

Efficacy of Cream-Based Novel Formulations of Hyaluronic Acid of Different Molecular Weights in Anti-Wrinkle Treatment

Tatjana Pavicic,^b Gerd G. Gauglitz,^b Peter Lersch,^a Khadija Schwach-Abdellaoui,^c Birgitte Malle,^c Hans Christian Korting,^b Mike Farwick^a

^aEvonik Goldschmidt GmbH, Essen, Germany

^bDepartment of Dermatology and Allergology, Ludwig Maximilians University, Munich, Germany

^cNovozymes Biopharma DK A/S, Bagsvaerd, Denmark

ABSTRACT

Background: Due to its strong water-binding potential, hyaluronic acid (HA) is a well-known active ingredient for cosmetic applications. Native HA is proposed to help the skin to retain and maintain elasticity, turgor and moisture.

Objective: To observe the efficacy of topical application of 0.1% hyaluronan formulations of different molecular weights (MW) (50, 130, 300, 800 and 2000 kDa, respectively) in the periocular area as anti-wrinkle treatment.

Material and Methods: Seventy-six female subjects between 30 and 60 years of age with clinical signs of periocular wrinkles applied one of the formulations twice-daily to the area of interest in a randomized fashion for 60 days. Around the other eye, a vehicle control cream was applied. Measurements of skin hydration and skin elasticity were performed before treatment, 30 and 60 days thereafter. At similar time points negative replicas were taken and evaluated by semi-automated morphometry.

Results: All HA-based creams utilized in this study demonstrated a significant improvement in skin hydration and overall elasticity values (R2) when compared to placebo. Measurements of wrinkle depth using mean roughness (Ra) and maximum roughness (Rz) values revealed significant improvement in the 130 and the 50 kDa HA group after 60 days of treatment compared to placebo-treated area.

Conclusion: Topical application of all 0.1% HA formulations used in this study led to significant improvement in skin hydration and elasticity. Application of low-molecular-weight (LMW) HA was associated with significant reduction of wrinkle depth, which may be due to better penetration abilities of LMW HA.

J Drugs Dermatol. 2011;10(9):990-1000.

INTRODUCTION

Evidence of the clinical signs associated with skin aging often first appears in the periorbital area and includes wrinkles, eyelid bags, circles around the eye, or a "tired" appearance.¹ Very few cosmetic preparations were shown to improve this situation using objective quantitative methods.

The physiological changes observed in chronologic aging skin are barrier function impairment, xerosis, loss of elasticity, slower turnover of epidermal cells and atrophy. There is a progressive reduction in water-binding capacity and changes in cutaneous permeability for chemical substances and increased production of free radicals. Moisturizers decelerate the loss of water from the surface of skin, maintain an appropriate level of skin humidity and minimize the aspect of fine wrinkles.²

The glycosaminoglycan hyaluronic acid (HA) is a major component of the extracellular matrix of the skin and plays an

important role in the metabolism of the dermis.³ HA is especially important in the skin because these macromolecules are highly hydrophilic and can bind up to 1,000 times their volume in water. In the skin, this property is likely to be relevant in controlling tissue hydration.⁴ Due to these characteristics, HA is often used as a moisturizing agent in cosmetic formulations.

In the skin, HA might also act as a scavenger of free radicals and antioxidants under physiological conditions. Spectroscopic studies even indicate that a double bond in the D-glucuronic acid unit can form a complex with reactive oxygen species and reduce the toxicity of radicals.⁵ Furthermore, it plays a major role in the exchange between fixed tissue cells and blood and in cell migration. Recent studies suggest that HA, via the CD44 receptor, is capable of increasing cell differentiation and cell motility.⁶ HA also has anti-inflammatory properties and promotes wound healing.⁷

Native HA has been employed for several years to help the skin regain elasticity, turgor and moisture.⁸ In a clinical study, an increase in elasticity and turgor following repeated injections of HA could be demonstrated, but this treatment approach is discussed controversially.

Most HA preparations used either as topical formulations, wound dressings, native HA injections or as fillers contain non-animal-based HA molecules produced by bacterial fermentation from a specific strain of Streptococci.⁹

In this study, for the first time, the HA molecules used were produced by *Bacillus subtilis*, thus also representing a non-animal derived raw material generally recognized as safe (GRAS).

Furthermore, according to our literature search using PubMed, this is the first publication addressing the effects of cream preparations containing hyaluronic acid of different molecular weights on skin hydration, skin elasticity and periorbital wrinkles in a group of volunteers using intraindividual comparison to the corresponding placebo cream.

