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Direct evidence for bioconversion of vitamin E acetate into vitamin E: An *ex vivo* study in viable human skin

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Synopsis

For better stability, vitamin E is commonly used as the non-active esterified pro-drug. Such esters are postulated to be hydrolyzed to the free active form by skin-related esterases. So far, successful conversion of esterified vitamin E to free vitamin E (tocopherol) has been mainly delineated from observed biological effects. Quantitative evidence in human skin is poor. *In vitro* and *in vivo* studies on human and animal skin have proved ambiguous. Formulation-based effects may have added to this controversy.

In the present study, comparable amounts of vitamin E acetate (i) in oil (Mygliol-812N), (ii) surfactant-solubilized in water, (iii) encapsulated in liposomes, or (iv) encapsulated in Nanotopes™ were applied to human skin mounted in modified Franz-perfusion chambers that permit emulation of both open or occlusive conditions. The distribution of vitamin E_{total} (vitamin E acetate + vitamin E) was assessed on the skin surface, in the horny layers, and in the underlying skin by high-pressure liquid chromatography (HPLC), with a recovery higher than 90%.

Vitamin E acetate in Mygliol deposited exclusively on the surface and in the stratum corneum. In contrast, solubilized or encapsulated vitamin E acetate deposited also in the underlying skin. Nanotopes™ performed best, followed by liposomes and solubilized vitamin E acetate. Non-occlusive application favored deposition in the skin relative to occlusive application.

Conversion of vitamin E acetate to vitamin E was not observed on the skin surface or in the horny layers, while in the underlying skin up to 50% of the vitamin E_{total} was deacetylated.

INTRODUCTION

Together with vitamin C, vitamin E is the most common non-enzymatic radical scavenger involved in protecting living tissue against oxidative stress and radical damage. Incidentally, the human epidermis contains much less vitamin E than other human tissue (1–4). Indeed, topical supplementation of the skin with vitamin E has led to a

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range of cosmetic effects, such as skin smoothening, moisturizing, prevention of premature skin-aging, suppression of UV-induced erythema, or improved wound healing (4).

Vitamin E is readily oxidized when exposed to atmospheric conditions or light (5). In consequence, free vitamin E is only rarely used in cosmetic formulations. It is replaced by stable esterified pro-drugs, usually vitamin E acetate (6). However, such esters are biologically inactive. If applied topically, vitamin E acetate has been reported to be hydrolyzed in the skin to free vitamin E by esterases (7–9). Yet, despite the many reports documenting their biological activity and implying a conversion of the pro-drug, direct quantitative evidence for a conversion of vitamin E acetate to vitamin E in skin is poor and ambiguous (10,11).

Vitamin E acetate and vitamin E are both lipophilic compounds. They dissolve in the oil phase of a formula, from where they are expected to be poorly liberated. To increase bio-availability they are commonly formulated in the water phase of a cosmetic formula by means of solubilizers or by specific carriers (6).

In the present study, we compared the penetration behavior of vitamin E acetate under non-occlusive and occlusive conditions. In turn, the pro-drug was either dissolved in oil, solubilized in water, or encapsulated in two different carrier systems, i.e., liposomes or Nanotopes™, the latter being detergent-resistant unilamellar vesicles with a diameter of 20–40 nm (12). The amount of both vitamin E acetate and vitamin E was quantified in three compartments: at the skin surface, in the horny layers, and within the underlying skin.

MATERIALS AND METHODS

SKIN BIOPSIES

Human skin was obtained after informed consent from otherwise healthy donors having undergone plastic surgery for stomach reduction. Upon excision, the skin flaps (ellipsoid, 5 × 20 cm) were surgically liberated of the adhering subcutaneous fat layer, cut to the size of the penetration chamber, and immediately stored in PBS (phosphate-buffered saline, pH 7.2–7.4) at 36°C.

