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Therapeutics

Randomized, placebo-controlled, double blind study on the clinical efficacy of a cream containing 5% α-lipoic acid related to photoageing of facial skin

H.BEITNER
Department of Dermatology, Karolinska Hospital, 17176 Stockholm, Sweden

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Summary

Background α-lipoic acid (LA) or the reduced form dihydrolipoate (DHLA) is a potent scavenger with anti-inflammatory properties. Previous uncontrolled studies with topical treatment with 5% LA-containing creams indicate a beneficial effect on photoageing skin.

Objective The purpose of this study was to investigate whether a cream containing 5% LA showed any advantages concerning a number of the criteria associated with ageing of the facial skin, compared with an identical cream lacking LA.

Material and methods Thirty-three women, mean age 54·4 years, were included in this controlled study. After randomization half the face was treated twice daily for 12 weeks with the LA cream and the other half with the control cream. The following methods of assessment were used: self-evaluation by the test subjects, clinical evaluation, photographic evaluation and laser profilometry. Profilometry was performed before the start of treatment and at the end.

Results All four methods of assessment showed a statistically significant improvement on the LA-treated half of the face. Laser profilometry, the most objective method used, showed an average decrease in skin roughness of 50·8% (44·9–54·0) on the LA-treated side, compared with 40·7% (32·4–48·7) on the placebo-treated half of the face $P < 0{\cdot}001$ (Wilcoxon matched pairs test).

Conclusions It is indicated that 12 weeks of treatment with a cream containing 5% LA improves clinical characteristics related to photoageing of facial skin.

Key words: α-lipoic acid, antioxidant, intrinsic ageing, photoageing

Ageing of the skin consists of a combination of intrinsic ageing and photoageing. Intrinsic or chronological ageing is mainly a result of genetically predisposed factors resulting in a thinner epidermis, increased fragility, a decrease in dermal thickness and vascularity, a reduction in the number of dermal fibroblasts and in their ability to synthesize, and a decreased response to growth factors. No topical treatment exists that significantly affects intrinsic ageing.

Photoageing results in clumping of melanocytes, elastosis and atrophy of the dermis with loss of collagen. The corresponding clinical manifestations are mottled hyperpigmentation, wrinkles, change of the superficial texture resulting in a coarseness of the skin and the formation of comedones. Photoageing can be modified by retinoic acid in combination with effective sun protective measures.[1]

α-lipoic acid (LA) was isolated in 1951 by Reed and coworkers.[2] LA, or the reduced form dihydrolipoate (DHLA), is a potent scavenger of hydroxyl radicals, superoxide radicals, peroxyl radicals, singlet oxygen and nitric oxide. LA also plays an important role in the mitochondrial dehydrogenase processes and as a modulator of the inflammatory response. LA is insoluble in water, but soluble in organic solvents. The small molecular weight of 206·3 in combination with the solubility characteristics suggests the possibility of LA being absorbed by the skin and, in the skin, exercising pharmacological activities.[3] Penetration of

Correspondence: Assoc. Prof. Harry Beitner.
E-mail: harry.beitner@ks.se

the skin has been shown in hairless mice. Kinetics of cutaneous and subcutaneous distribution after topical application of LA on hairless mice demonstrates swift penetration through the epidermis and, after 4 h, LA is distributed in the dermis and the subcutaneous tissue.[4] The effect of LA on a cellular level indicated at first that this could be considered to be a vitamin. However, both animals and humans can synthesize LA, although the exact mechanism is not yet fully understood.[5] Thus, LA is at present considered to be a coenzyme in the citric acid cycle of the mitochondria. Ketoglutarate dehydrogenase and pyruvate dehydrogenase contain LA.[6] Oxidative damage of the DNA, particularly the mitochondrial DNA (mtDNA), accumulates with increasing age. It has been shown that age-related decay of mitochondrial function can be improved by the addition of LA.[7–9]

Other compounds in the creams tested that might exercise a pharmacological effect are coenzyme Q_{10} (Q_{10}) and acetyl-L-carnitine (AC). Q_{10} is synthesized both in animals and humans.[9] Most of Q_{10} is absorbed in the stratum corneum, but in an ethanol solution 20% is absorbed in the basal layers of the epidermis and 2% in the papillary dermis.[10] Q_{10} is, in its reduced form, ubiquinol, an antioxidant present in all biological membranes.