PATIENTS, MATERIALS & METHODS

Study Cream Samples

Placebo cream represents an oil-in-water emulsion containing: aqua, hydrogenated polydecene, steareth-2, cetearyl alcohol, steareth-21, phenoxyethanol, methylparaben, butylparaben, iso-butylparaben, diazolidinyl urea and disodium EDTA (according to the INCI declaration).

HA cream formulations contain the same ingredients as placebo cream and additionally 0.1% sodium hyaluronate of different molecular weights (50, 130, 300, 800 and 2000 kDa, respectively).

HA used in these formulations was obtained through a protein-free process of fermentation based on a novel fermentation strain of the species *Bacillus subtilis*. Minimal medium with sucrose as the carbon source was used for culture and HA-macromolecules secreted into the medium without any cell association were obtained through a water-based recovery process. The HA-macromolecules generated this way are characterized by very high purity without any cell wall impurities, endo- or exotoxins, haemolytic activity and very low protein levels. The structure of *Bacillus* HA is identical to that of natural HA, which has been confirmed by enzymatic hydrolysis and MALDI-TOF analysis, Fourier-Transformation-Infrared- (FTIR-) and High-Performance-Liquid-Chromatography- (HPLC-) Spectroscopy for monomer composition.

Study Design

Patients included in this study were divided into five groups. The inclusion criteria were Caucasian race, age between 30 and 60 years, either sex, and healthy physical state. The exclusion

criteria were taking topical or systemic drugs that could affect the results of the test, pregnancy or breast-feeding, presence of skin diseases, history of intolerance to drugs and/or cosmetic products as well as not fulfilling inclusion criteria. For the entire duration of the study, the subjects were instructed not to use other products on the tested areas and to avoid exposure to UV radiation. Participation in the study was terminated earlier than foreseen either by decision of the subject or because of reasons correlated with treatment (exceptional irritant or allergic reactions). At the beginning of the study, each subject signed an informed consent declaration. Twelve women were treated either with the 0.1% formulation of HA of 50, 130 or 300 kDa and 20 women with the 0.1% formulation of HA of 800 and 2000 kDa, respectively.

In each group, the volunteers were randomized to apply twice-daily a topical cream formulation with HA of defined, yet differing molecular weights (50, 130, 300, 800 or 2000 kDa) to the periocular area of one side of the face and the placebo cream as a control to the other side for two months. The side of application (left or right) on the face of the two creams was randomized.

The study was carried out in compliance with quality assurance system requirements, according to the principles of good laboratory practice (GLP) and good clinical practice (GCP), as well as the principles established by the World Medical Association in the Declaration of Helsinki.

The study investigations were carried out in a bioclimatic room (24°C; 50% room humidity) in order to keep the temperature and the humidity during the measurements constant. The patients were asked not to wash their face for at least three hours before performing the measurements. Instrumental measurements of skin hydration and elasticity were taken in the left and right periocular areas, marked out in a reproducible way, at the beginning of the study, after one month of treatment and at the end of the study. In the same areas, the micro-relief of stratum corneum was assessed by the image analysis of plastic replicas of the skin surface. Furthermore, digital photographs of the investigated areas were taken. The study was carried out in two subtrials: the measurements for 50, 130 and 300 kDa HA topical cream formulations were performed together and for 800 and 2000 kDa HA cream separately.

The data obtained were then recorded and later analysed and statistically compared.

Measurements

Skin Hydration

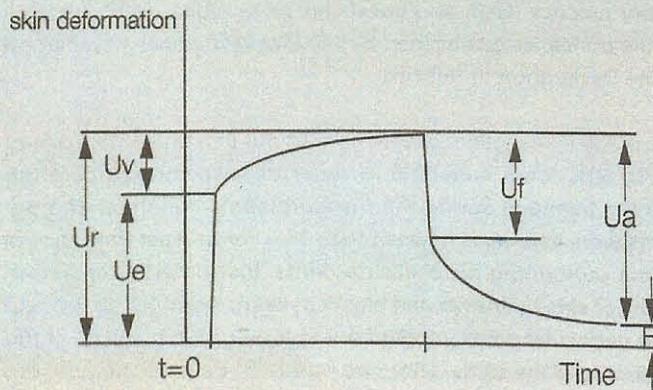
The measurements of skin hydration were performed using the Corneometer CM 825 (Courage & Khazaka, Cologne, Germany) as recommended by the manufacturer and described in former publications.^{10,11}