TEST FORMULATIONS

Final concentrations of 2% vitamin E acetate were dissolved in Mygliol-812N (caprylic/capric acid triglyceride; Condea Chemie GmbH, [Brunsbüttel, Germany]), solubilized in water by Solubilisant-LRI (PPG-26-buteth-26 and PEG-40 hydrogenated castor oil; LCW-Wackherr [Saint Ouen L'Aumône, France]), encapsulated in liposomes made from soybean phosphatidyl choline with an average mean diameter of 200 nm (Mica Products GmbH [Badenweiler, Germany]), or encapsulated in Nanotopes™ with a mean diameter of about 25 nm (Tinoderm E, Ciba Specialty Chemicals [Basel, Switzerland]).

PENETRATION

Skin flaps were mounted within 1 h after excision in Franz-like perfusion chambers according to Maibach (13), which had been modified as described (14). The skin sup-

ported by a grid was fitted in the permeation chamber (surface area 28 cm^2). Its underside was in contact with degassed PBS, avoiding air bubble formation. The chamber was covered with a bell-shaped lid equipped with cocks and enclosing a volume of 46 cm^3, thus permitting the maintenance of controlled conditions during permeation experiments, e.g., to mimic open or occlusion-like conditions. The maintenance of enzymatic activity in the excised skin was monitored as described (13,14).

Test solutions of 300 µl were applied to the surface of the mounted skin and evenly spread with a glass spatula. Experiments were carried out under controlled conditions at 23°C and at a maintained humidity of 40%. All experiments were carried out in triplicate and with the skin from three different donors. Experiments were rejected if vitamin E_{total} was detected in the PBS media (>100 ng/ml) within 10 min after application.

ASSESSMENT OF VITAMIN E DISTRIBUTION IN SKIN

The distribution of both vitamin E acetate and vitamin E was monitored 8 h after application. Vitamin E_{total} was collected from the skin surface by wiping with a cotton sponge (1× dry, 1× with 200 µl methanol, 1× dry). The horny layer was essentially removed by 20 strippings using adhesive tape (Tesa-Film® [Beiersdorf, Hamburg, Germany]), and the remaining skin was frozen and sectioned. Cotton sponges, tape strips, and skin sections were extracted with methanol/phosphate buffer (50/50, v/v).

QUANTIFICATION OF VITAMIN E ACETATE AND VITAMIN E BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

Concentrated extracts were separated by HPLC using a reverse-phase column (Luna C18, 5 µm, 150-mm length, 4.6 mm Ø, Penomenex® [Aschaffenburg, Germany]) and a mobile phase of methanol/acetonitrile (25/75, v/v). Injection volumes were 100 or 250 µl, and the sensitivity of detection was 25–50 ng/ml.

RESULTS

Four formulations, each containing 2% vitamin E acetate, i.e., dissolved in Mygliol-812N (EM), solubilized in water (ES), or encapsulated in liposomes (EL) or in Nanotopes™ (ET), were tested. Of each formulation 11 mg/cm^2 was applied to the skin, corresponding to 220 µg/cm^2 of vitamin E acetate. The skin was then maintained for 8 h under defined non-occlusive or occlusive conditions, respectively. Then the amount of vitamin E acetate and vitamin E (tocopherol) was determined for the three compartments (skin surface, horny layer, underlying skin). In all experiments, the recovery rate of vitamin E_{total} (vitamin E acetate + vitamin E) exceeded 90%. The vitamin E acetate preparations contained ≤2% of vitamin E prior to application.

DISTRIBUTION OF VITAMIN E_{TOTAL} UNDER NON-OCCLUSIVE CONDITIONS

Under non-occlusive conditions and when applied in the oil phase (EM), 178.3 (±8%, SD = 3) µg/cm^2 vitamin E_{total} was detected on the skin surface and 28.2 (±31%) µg/cm^2