AC has a very important function in the citric acid process by enhancing the uptake of acetyl coenzyme A (acetyl CoA) in the reduction of fatty acids. Whether skin penetration occurs is not known, but the binding of AC to fatty acids is a characteristic that might facilitate skin penetration. In bovines, AC penetrates the cornea in calf eyes.[11] AC is usually administered orally, 1–3 g day^{-1}, or intravenously, 1·5–2 g day^{-1}. No serious adverse events have been reported.

The purpose of this study was to investigate whether a cream containing 5% LA showed any advantages concerning a number of criteria associated with ageing of the facial skin, compared with the same cream lacking LA.

Materials and methods

Thirty-three females (age 40–75, mean age 54·4 years) were included in the study. In order to standardize conditions during the test period they were asked to replace the different kinds of soap in use with Lactacyd® a mild lactic acid soap (pH 3·5; GlaxoSmithKline, Uxbridge, U.K.). The test subjects were also asked to avoid getting a sun tan. However, all were provided with and instructed how to use Coppertone® (Schering-Plough, Farum, Denmark) lotion, sun protection factor 15, on a daily basis in the case of visits to countries with a sunny climate. Regarding make-up, the test subjects were advised to continue their daily routines, with the exception being on days scheduled for test controls, on which the use of cosmetics was restricted.

Test procedure

The test procedure was done according to good clinical practice (GCP) guidelines. The study consisted of four parts: self-evaluation by the test subjects on four occasions, recorded and handed to the study nurse before the clinical evaluation, five clinical evaluations by the same physician, an evaluation based on standardized photographs and laser profilometry before and after the period of treatment. The clinical evaluation took place at the start of the study and after 2, 4, 8 and 12 weeks of treatment. At each control visit the following clinical characteristics were evaluated: fine lines, deep lines, pigmentation, bags under the eyes, telangiectasia, pore size, skin colour, visible dryness or scaling, elevated uneven structures, i.e. tactile roughness, degree of elasticity or flaccidity and thickness of skin. The clinical evaluation included protocols for assessment by the clinician according to an 11 grade scale, ranging from −1 (worse), 0 (no change) to 9 (very pronounced improvement). An assessment of adverse events was also performed. At inclusion and after 12 weeks of treatment standardized photographs and silicon impressions for laser profilometry were obtained.

Treatment creams

Creams contained identical vehicle, 0·3% coenzyme Q_{10} and 0·03% AC. The active cream also contained 5% LA, but was otherwise identical in colour, smell and consistency to the control cream. Two coded 50 g tubes, marked right and left, respectively, for use twice daily on each half of the face during the 12-week treatment period, were distributed at the start and the first visit. At the second and third control visits the number of tubes handed to the subjects was doubled. Whether the right or left side should receive active treatment was chosen at random according to a prepared coded list in order to ensure that the test was blind. At each control used tubes were collected in order to allow calculation of the amount of cream used per month of treatment.

Photography

All test subjects were photographed by the same photographer in the same place, using identical equipment and film, before and after treatment, to ensure maximum standardization. Each subject had a frontal view, left and right profile taken on each occasion. The two sets of photographs were evaluated independently of each other. The investigator used a Nikon magnifying glass with 8 × enlargement at a standard distance of 4·7 cm and on the same light box. Lines, pigment disorders, other colour changes in the skin, telangiectasia, skin texture and pouches under the eyes were evaluated using an 11 grade scale ranging from −1 (worse) to 9 (very pronounced improvement).

Laser profilometry

The periorbital region was cleansed with alcohol before the application of adhesive rings (Dermatest, Munich, Germany) and silicone impression material (Orbis Dental, Hannover, Germany). Precise application of the adhesive replica locating rings was aided with a ruler graded in two directions to ensure consistent distances from reference points of the lateral orbital canthus and superior auricular tragus. The centre spot of the adhesive rings was marked with a marker pen. The orientation of the ring tap was inward facing, toward the eye.

With the subject supine, a thin layer of the silicon impression material was applied over the bounded area of the ring and allowed to polymerize over a 3–4-min period, after which the ring was lifted from the skin, together with the replica. Each specimen was labelled with the date and the subject's identification number, and the side of the face. The specimens were stored in individual Petri dishes until analysed by laser profilometry. Precise instructions on how to accomplish this and an evaluation of the reproducibility of the method have been described elsewhere and the method is certified according to DIN-standard 4768 ff.[12]

Statistical method and considerations

The power analysis was based on an estimate of the proportion of subjects showing improvement on the active side. Using a χ^2-test for proportions in a group of 28 subjects a power of 80% is achieved to detect the difference between the null hypothesis $P = 0{\cdot}5$ (active side preferred) and a hypothetical improvement $P = 0{\cdot}75$ for the active side. A statistically significant difference in efficacy is achieved if the difference from the null hypothesis (no difference between active and control side) reaches $P < 0{\cdot}05$. A single tailed $P < 0{\cdot}1$ indicates a trend, but is not conclusive. All clinical variables were compared before and after 12 weeks of treatment. The difference between the active and placebo side was statistically analysed using the Wilcoxon matched pairs rank test (WT). Files were closed 27 May 2001.