Skin Elasticity

Skin elasticity was assessed with a Cutometer SEM 575 (Courage & Khazaka), which measures the vertical deformation of the skin when sucked into the loop of a measuring probe and reversed into its original condition. The skin surface is sucked into the opening of a measuring probe, in which a constant level of depression is created (350 mbar) for an established time (one second). The air depression is then annulled and the released skin can return to its original position. Three measurement cycles were performed on the same spot. An optical system measures the variations in electrical capacitance. They are proportional to the rise of the skin surface which has been measured (expressed in mm). The skin rises were shown on Cartesian axes, where the deformation of the skin (expressed in mm) is a function of time (expressed in seconds). Accordingly, the three suction/release cycles were represented as three successive curves, which allow the measurement of the deformation parameters relating to the elastic features of the skin.

In the final calculation of the results, the following parameters were considered (Figure 1):

FIGURE 1. Skin deformation in mm as a function of time as measured with the Cutometer.



Ua = Total deformation recovery at the end of the stress-off period;

Uf = Total extensibility of the skin;

Uv = Viscoelastic creep occurring after the elastic deformation;

Ur = Elastic deformation recovery due to the stress-off period;

Ue = Elastic deformation of the skin due to the application of stress;

R = Amount of deformation not recovered by the end of the stress-off period.

In the final calculation of the results, the parameter:

Uf (maximal deformation of the skin) was referred to as **R0** parameter;

Ua / Uf ratio referred to as parameter **R2** representing the overall elasticity;

Uv / Ue ratio referred to as **R6** parameter representing the viscoelastic ratio.

Wrinkle Depth

The anti-wrinkle effects of the various preparations were analyzed using replicas with the help of a profilometer equipped with an image analyzer. In order to obtain negative imprints of the skin surface (skin replicas), the following materials were used: a fast-hardening synthetic polymer (Silflo, Flexico Developments Ltd, London, UK) and adhesive discs (24x40, 3M, Neuss, Germany).

The adhesive discs were put onto the subject's skin in order to delimit the investigated area and to avoid skin stretching during the polymer application. A small amount of polymer was then spread into the internal circular area of every disc and left in situ for a few minutes until it became dry. The disc was then removed and processed further.

For the calculation of **Ra**, the mean roughness value and **Rz**, the maximum roughness value, especially describing the deep wrinkles, the silicon replicas of the cutaneous surface were lightened by a grazing light source with a defined incident angle (35°), with the purpose of generating shadows which are wider when furrows are higher. The main wrinkles must be oriented perpendicularly to the incident light. Using the High Performance Charge Coupled Device (CCD) camera (COHU, Inc., Electronics Division, San Diego, CA, USA) an image of the skin replica covering an area of 12x9 mm was taken.

The anti-wrinkle efficacy of a treatment can be judged by a decrease of **Ra** and/or **Rz** values at the end of the treatment.

Digital photographs of the periocular area were also taken at the beginning of the test and after one respectively two months of treatment. The images were taken by means of a Nikon Coolpix 5000 digital camera (Nikon Corporation, Tokyo, Japan).

Statistical Analysis

The mean value of three corneometry readings taken in contiguous spots of the same area was considered for further calculations. Mean values and standard deviations were calculated for initial and final instrumental values (addressing hydration, elasticity and image analysis) recorded in the two areas (verum, placebo) at the three checks.

Furthermore, the variation of the parameter was calculated as a difference between the mean values obtained at the end of the treatment or after one month and the mean values at the beginning of the treatment for the left and right periocular area. This difference is reported as percentage of variation, too.

The basal (T_0), intermediate (T_{30}) and final values (T_{60}) of the two considered areas (one treated with the HA topical cream formulation and another with the placebo cream) were compared to each other by means of the paired samples t-test. The groups of data were considered significantly different for a

TABLE 1.

The Effects of Topical HA Formulations on Skin Hydration (Corneometric Units) in Comparison to an Untreated Area: Mean Values

	T_0	T_{30}	T_{60}	Variation ($T_{30}-T_0$)	Variation ($T_{60}-T_0$)
50 kDa HA	59.8±10.5	64.5±6.7	69.2±6.7	+4.7 (7.9%)	+9.4* (15.8%)
130 kDa HA	59.2±10.8	64.8±10.0	65.0±8.0	+5.6* (9.5%)	+5.8* (9.8%)
300 kDa HA	55.8±9.4	60.7±8.1	63.5±8.5	+4.9 (8.8%)	+7.7* (13.8%)
800 kDa HA	52.7±6.0	55.9±5.6	55.4±5.5	+3.2* (6.1%)	+2.7** (5.1%)
2000 kDa HA	55.7±7.1	52.8±9.1	57.3±9.1	-2.9 (-5.2%)	+1.6 (2.9%)

*P≤0.05; **P≤0.01.

probability value of $P\leq 0.05$ ($(T_{30}-T_0)$ treated area vs. $(T_{30}-T_0)$ placebo area and $(T_{60}-T_0)$ treated area vs. $(T_{60}-T_0)$ placebo area).