in the horny layer (more than 80% of it in the first five strips [unpublished result]); only 0.1 (±171%) μg/cm^2 was recovered from the underlying skin. In contrast, when applying ES, 97.2 (±5%) μg/cm^2 of vitamin E_{total} deposited at the skin surface, 46.5 (±8%) μg/cm^2 in the horny layer, and 58.2 (±9%) μg/cm^2 in the underlying skin. For encapsulated vitamin E acetate distributed in the horny layer and in the underlying skin, the following amounts were detected. For EL: surface 68.3 (±8%) μg/cm^2, horny layer 52.6 (±8%) μg/cm^2, underlying skin 79.7 (±3%) μg/cm^2. For ET: surface 54.4 (±3%) μg/cm^2, horny layer 53.2 (±4%) μg/cm^2, viable skin 97.3 (±4%) μg/cm^2. The relative distribution of vitamin E_{total} under non-occlusive conditions is illustrated in Figure 1 and summarized in Table I.

DISTRIBUTION OF VITAMIN E_{TOTAL} UNDER OCCLUSIVE CONDITIONS

Under occlusive conditions, with EM, 166.9 (±7%) μg/cm^2 vitamin E_{total} was found on the skin surface and 38.2 (±36%) μg/cm^2 in the horny layer, again concentrated in the first five strips. No vitamin E_{total} was detected in the underlying skin. In contrast, after application in ES 108.8 (±5%) μg/cm^2 of vitamin E_{total} was localized at the skin surface, 61.9 (±8%) μg/cm^2 in the horny layer, and 35.7 (±7%) μg/cm^2 in the underlying skin. A preference of encapsulated vitamin E_{total} for the horny layer and for the underlying skin was observed also under occlusive conditions, yet to a lesser extent. For EL: surface 91.2 (±7%) μg/cm^2, horny layer 74.0 (±3%) μg/cm^2, underlying skin 43.4 (±15%) μg/cm^2. For ET: surface 65.2 (±7%) μg/cm^2, horny layer 68.2 (±5%) μg/cm^2, under-

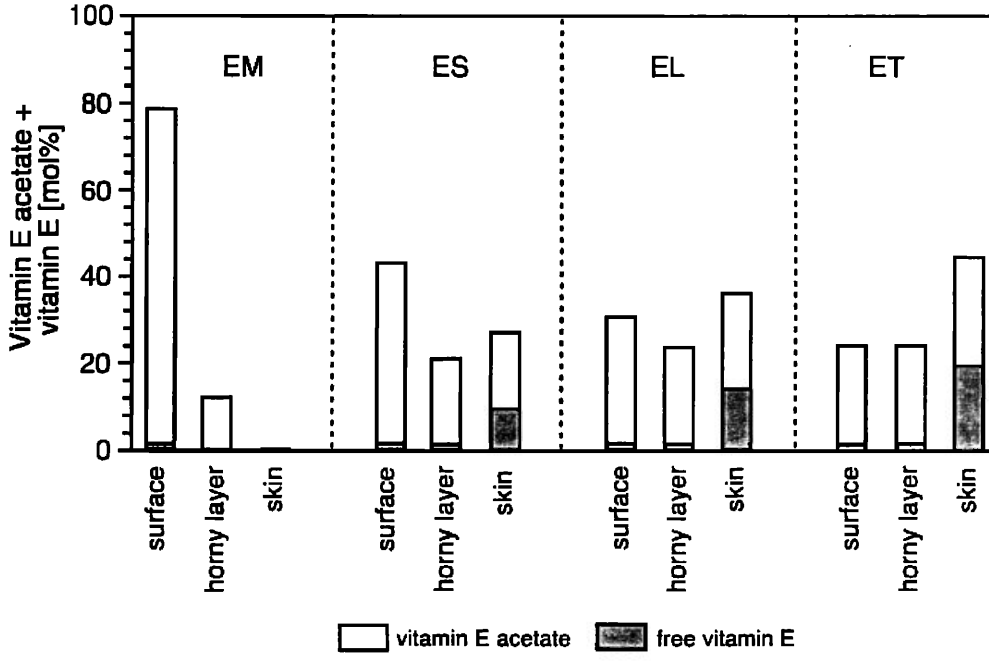

Figure 1. Influence of formulation on distribution of vitamin E acetate and vitamin E in human skin. Application on excised human skin under air exchange (non-occlusive conditions). Vitamin E acetate was dissolved in Mygliol (EM), solubilized in water (ES), and encapsulated in liposomes (EL) and in Nanotopes™ (ET).