Ethical considerations

All subjects included in the study were recruited through an advertisement in a local newspaper. They were informed about the study both in writing and verbally. Subjects who met the conditions for inclusion signed a written consent form before being allowed to enter the study. The study was approved by the ethical committee of the Karolinska Hospital.

Results

Of those recruited, 32 of 33 subjects completed the 12-week treatment period according to the clinical protocol. One subject, for unknown reasons, did not attend for the first clinical control and the results of the remaining 32 subjects were analysed. None had to leave the study for adverse events or serious side-effects. Eighty-one per cent ($n = 26$) belonged to skin type III, 13% ($n = 4$) to skin type II and 6% ($n = 2$) to skin type I. Side-effects were reported by the test subjects and mainly occurred during the first 4 weeks of treatment (Table 1). The most common side-effects reported were burning and warmth in the skin. This occurred immediately upon application of the cream and lasted a few minutes. These symptoms eventually

Table 1. Protocol of side-effects, recorded on the active side (left) and vehicle side (right) by the subjects included in the trial. A test subject could report more than one side-effect

Side-effects reported by test subjects	2 weeks	4 weeks	8 weeks	12 weeks
A. No side-effect	4, 27	12, 30	16, 29	22, 29
B. Causes a feeling of warmth in the skin	11, 2	6, 0	4, 1	2, 0
C. Causes burning	13, 0	9, 0	5, 1	4, 0
D. Causes stinging	13, 1	10, 2	6, 1	5, 1
E. Causes redness	4, 1	1, 0	2, 0	0, 1
F. Causes desquamation	1, 0	0, 0	2, 1	1, 0
G. Causes dryness	2, 1	2, 0	3, 1	2, 1
I. Causes itching	1, 0	1, 0	1, 0	1, 0
J. Causes a rash	2, 0	2, 0	1, 1	1, 1

decreased and, in most cases, disappeared, during the treatment period.

Two subjects initially reported the development of a rash related to the application of the cream. The rash disappeared soon after application and was never observed at a clinical control visit. No allergic reactions were documented during the test period.

An indirect estimate of compliance was achieved by weighing the remains of the cream left in the tubes collected at each control visit. The mean use was 31·8 g month^{-1} on the active side and 32·1 g month^{-1} on the placebo half of the face.

In the clinical overall assessment the 11 characteristics in Table 1 were evaluated according to the scale presented in the same table. Slight improvement was defined as a higher point on the scale for at least three different variables; improvement as higher points in five variables and much improvement in seven or more variables. The half of the face treated with 5% LA showed an improvement and the difference was statistically significant, $P = 0{\cdot}03$ (Fig. 1). Furthermore, in the clinical evaluation a significant improvement of fine lines, $P = 0{\cdot}01$, was observed on the LA-treated side (Figs. 2 and 3).

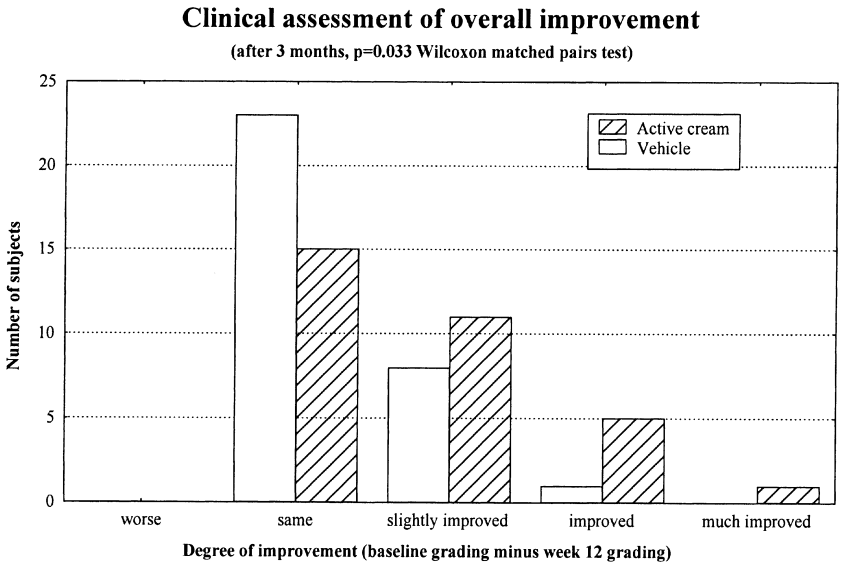

Figure 1. The result of the overall clinical assessment after three months of treatment. Slight improvement was defined as a higher point on the scale in at least three different variables. Improvement, as higher points in five variables and much improvement in seven or more variables.