RESULTS

Skin Hydration

In comparison to the values found before treatment with 800 kDa HA cream formulation, there was an increase in skin hydration of 6.1 percent after one month of treatment ($P<0.05$) and also at the end of the study of 5.1 percent ($P<0.01$). In the areas treated with HA formulations of 2000, 300, 130 or 50 kDa, respectively, an increase, equal to 2.9 percent, 13.8 percent, 9.8 percent and 15.8 percent in the mean basal skin hydration was achieved after two months of application when compared to the beginning of the study ($P<0.05$, except for the 2000 kDa HA formulation; Table 1).

In comparison to the placebo-treated area in the same subject, significant differences in skin hydration could be reported for cream-based HA formulations of all sizes except 50 kDa. While treatment with a 130 kDa HA cream formulation resulted in a significantly increased hydration at one and two months thereafter, application of cream formulations containing 300, 800 and 2000 kDa HA ($P<0.05$), respectively, was only associated with significantly increased hydration at the two month time point (Figure 2a-e).

Skin Elasticity

The values for maximal skin deformation (R_0) showed a prominent decrease for 800 kDa HA cream after one month and two months of treatment, equal to 7.2 percent ($P<0.01$), as well as for 50 kDa HA cream (-21.8%) after one month ($P<0.05$) when compared to the values before treatment. All the other treated areas did not show any major changes in R_0 values ($P>0.05$; Table 2a). When compared to the placebo-treated areas, only the treatment with 800 kDa HA cream resulted in prominent changes after one as well as after two months ($P<0.05$; Table 2b).

After one month (T_{30}) in all areas treated with HA-based creams an increase in the overall elasticity values ($R_2 = U_a / U_f$ ratio),

equal to 14.5 percent for 50 kDa, 20.0 percent for 130 kDa, 8.9 percent for 300 kDa, 6.4 percent for 2000 kDa (all $P<0.05$) and 6.1 percent for 800 kDa ($P<0.01$) was measured in comparison to the start of the study. At the end of the study (T_{60}) the areas treated with 50, 800 and 2000 kDa showed an increase ($P<0.05$) and even more for 130 and 300 kDa HA creams ($P<0.01$) in the R_2 values (Table 3). In comparison to the placebo-treated area, treatment with HA formulations of different molecular weights revealed significant increased R_2 values for all HA fragments examined. While application of 50, 130, 800 and 2000 kDa HA cream formulations resulted in significantly higher R_2 values at both time points ($P<0.05$), the 120 kDa group only showed significant differences at the one-month time point (Figure 3a-e).