Table I
Relative Amount (mol %) of Topically Applied Vitamin E_{total} Recovered From Different Sites of Human Skin *In Vitro*

		Surface		Horny layer		Underlying skin	
	Formulation	Non-occlusive	Occlusive	Non-occlusive	Occlusive	Non-occlusive	Occlusive
EM	Vitamin E_{total}	79.2	74.2	12.6	17.0	0.4	—
	Free vitamin E	1.2	1.2	0.1	0.1	—	—
ES	Vitamin E_{total}	43.2	48.4	20.7	27.6	26.6	16.6
	Free vitamin E	1.3	1.4	0.9	1.3	9.3	8.1
	% Conversion					34.8	48.9
EL	Vitamin E_{total}	30.6	40.6	23.6	33.0	36.8	20.2
	Free vitamin E	1.3	1.9	1.3	1.7	14.5	10.5
	% Conversion					39.4	51.2
ET	Vitamin E_{total}	24.5	29.5	24.0	31.1	45.3	31.7
	Free vitamin E	1.1	1.5	1.1	1.5	19.5	15.6
	% Conversion					43.1	49.2

Topical application of vitamin E acetate onto excised human skin. Vitamin E acetate was dissolved in Mygliol (EM), solubilized in water (ES), and encapsulated in liposomes (EL) and in Nanotopes™ (ET). The amount of vitamin E acetate and vitamin E was quantified by HPLC in the respective extracts. All experiments were carried out in triplicate.

lying skin 66.3 (±7%) μg/cm^2. The relative distribution of vitamin E_{total} under occlusive conditions is illustrated in Figure 2 and summarized in Table I.

BIOCONVERSION OF VITAMIN E ACETATE TO VITAMIN E

The extent of conversion of vitamin E acetate to vitamin E was calculated from the molar ratio of vitamin E_{total} recovered in each compartment as vitamin E, i.e., on the skin surface, in the horny layer, and in the underlying skin. Consequently, the absolute amount of vitamin E depends on both the amount of vitamin E_{total} transported to a compartment and the local activity of ester hydrolysis.

At the skin surface or in the horny layer, the vitamin E determined in all instances essentially corresponded to the less than 2% of the vitamin E contained in the vitamin E acetate formulations prior to application, indicating the absence of relevant esterase activity.

In the underlying skin, however, substantial amounts of free vitamin E were detected. If applied as ES, EL, or ET, 26–45% of vitamin E_{total} was recovered from the underlying skin under non-occlusive conditions (Figure 1) and 16–30% under occlusive conditions (Figure 2). Findings are summarized in Table I. Accordingly, the highest absolute concentration of vitamin E in the skin was obtained after application of ET under non-occlusive conditions (ET: 20%; EL: 15%; ES: 9%; Figure 1), as well as under occlusive conditions (ET: 16%; EL: 11%; ES: 8%; Figure 2).

The conversion rate of vitamin E acetate to vitamin E, i.e., the molar amount of vitamin E_{total} transformed to vitamin E, was calculated as 35% (ES), 39% (EL), or 43% (ET) under non-occlusive conditions. Though the amount of vitamin E acetate deposited