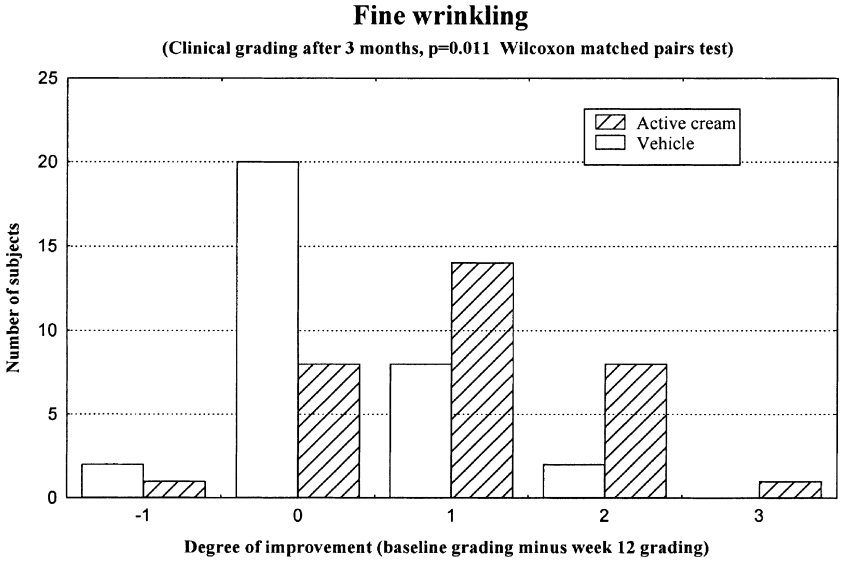

Figure 2. The clinical evaluation showed a significant improvement of fine lines, $P = 0{\cdot}011$. Worse (= −1), no change (= 0), slight improvement (= 1), improvement (= 2) and much improvement (= 3).

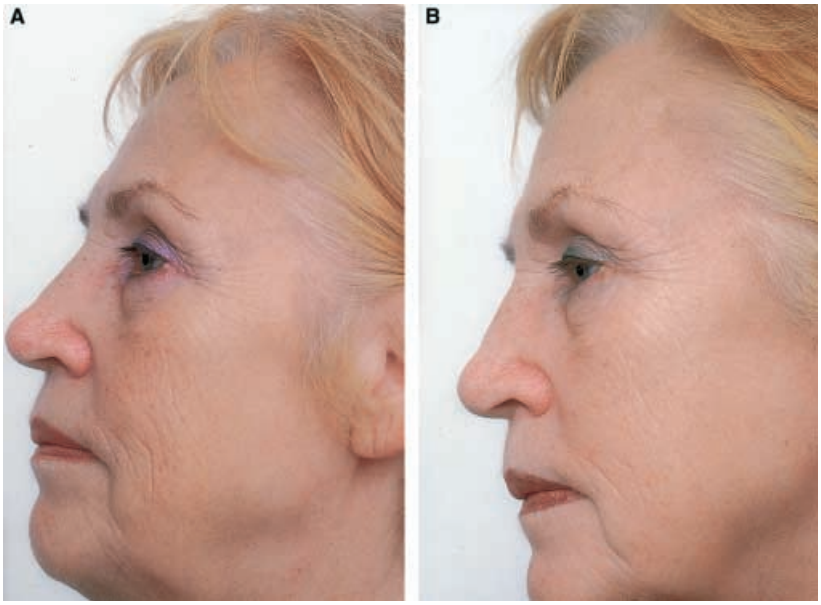

Figure 3. (A) Test subject, with moderate signs of photoageing. A generalized mottling of skin pigment combined with an increase in discrete solar lentigines is seen. The skin appears yellow, coarse with deep wrinkles. (B) After 12 weeks of treatment with the active cream a change in the overall appearance of the skin is seen.