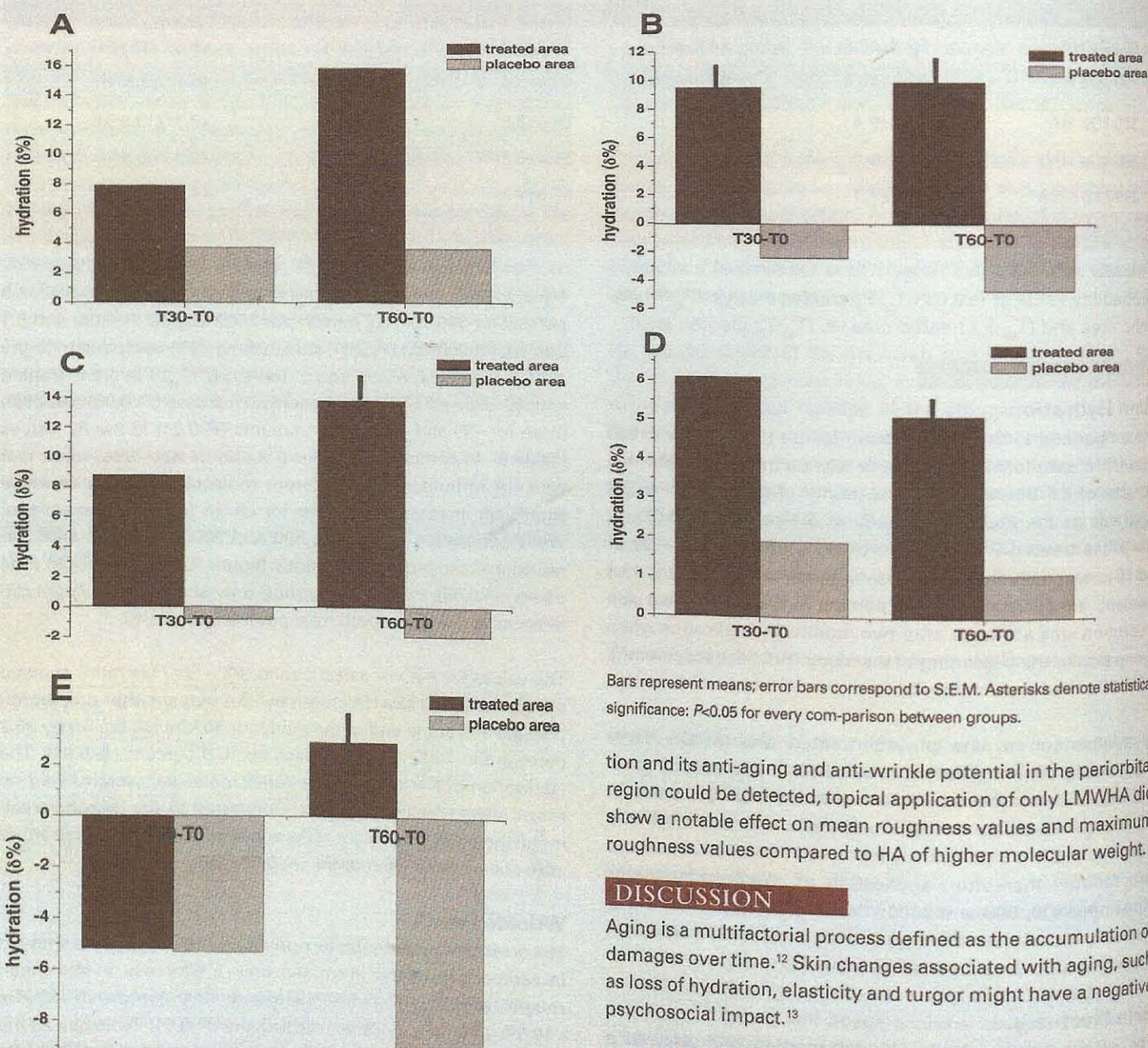
The values for the viscoelastic ratio ($R_6 = U_v / U_e$ ratio) showed a decrease for 50 kDa HA cream by 20.2 percent after one month ($P<0.05$) and at the end of the study for 50 kDa HA cream by 36.2 percent and 2000 kDa HA cream by 18.8 percent ($P<0.01$). The application of HA creams with other molecular weights had no major effect ($P>0.05$; Table 4a). Compared to the placebo treatment, there were no major differences between R_6 values either after one or after two months ($P>0.05$; Table 4b).

Wrinkle Depth

The areas treated with the formulation of HA of 800 kDa showed in comparison to the untreated area a decrease in the mean roughness values (R_a) after one month (-8.4%) and with 130 kDa (-10.8%) after two months, respectively ($P<0.01$; Table 5a). At the end of the study, the application of cream with HA of 800 kDa (-9.9%) as well as 300 kDa (-17.7%) resulted in a clear decrease ($P<0.05$) in R_a values compared to the start values (Table 6a). Compared to the placebo treatment, only the topical application of 130 kDa HA-based cream resulted in a significantly lower R_a value after two months of treatment ($P<0.05$; Figure 4a).

In comparison to the values for untreated areas, the maximum roughness values (R_z = wrinkle depth) showed a major decrease after the application of 50, 130 and 800 kDa HA cream after one (-10.2%, -5.3% and -4.9%) as well as after two months (-11.0%, -8.1% and -6.2%; $P<0.05$). No such changes were detected in the

FIGURE 2. The effects of topical HA formulations containing **A) 50, B) 130, C) 300, D) 800** and **E) 2000 kDa HA**, respectively, on skin hydration (reported as percentage of variation) in comparison to a placebo-treated area.



Bars represent means; error bars correspond to S.E.M. Asterisks denote statistical significance: $P<0.05$ for every comparison between groups.

tion and its anti-aging and anti-wrinkle potential in the periorbital region could be detected, topical application of only LMWHA did show a notable effect on mean roughness values and maximum roughness values compared to HA of higher molecular weight.

