_{10} + CoQ_{10}H_2$), without any significant consequence on the amounts of CoQ_{10} in SSL.

The exposure of SSL to increasing amounts of UV irradiation led to the depletion mainly of CoQ_{10} and vit. E. A dose of 4 MED ($5.6\,J/cm^2$) was able to deplete 84.2% vit. E and 70% CoQ_{10} and only 13% squalene. When isolated, i.e. in the absence of other lipid fractions and lipophilic antioxidants, squalene was depleted by UV irradiation approximately at the same rate as CoQ_{10} (see Table III). Unsaturated fatty acids and cholesterol resulted unaffected even following 10 MED UV light exposure, which produced 26% decrease of squalene (see legend of Table III).

DISCUSSION

When discussing cutaneous antioxidants, sebum needs to be considered in addition to dermis and epidermis for which a considerable amount of data already exists.[16–24] A depletion of antioxidants produced by UV irradiation on dermis and epidermis has been reported.[17,18] Our group previously investigated enzymatic and nonenzymatic antioxidants in the viable layers of epidermis of healthy individuals and patients affected with active vitiligo[19] in whom a significant depletion of $CoQ_{10}H_2$, vit. E, enzymatic antioxidants and polyunsaturated fatty acids was found.

Extensive work has been carried out on the antioxidant network of the stratum corneum (SC) by Thiele and coworkers,[13,20–24] and their research is particularly close to our issue, which regards sebum lipids and their reactivity towards environmental oxidative insult. Those studies highlight the role of α-tocopherol as the major antioxidant in the human SC: its depletion together with the presence of carbonyl groups in SC keratins are early and sensitive biomarkers of environmentally induced oxidation.[22–24] Moreover, a strong correlation was

TABLE III Depletion of lipophilic antioxidants in SSL following exposure to increasing levels of UV light irradiation

UV light exposure (MED)	Vitamin E ($ng/10\,cm^2$)	CoenzymeQ_{10} ($ng/10\,cm^2$)	Squalene ($\mu g/10\,cm^2$)	Isolated squalene ($\mu g/10\,cm^2$)	Cholesterol ($\mu g/10\,cm^2$)
0	196 ± 17	173 ± 15	139 ± 16	139 ± 16	1.8 ± 0.2
1	112 ± 20*	132 ± 24*	139 ± 13	106 ± 8*	1.8 ± 0.3
2	72 ± 23*	92 ± 17*	126 ± 13	75 ± 12*	1.7 ± 0.2
4	31 ± 10*	52 ± 14*	121 ± 15**	46 ± 8*	1.7 ± 0.3
6	14 ± 6*	33 ± 10*	116 ± 17**	32 ± 5*	1.7 ± 0.4
8	8 ± 4*	21 ± 7*	111 ± 17**	20 ± 4*	1.6 ± 0.3
10	1 ± 1*	13 ± 5*	103 ± 15*	16 ± 4*	1.6 ± 0.4

Each result represents the mean of three experiments. Diunsaturated fatty acids ($C18{:}2_{\Delta5,8}$, $C20{:}2_{\Delta7,10}$, $C18{:}2_{\Delta9,12}$) as well as monounsaturated fatty acids of triglycerides and FFA pooled fractions were unaffected even following 10 MED UV light exposure. $*p < 0.001$ and $**p < 0.01$, respectively vs. 0 MED. Isolated squalene is squalene irradiated in the absence of CoQ_{10} and vit. E.

found between vit. E and the levels of co-secreted squalene, suggesting that sebaceous gland secretion is a major physiological route of vit. E delivery to skin.[13]