In the photographic evaluation the following clinical characteristics were excluded: dryness, elevated uneven structures of the face identified by touch, degree of elasticity and evaluation of skin thickness. Slight improvement was defined as an increased grading in the 11-point scale of two variables, improvement in three variables and much improvement in four or more variables. The improvement demonstrated on the half of the face treated with active cream was statistically significant, $P = 0{\cdot}025$ (Fig. 4). Besides improvement of fine lines, $P < 0{\cdot}031$, the photographic evaluation also showed decreased pigmentation, $P < 0{\cdot}007$, and indications of decreased under-eye bags and puffiness, $P < 0{\cdot}09$, and decreased pore size, $P < 0{\cdot}08$.

In the self-assessment by subjects included in the study, 78% claimed the active side (25 of 32) showed different degrees of improvement, while 31% claimed the placebo-treated side (10 of 32) had improved. The difference tested was statistically significant, $P = 0{\cdot}002$ (Fig. 5).

The laser profilometry, measuring skin roughness lateral to the periorbital area, predominately the depth of fine lines, demonstrated a significant improvement on the LA-treated side. After 3 months the median

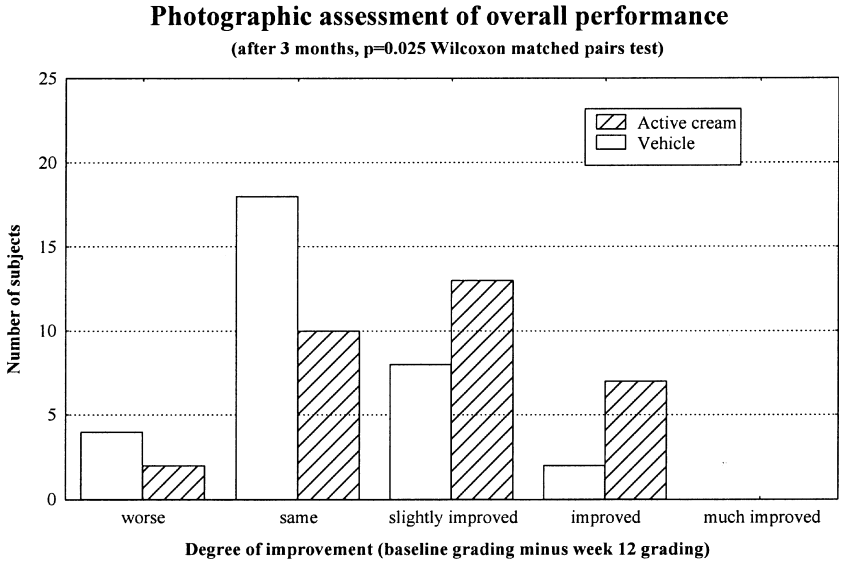

Figure 4. Slight improvement in the overall photographic evaluation was defined as an increased grading in the 11 grade scale of two variables, improvement in three variables and much improvement in four or more variables. The improvement demonstrated on the active half of the face was statistically significant.

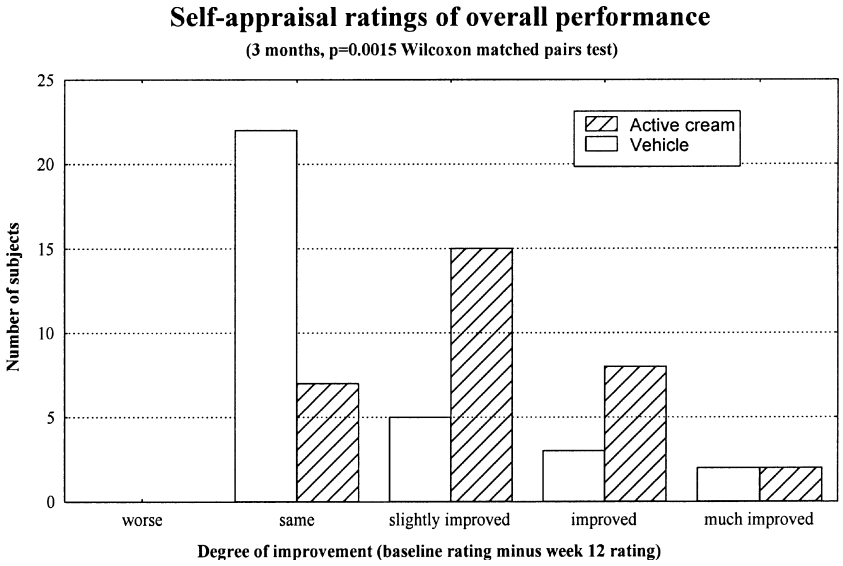

Figure 5. In the self-assessment by the subjects included in the study 25 of 32 claimed the active side showed different degrees of improvement, while 10 of 32 claimed the placebo-treated side had improved. None reported a deterioration of the treated skin.