DISCUSSION

Aging is a multifactorial process defined as the accumulation of damages over time.¹² Skin changes associated with aging, such as loss of hydration, elasticity and turgor might have a negative psychosocial impact.¹³

It is well known that skin hydration is highly related to the content and distribution of dermal glycosaminoglycans (GAGs), especially HA. Photoaged skin has been shown to be characterized by reduced levels of HA and elevated levels of chondroitin sulphate proteoglycans, resulting in a paradoxical GAGs increase in photoaged skin compared with young or intrinsically aged skin.¹⁴ Meyer and Stern¹⁵ reported that with advancing age, hyaluronan polymers became progressively more tissue-associated. The proportion of hyaluronan released after papain digestion increased from seven percent of the total in fetal to 23 percent of the total in senescent skin. The enhanced bonding with other tissue constituents is pre-

areas treated with other HA formulations either after one or two months (Table 5b). In comparison to the placebo-treated area, only the treatment with 50 kDa HA cream resulted in significantly smaller Rz values and clinical improvement at one and two months of treatment ($P<0.05$; Figures 4b and 5a-c).

The effects of cream-based HA formulations of different molecular weights on different parameters after one month and two months of treatment compared to placebo are summarized in Table 6a and b. Even though no close correlation between the molecular weight of the respective HA molecules in the formula-

TABLE 2a.

The Effects of Cream-Based HA Formulations of Different Molecular Weights on Maximal Skin Deformation (R_0) in Comparison to Untreated Area: Mean Values

	T_0	T_{30}	T_{60}	Variation ($T_{30}-T_0$)	Variation ($T_{60}-T_0$)
50 kDa HA	0.160±0.048	0.125±0.047	0.166±0.049	-0.035* (-21.8%)	0.006 (3.8%)
130 kDa HA	0.133±0.043	0.135±0.034	0.134±0.027	+0.002 (1.5%)	+0.001 (0.7%)
300 kDa HA	0.118±0.028	0.114±0.036	0.118±0.024	-0.004 (-3.4%)	0.000 (0%)
800 kDa HA	0.166±0.044	0.154±0.040	0.154±0.052	-0.012** (-7.2%)	-0.012* (-7.2%)
2000 kDa HA	0.153±0.067	0.141±0.053	0.147±0.050	-0.012 (-7.8%)	-0.006 (-3.9%)

* $P\leq 0.05$; ** $P\leq 0.01$.

TABLE 2b.

The Effects of Topical HA Formulations on Skin Elasticity Parameter Maximal Skin Deformation (R_0) in Comparison to Placebo-Treated Area

($T_{30}-T_0$) verum-treated area versus ($T_{30}-T_0$) placebo-treated area
($T_{60}-T_0$) verum-treated area versus ($T_{60}-T_0$) placebo-treated area

	T_{30}	T_{60}
50 kDa HA vs. placebo	$P>0.05$	$P>0.05$
130 kDa HA vs. placebo	$P>0.05$	$P>0.05$
300 kDa HA vs. placebo	$P>0.05$	$P>0.05$
800 kDa HA vs. placebo	$P<0.05$ (*)	$P<0.05$ (*)
2000 kDa HA vs. placebo	$P>0.05$	$P>0.05$

* $P\leq 0.05$; ** $P\leq 0.01$.

TABLE 3.

The Effects of Topical HA Formulations on Skin Elasticity Parameter Overall Skin Elasticity (R_2) in Comparison to Untreated Area: Mean Values

	T_0	T_{30}	T_{60}	Variation ($T_{30}-T_0$)	Variation ($T_{60}-T_0$)
50 kDa HA	0.508±0.0151	0.582±0.164	0.575±0.133	+0.074* (14.5%)	+0.067* (13.2%)
130 kDa HA	0.469±0.097	0.563±0.149	0.583±0.128	+0.094* (20.0%)	+0.114** (24.3%)
300 kDa HA	0.529±0.118	0.576±0.134	0.598±0.152	+0.047* (8.9%)	+0.069** (13.0%)
800 kDa HA	0.678±0.062	0.720±0.076	0.717±0.071	+0.042** (6.1%)	+0.039* (5.8%)
2000 kDa HA	0.640±0.152	0.681±0.138	0.684±0.135	+0.041* (6.4%)	+0.044* (6.9%)

* $P\leq 0.05$; ** $P\leq 0.01$.

sumably mediated through hyaluronan-binding proteins and alterations in the localization of hyaluronans with a steady decline of HA in the upper epidermal layer and concomitant increases in the basal layer of the epidermis and the upper portions of the papillary dermis. The consequence of these age-dependent changes of hyaluronans is probably a declined water binding capacity.