SSL mantle the epidermis, thus representing the ultimate barrier of the body against exogenous oxidative insults, above all from the harmful UV rays. Most of the lipids in the skin surface come from the sebaceous glands, which excrete an oily–waxy material known as sebum. Its amount in a particular skin area cannot be correlated exactly with the number of sebaceous glands present (these are absent only in the palms and soles), since sebum is able to flow, through the stratum corneum, from sites with a high density of glands to areas of low density. Our study deals essentially with the sebum from the upper chest, as the contribution of epidermal lipids is negligible. This is shown by the lack of appreciable amounts of typical fatty acids, namely oleic and linoleic acid (see legend of Table I) which are known to be of epidermal origin.[25] Total SSL also vary according to sex and age:[8,11,12] they are constantly, although not significantly, higher in males than in females and they peak at maturity, in concurrence with the maximum activity of the sebaceous glands. They are significantly lower in childhood and old age, when gland activity is reduced. The same behaviour was found, in the present work, for monounsaturated and diunsaturated, as well as branched monounsaturated fatty acids of triglycerides and FFA pooled fractions (Table I). The decreased amounts of diunsaturated fatty acids (approximately 2.5–3%, and represented exclusively by C18:$2_{\Delta5,8}$, C20:$2_{\Delta7,10}$ with traces of C18:$2_{\Delta9,12}$) coupled to both lack of other polyunsaturated fatty acids and the high percentage of saturated and monounsaturated fatty acids (Table I), allow sebum to be widely resistant to oxidative attack. The resistance is further enhanced by the presence of squalene, a peculiar sebum fraction present in large amounts in human SSL (approximately 12% to total SSL), but completely absent from skin lipids of non human primates.[26] This open chain triterpenoid hydrocarbon, containing 30 carbon atoms and six double bonds, is normally cyclised to form a tetracyclic steroid skeleton in two steps: squalene epoxidase catalyses the oxidation of squalene to form 2,3-oxidosqualene, which is converted, through a complex process, by squalene oxido-cyclase to lanosterol, the precursor of cholesterol.[27] Literature does not report the presence of significant quantities of this polyunsaturated hydrocarbon in the sebum of any animal species except for the otter, beaver, and the kinkayou.[28] Probably it exerts a powerful water-repellent function in these three aquatic animals,[28] but one is led to wonder why other aquatic animals and birds have evolved different cutaneous lipid fractions such as wax esters and wax diesters with this essential property. It is also unknown why only man, in the primate superfamily, displays squalene in sebum. A reasonable explanation could be that the natural deficiency, in human skin, of squalene epoxidase and squalene oxido cyclase activity has constituted a strategic step in human evolution, as squalene is capable of counteracting reactive oxygen species induced by UV irradiation on the skin, thus behaving as an indirect natural filter.[29,30] The skin of monkeys, unlike that of humans, is covered by a large quantity of hair, which has a protective effect against UV rays, so that the above-mentioned enzymatic activities are fully efficient in synthesizing cholesterol.[26] In the hairless human skin the protective function is carried out by squalene, in association with the stratum corneum and melanin. When isolated, squalene is easily oxidizable by UV rays and other oxidant species which generate a wide range of oxidation byproducts of varying polarity and reactivity[31] while its oxidation is less rapid when mixed with other SSL constituents.

The present data give further insight regarding this peculiar behaviour: sebum contains vit. E and CoQ_{10} (Table II), which are well known to act as a lipophilic antioxidant duet in biological membranes and in plasma lipoproteins.[32] It was not possible to isolate $CoQ_{10}H_2$, which is probably oxidized to CoQ_{10} during the collection of sebum and extraction procedure. Vitamin A, lycopene and beta-carotene were also undetectable, therefore the antioxidant capacity of sebum appears to rely on vit. E and CoQ_{10}. Vitamin E derives from exogenous sources, while CoQ_{10} has a mainly endogenous origin. This is also indicated by the lack of correlation between the plasma and sebum levels following the administration of 200 mg of CoQ_{10} daily for 10 days to human volunteers (See "Results" section). CoQ_{10} and squalene follow the same biosynthetic pathway starting from acetyl CoA via mevalonate up to farnesyl pyrophosphate, which also acts as a precursor of dolichol. This pathway, as mentioned above, leads in most tissues to cholesterol biosynthesis, but in human sebaceous glands mainly ends up with squalene, and produces, at the same time, small amounts of CoQ_{10}. Similarly to total SSL, we also found that squalene, vit. E and CoQ_{10} were significantly low in childhood and in old age, thus testifying a functional interplay among these three molecules. In spite of their diminished levels (Table II), vit. E and CoQ_{10} work synergically to inhibit the UV induced depletion of squalene and other unsaturated lipid molecules of SSL (Table III). The exposure of SSL to increasing doses of UV irradiation leads preferentially to decreased levels of vit. E and CoQ_{10}. Four MED (5.6 J/cm^2) are able to deplete 84.2% vit. E and 70% ubiquinone, and only 13% squalene (Table III). Diunsaturated and monounsaturated fatty acids, and cholesterol are