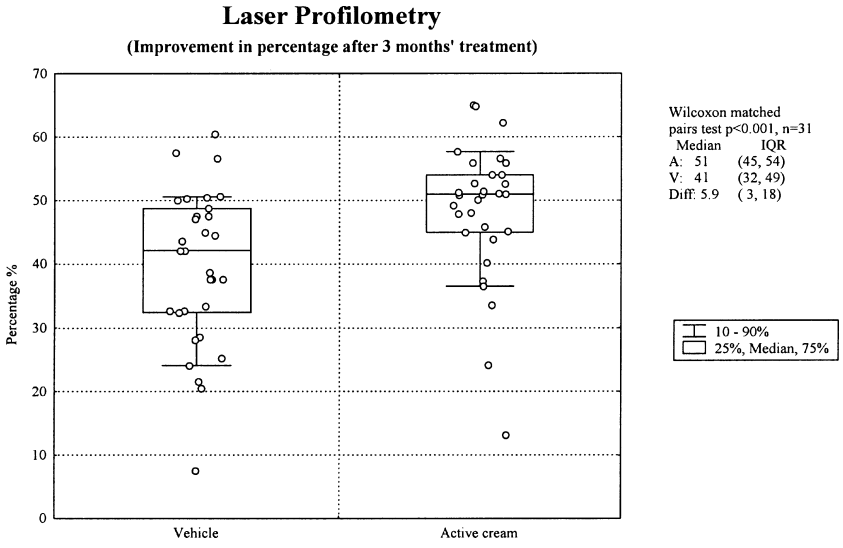

Figure 6. Each ring represents a test subject and indicates in percentage terms the difference between the laser profilometry measurements before start of treatment and after 3 months. At the end of the study period the median improvement on the α-lipoic acid-treated side was 51% (45–54) and 41% (32–49) on the vehicle treated half of the face, $P < 0{\cdot}001$.

improvement on the LA side was 51% (44·9–54·0) and 41% (32·4–48·7) on the placebo-treated half of the face, $P < 0{\cdot}001$ (Fig. 6). The results are based on measurements from 62 silicon impressions. Because of technical difficulties, two specimens from one test subject were lost from follow-up. Age compared with the results from the laser profilometry indicated a weak correlation between degree of improvement on the active side and increasing age. However, this association was not statistically significant.

Discussion

In recent years there has been increased interest in LA, related to its sometimes dramatic protective effect in tissues under extreme oxidative stress created under conditions of critical ischaemia.[13] DHLA has been shown to suppress UVB erythema when applied topically immediately after UV exposure.[14] In an uncontrolled study, after 12 weeks of treatment with 5% LA cream, a 50% reduction in the depth of medium vertical lines on the upper lip and a significant reduction of fine lines in the periorbital region was reported.[15] In that study the evaluation was done only by assessment of photographs.

Thus, the results of this controlled study support earlier observations that 5% LA might be effective in reducing photo-induced changes in skin (Fig. 3). Possible mechanisms causing this effect can be the antioxidant properties of LA and its acting as a

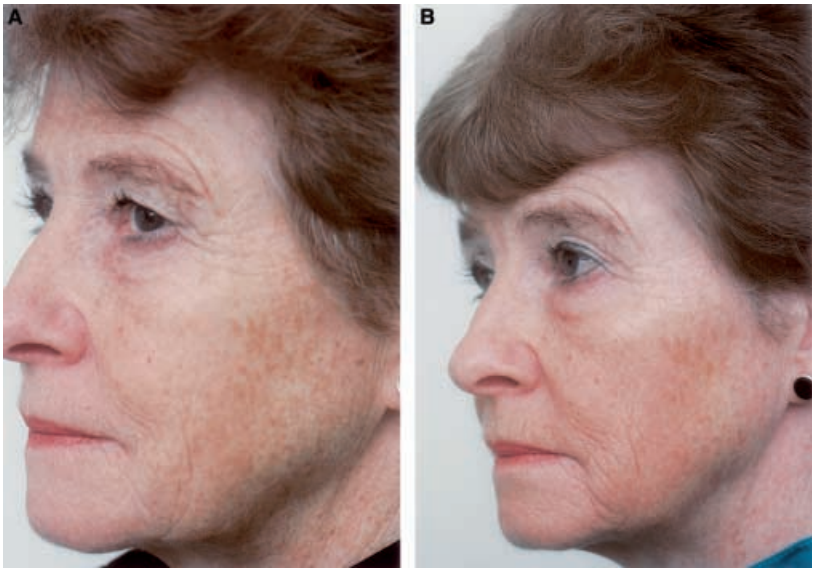

Figure 7. (A) Profile of a test subject with light to moderate signs of photoageing before treatment. (B) Corresponding region after 12 weeks of treatment. Observe the change in lentigines, appearance of under the eye bag and reduction in fine lines.