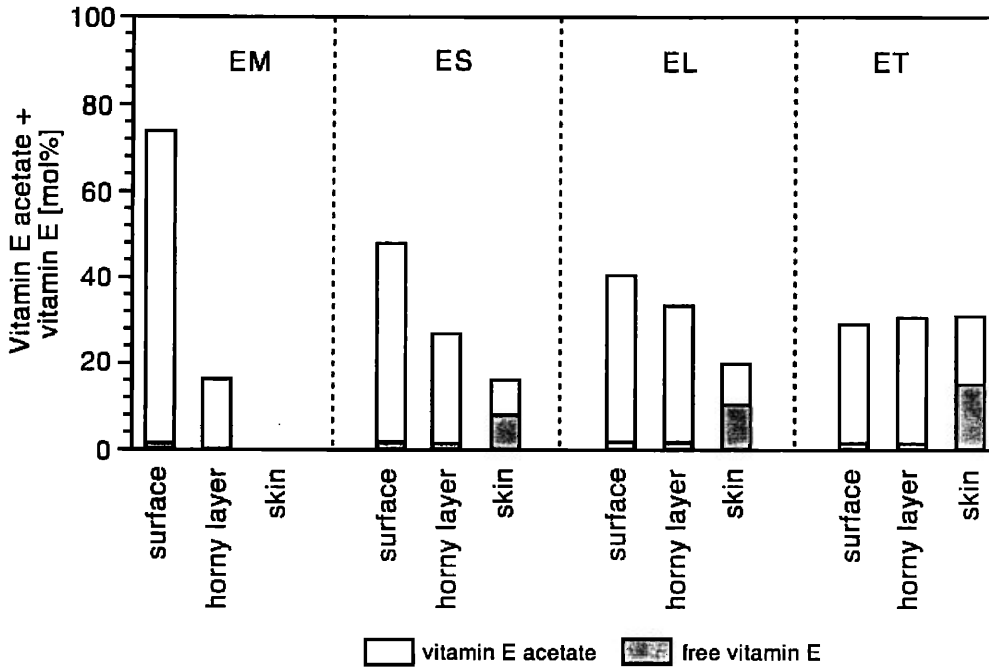

Figure 2. Influence of formulation on distribution of vitamin E acetate and vitamin E in human skin. Application on excised human skin under air occlusive conditions. Vitamin E acetate was dissolved in Mygliol (EM), solubilized in water (ES), and encapsulated in liposomes (EL) and in Nanotopes™ (ET).

under occlusive conditions was smaller, conversion amounted to about 50% in each of the three formulations (see Table I).

DISCUSSION

Vitamin E is known as nature's major lipid-soluble free-radical scavenging antioxidant (4). Due to its instability in the presence of oxygen or light, the use of free vitamin E as a cosmetic active is very limited. Therefore, it has been replaced by stable, oil-soluble precursors, i.e., mainly vitamin E acetate or palmitate. These derivatives are claimed to be converted in the skin to the active vitamin E (4,5).

The present study corroborated that vitamin E acetate is a suitable precursor for vitamin E in topical applications. Quantitative analysis demonstrated that vitamin E acetate was only available in the living skin if formulated into a water phase and not in an oil phase.

Lipophilic substances are incorporated into the water phase either by means of solubilizers or with carrier systems such as liposomes or Nanotopes™. In the present study, Nanotopes™ deposited more vitamin E acetate in skin than liposomes or the solubilizer under both non-occlusive and occlusive conditions. This consistently better performance of ET may relate to the size of Nanotopes™, which is about ten times smaller than that of conventional liposomes (12).

Bioconversion of vitamin E acetate to vitamin E was localized exclusively in the viable skin (most probably in the epidermal layer [Artmann, unpublished]). Hydrolysis was

absent on the skin surface as well as in the horny layer. These findings are in accordance with Landmann (15), who reported extracellular esterases being shed by lamellar bodies in the epidermis.

As demonstrated, the galenic formulation of the vitamin E acetate, as of lipophilic substances in general, is crucial for its skin availability. Beyond the physical and chemical properties of the active, the type of formulation and the phase in which the active is dissolved also determine its fate and the biological activity. In the present study, more than 95% of vitamin E acetate remained either on the skin surface or deposited in the horny layer when applied in an oil. In contrast, when applied in the water phase, up to 50% of vitamin E_{total} was made available to the viable skin, the zone of cosmetic interest. In the best case, up to 20% of the initial amount of vitamin E acetate was recovered as free vitamin E. Controversial findings on the activation of the pro-drug may well be due to differences in formulating vitamin E acetate (7–11).

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