unaffected even following 10 MED UV exposure, which produces a 26% squalene loss. Regarding this point, Podda *et al.*[33] showed that $CoQ_{10}H_2$ and CoQ_{10} were the most sensitive molecules to UV exposure. Three (4.2 J/cm^2) and 6 MED (8.4 J/cm^2) UV light dose led to a complete disappearance of $CoQ_{10}H_2$ and CoQ_{10}, respectively. Vitamin E was a little less susceptible to depletion, as it required a higher dose of irradiation for its disappearance. These findings differ slightly from our present results. In fact in our sebum extract vit. E is probably a little more vulnerable than ubiquinone. A reasonable explanation for this behaviour lies in the fact that in the "skin equivalents" used by Podda *et al.* also ubiquinol was present, which may spare vit. E by regenerating it from α-tocopheryl radical. In the present study we did not find $CoQH_2$, which had probably been oxidised during sebum collection and lipid extraction, therefore ubiquinone and vit. E were two of the targets of UV irradiation. Even though ubiquinol is more reactive than vit. E in systems such as human circulating lipoproteins and "skin equivalents", also the oxidised form of coenzyme Q displays a certain reactivity towards radicals, as we showed in a recent paper.[34] The role of coenzyme Q as a cutaneous antioxidant was highlighted by Hoppe *et al.* and, similarly to what we found in sebum, also in the epidermis there was a decrease of CoQ_{10} with age.[35]

Present data confirm that sebum and its lipophilic antioxidant factors play a synergic, active role in the defensive mechanisms of the outermost skin layers.