modulator of the inflammatory reaction. Oxidative damage to DNA, cells and tissue increases with age. In particular, damage to mtDNA occurs with an increased frequency. It has been demonstrated that addition of LA systemically to older rats improves the function of mitochondria.[16–18] Administration of LA to older rats significantly increases the intracellular amount of nucleic acids and protein.[16] This increase in intracellular proteins may enhance the production of enzymes necessary to transform retinols to retinoic acid. It has been shown that cellular reactive oxygen intermediates may affect fibroblast proliferation and collagen gene expression.[19] A recent report demonstrated that topically applied vitamin C enhances the mRNA level of collagens I and III and their processing enzymes and tissue inhibitors of matrix metalloproteinase 1 in human dermis. In the same study the level of accumulated solar damage estimated in the placebo-treated side of the test subjects did not correlate with responsiveness to the topically applied vitamin C. This suggested that a reduced biosynthetic activity related to chronological ageing and/or a low tissue concentration of the vitamin are the likely targets of topical treatment.[20] LA can replace both vitamins C and E.[21] DHLA has the capacity to reshape ascorbic acid from dihydro-ascorbic acid and α-tocopherol.[22] Strong evidence indicates that antioxidants including LA can act as an anti-inflammatory agent by reducing the production of transcription factors such as nuclear factor-κB and indirectly affect the gene expression of inflammatory cytokines such as tumour necrosis factor-α, interleukin (IL)-1, IL-2, IL-6 and IL-8.[23] The tested creams probably also have epidermal moisturizing affects. Results indicate that addition of LA further improves properties related to skin roughness (Fig. 5).

It must be noted that in most biochemical systems feedback mechanisms occur. Thus, a continuous supply might induce mechanisms reducing further beneficial effects of the treatment. Accordingly, the long-term effect of topically adding a compound such as LA is at the moment unclear. The length of treatment necessary to ensure maximum improvement is still being investigated. At present we do not know which is the ideal treatment period. In order to investigate whether additional improvement in skin texture occurs, a 6-month follow-up study is in progress. As photoexposure is an ongoing event, it has to be anticipated that repeated treatment courses are necessary in order to maintain the results obtained by the LA treatment.

The 5% concentration of LA cream used in this study is mainly based on empirical data. As no serious side-effects occurred and none of the subjects withdrew because of inconvenience caused by the treatment, 5% LA seems to be a suitable therapeutic agent. Whether this concentration is optimal regarding clinical effect in relation to risks still has to be determined.

From a toxicological point of view topical application of 5% LA is safe. The LD_{50} for LA in rats are 1 g kg^{-1}, in dogs 400–500 mg kg^{-1}.[20,24] In systemic treatment of humans the most common dose range is 200–

600 mg day^{-1}, equivalent to 3–9 mg kg^{-1} day^{-1}. The median amount of the 5% LA cream used by the test subjects in this study was approximately 1 g on half the face per day, corresponding to1·5 mg kg^{-1} day^{-1} of LA for the whole face. With an average weight of 65 kg, this represents a dose of approximately 100 mg day^{-1} of LA. Thus, full face topical treatment with a 5% LA cream applied twice daily does not reach the lower range of doses used for systemic treatment. Concerning clinical safety, allergic skin reactions are among the few side-effects previously reported[25] after systemic administration. In this study we have noticed that local irritation on application of 5% LA is common during the first weeks of treatment, but eventually reduces and disappears in the vast majority of cases (Table 1). However, the side-effects commonly seen in patients treated with retinoic acid, such as a scaly erythema combined with swelling of the skin, seem to be a rare event in topical LA-treated subjects.

In this study we have used four different assessment methods to document the effect of topical LA treatment on the skin. Two of these methods are subjective, the photographic evaluation has to be considered as a semiobjective and the laser profilometry as an objective method. The global assessment of the results obtained, independently of the method used, show a similar pattern. In this study no age-dependent pattern of improvement was detected statistically. This was probably explained by the relatively few subjects in each age group.

In conclusion, 12 weeks of 5% LA in the tested cream vehicle seems to improve several clinical characteristics related to photoageing of the facial skin. Further studies to elucidate possible mechanisms, optimal dosage and treatment periods are warranted.