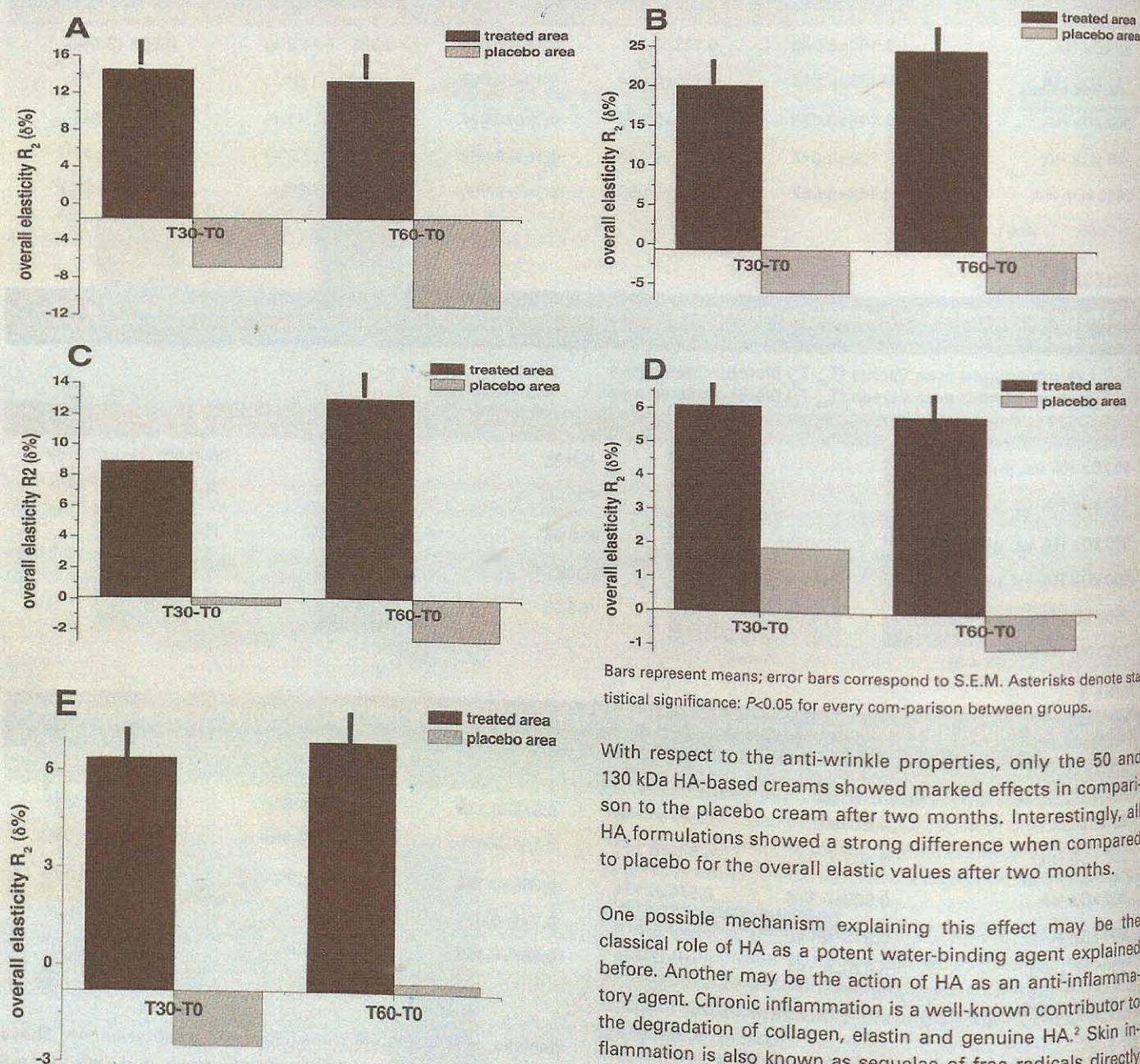
Furthermore, confocal laser microscopy reveals that GAGs in photodamaged skin are abnormally deposited on elastic material in the superficial dermis, rather than diffusely scattered as in young skin.¹⁴ This aberrant localization may interfere with normal water binding capacity of GAGs, es-

specially of HA, despite their increased general levels. These factors likely contribute to increased xerosis and withered appearance of aged skin.¹⁶