References

[1] Nicolaides, N. (1974) "Skin lipids: their biochemical uniqueness", *Science* **186**, 19–26.
[2] Lewis, C.A. and Hayward, B.J. (1971) "Human skin surface lipids", The Modern Trends Series—Modern Trends in Dermatology, 4th ed. (Butterworth and Co., London), pp 89–121.
[3] Downing, D.T. and Strauss, J.S. (1974) "Synthesis and composition of surface lipids of human skin", *J. Invest. Dermatol.* **62**, 228–244.
[4] Vantrou, M., Venencie, P.Y. and Chaumeil, J.C. (1987) "Skin surface lipids in man: origin, synthesis and regulation", *Ann. Dermatol. Venereol.* **114**, 1115–1129.
[5] Downing, D.T., Stewart, M.E., Wertz, P.W., Colton, 6th, S.W. and Strauss, J.S. (1983) "Skin lipids", *Comp. Biochem. Physiol.* **76**, 673–678.
[6] Stewart, M.L. (1992) "Sebaceous gland lipids", *Semin. Dermatol.* **11**, 100–105.
[7] Clarys, P. and Barel, A. (1995) "Quantitative evaluation of skin surface lipids", *Clin. Dermatol.* **13**, 307–321.
[8] Nazzaro-Porro, M., Passi, S., Boniforti, L., *et al.*, (1979) "Effects of aging on fatty acids in skin surface lipids", *J. Invest. Dermatol.* **73**, 112–117.
[9] Passi, S., Picardo, M., Morrone, A., De Luca, C. and Ippolito, F. (1991) "Skin surface lipid in HIV sero-positive and HIV sero-negative patients affected with seborrheic dermatitis", *J. Dermatol. Sci.* **2**, 84–91.
[10] Passi, S., Grandinetti, M., Stancato, A. and De Luca, C. (1996) "Invecchiamento Cutaneo", *Chronica Dermatologica* **6**, 193–216.
[11] Cotterill, J.A., Cotterill, W.J., Williamson, B. and Bulusu, L. (1972) "Age and sex variation in skin surface lipid composition and sebum excretion rate", *Br. J. Dermatol.* **87**, 333–340.
[12] Pochi, P.E. and Strauss, J.S. (1974) "Endocrinological control of the development and activity of the human sebaceous gland", *J. Invest. Dermatol.* **62**, 191–201.
[13] Thiele, J.J., Weber, S.U. and Packer, L. (1999) "Sebaceous gland secretion is a major physiologic route of vitamin E delivery to skin", *J. Invest. Dermatol.* **113**, 1006–1010.
[14] Edlund, P.O. (1988) "Determination of coenzyme CoQ_{10}, α-tocopherol and cholesterol in biological samples by coupled-column liquid chromatography with coulometric and ultraviolet detection", *J. Chromatogr.* **425**, 87–97.
[15] De Luca, C., Filosa, A., Grandinetti, M., Maggio, F. and Passi, S. (1999) "Blood antioxidant status and urinary levels of catecholamine metabolites in β-thalassemia", *Free Radic. Res.* **30**, 453–462.
[16] Shindo, Y., Witt, E. and Packer, L. (1993) "Antioxidant defence mechanisms in murine epidermis and dermis and their response to ultraviolet light", *J. Invest. Dermatol.* **100**, 260–265.
[17] Shindo, Y., Witt, E., Han, D., Epstein, W. and Packer, L. (1994) "Enzymic and non enzymic antioxidants in epidermis and dermis of human skin", *J. Invest. Dermatol.* **102**, 122–124.
[18] Fuchs, J., Hufflejt, M.E., Rothfuss, L.M., Wilson, D.S., Carcamo, G. and Packer, L. (1989) "Impairment of enzymic and non enzymic antioxidants in skin by UV irradiation", *J. Invest. Dermatol.* **93**, 769–773.
[19] Passi, S., Grandinetti, M., Maggio, F., Stancato, A. and De Luca, C. (1998) "Epidermal oxidative stress in vitiligo", *Pigment Cell Res.* **11**, 82–85.
[20] Thiele, J.J., Schroeter, C., Hsieh, S.N., Podda, M. and Packer, L. (2001) "The antioxidant network of the stratum corneum", *Curr. Prob. Dermatol.* **29**, 26–42.
[21] Thiele, J.J. (2001) "Oxidative targets in the stratum corneum: a new basis for antioxidative strategies", *Skin Pharmacol. Appl. Skin Physiol.* **1**, 87–91.
[22] Thiele, J.J., Traber, M.G. and Packer, L. (1998) "Depletion of human stratum corneum vitamin E: an early and sensitive marker of UV induced photo-oxidation", *J. Invest. Dermatol.* **110**, 756–761.
[23] Weber, S.U., Thiele, J.J., Cross, C.E. and Packer, L. (1999) "Vitamin C, uric acid and glutathione gradients in murine stratum corneum and their susceptibility to ozone exposure", *J. Invest. Dermatol.* **113**, 1128–1132.
[24] Thiele, J.J., Traber, M.G., Re, R., Espuno, N., Yan, L.J., Cross, C.E. and Packer, L. (1998) "Macromolecular carbonyls in human stratum corneum: a biomarker for environmental oxidant exposure", *FEBS Lett.* **422**, 403–406.
[25] Elias, P.M., Orozco-Topete, R., Lampe, M.A., *et al.* (1985) "Methods in epidermal lipid research", In: Skerrow, D. and Skerrow, C.J., eds, Methods in Skin Research (Wiley, New York), pp 381–404.
[26] De Luca, C., Fanfoni, G.B., Stancato, A. and Passi, S. (1997) "Significant differences in skin surface lipids between man and other primates", *Chem. Phys. Lipids* **88**, 125.
[27] Voet, D. and Voet, J.G. (1990) Biochemistry Lipid Metabolism, (Wiley, New York).
[28] Lindholm, J.S. and Downing, D.T. (1981) "Occurrence of squalene in skin surface lipids of the otter, the beaver and the kinkajou", *Lipids* **15**, 1062–1063.
[29] De Luca, C., Picardo, M., Breathnach, A.S. and Passi, S. (1996) "Lipoperoxidase activity of *Pityrosporum*: characterization of by-products and possible role in *Pityriasis versicolor*", *Exp. Dermatol.* **5**, 49–56.
[30] Kohno, Y., Egania, Y. and Itoh, S. (1995) "Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radicals by squalene in *n*-butanol", *Biochim. Biophys. Acta* **1256**, 52–56.
[31] Dennis, K.J. and Shibamoto, T. (1989) "Production of malondialdehyde from squalene, a major skin surface lipid, during UV irradiation", *Photochem. Photobiol.* **49**, 711–716.
[32] Stocker, R., Bowry, V.W. and Frei, B. (1991) "Ubiquinol-10 protects human low density lipoprotein more efficiently

against lipid preoxidation than does α-tocopherol", *Proc. Natl Acad. Sci. USA* **88**, 1646–1650.
[33] Podda, M., Traber, M.G., Weber, C., Yan, L.-J. and Packer, L. (1998) "UV-irradiation depletes antioxidants and causes oxidative damage in a model of human skin", *Free Radic. Biol. Med.* **24**, 55–65.
[34] Giorgini, E., Tommasi, G., Stipa, P., Tosi, G., Littarru, G.P. and Greci, L. (2001) "Reactivity of ubiquinones and ubiquinols with free radicals", *Free Radic. Res.* **35**, 63–72.
[35] Hoppe, U., Bergemann, J., Diembeck, W., Ennen, J., *et al.*, (1999) "Coenzyme Q_{10}, a cutaneous antioxidant and energizer", *BioFactors* **9**, 371–378.