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References

1 Lawrence N. New and emerging treatments for photoaging. *Dermatol Clin* 2000; **18**: 99–112.
2 Reed LJ, Debusk BGF, Gunsalus IC, Hornberger CS Jr. Crystalline α-lipoic acid: a catalytic agent associated with pyruvate dehydrogenase. *Science* 1951; **114**: 93–4.
3 Bayer W, Schmidt K, eds. *Vitamine in Prävention und Therapie*. Stuttgart: Hippokrates-Verlag, 1991: 267–71.
4 Freisleben H-J, Groth N, Fuchs J *et al.* Penetration of spin-labeled DHLA into the skin of hairless mice. *Arzneim Forsch* 1994; **9**: 1047–50.
5 Podda M, Rallis M, Traber MG *et al.* Kinetic study of cutaneous and subcutaneous distribution following topical application of (7,8-14C)rac-α-lipoic acid onto hairless mice. *Biochem Pharmacol* 1996; **52**(4): 627–33.
6 Carreau JP. Biosynthesis of lipoic acid via unsaturated fatty acids. *Methods Enzymol* 1979; **62**: 152–8.
7 Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. *Proc Natl Acad Sci USA* 1994; **91**: 10771–8.
8 Hagen TM, Ingersoll RT, Lykkesfeldt J *et al.* (R)-α-Lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. *FASEB J* 1999; **13**: 411–8.
9 Karlsson J, Diamant B. Kolesterolets okända syskon ubikinon ett instrument i antioxidantorkestern. *Läkartidningen* 1991; **88**: 512–4.
10 Hoppe U, Bergemann J, Diembeck W *et al.* Coenzyme Q10, a cutaneous antioxidant and energizer. *Biofactors* 1999; **9**: 371–8.
11 Swamy-Mruthinti S, Carter AL. Acetyl-L-carnitine decreases glycation of lens proteins: in vitro studies. *Exp Eye Res* 1999; **69**: 109–15.
12 Breuer M, Voss W. *Proving for Efficacy: Laser Profilometry.* Munster: Dermatest GmbH [available from Dermatest].
13 Panigrahi M, Sadguna Shivakumar BR *et al.* α-Lipoic acid protects against reperfusion injury following cerebral ischemia in rats. *Brain Res* 1996; **717**: 184–8.
14 Fuchs J, Milbradt R. Inhibierung des UVB-Erythems am Menschen durch das Antioxidans Dihydroliponsäure. In: *Thioctsäure-Neue Biochemische, pharmakologische und klinische Erkenntnisse zur Thioctsäure* (Borbe HHH, Ulrich H, eds). Frankfurt: PMI, 1989.
15 Perricone NV. Topical 5% alpha lipoic acid cream in the treatment of cutaneous rhytids. *Aesthetic Surg J* 2000; **20**: 218–22.
16 Arivazhagan P, Panneerselvam C. Effect of DL-alpha-lipoic acid on tissue nucleic acid contents in aged rats. *Pharmacol Res* 2000; **42**: 223–6.
17 Gerhard G, Benko FA, Allen RG *et al.* Mitochondrial DANN mutation analysis in human skin fibroblasts from fetal, young and old donors. *Mech Ageing Dev* 2000; **123**: 155–66.
18 Van Remmen H, Richardsson A. Oxidative damage to mitochondria and aging. *Exp Gerontol* 2001; **36**: 957–68.
19 Murell G, Francis N, Bromley L. Modulation of fibroblasts proliferation by oxygen free radicals. *Biochem J* 1990; **265**: 659–65.
20 Nusgens BV, Humbert P, Rougier A *et al.* Topically applied vitamin C enhances the mRNA level of collagens I and III, their processing enzymes and tissue inhibitors of matrix metalloproteinase 1 in the human dermis. *J Invest Dermatol* 2001; **116**: 853–9.
21 Rosenberg HR, Culik R. Effect of α-lipoic acid on vitamin C and E deficiencies. *Arch Biochem Biophys* 1959; **80**: 86–93.
22 Scholich H, Murphy ME, Sies H. Antioxidant activity of dihydrolipoate against microsomal lipid peroxidation and its dependence on α-tocopherol. *Biochem Biophys Acta* 1989; **1001**: 256–61.

23 Suzuki YJ, Aggarwal BB, Packer L. α-Lipoic acid is a potent inhibitor of NF-κB activation in human T cells. *Biochem Biophys Res Commun* 1992; **189**: 1709–15.
24 Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. *Free Radic Biol Med* 1995; **19**: 227–50.
25 Packer L, Tritschler HJ, Wessel K. Neuroprotection by the metabolic antioxidant α-lipoic acid. *Free Radic Biol Med* 1997; **22**: 359–78.