Correspondingly, many topical anti-aging products contain HA and many companies claim its efficacy, but so far unequivocal evidence for the efficacy in the treatment of facial wrinkles has been missing. Furthermore, HA has been considered to be unable to penetrate the skin upon topical application.¹⁷

In this *in vivo* study examining objectively the anti-aging and anti-wrinkle properties of HA-based creams of different HA molecular size, all HA-based creams showed an improvement of skin hy-

FIGURE 3. The effects of topical HA formulations containing **A) 50, B) 130, C) 300, D) 800 and E) 2000 kDa HA, respectively, on overall skin elasticity (R_2 , reported as percentage of variation) in comparison to a placebo-treated area.**

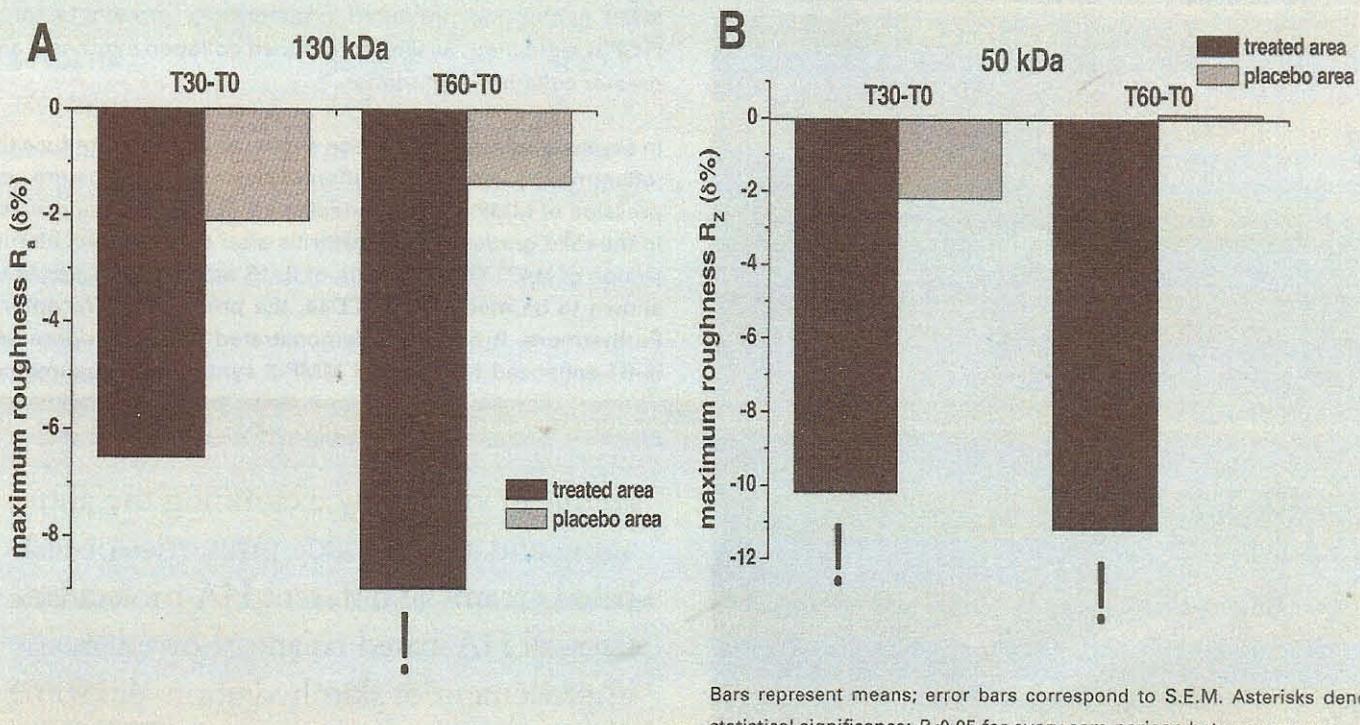


Bars represent means; error bars correspond to S.E.M. Asterisks denote statistical significance: $P<0.05$ for every comparison between groups.

With respect to the anti-wrinkle properties, only the 50 and 130 kDa HA-based creams showed marked effects in comparison to the placebo cream after two months. Interestingly, all HA formulations showed a strong difference when compared to placebo for the overall elastic values after two months.

One possible mechanism explaining this effect may be the classical role of HA as a potent water-binding agent explained before. Another may be the action of HA as an anti-inflammatory agent. Chronic inflammation is a well-known contributor to the degradation of collagen, elastin and genuine HA.² Skin inflammation is also known as sequelae of free radicals directly acting on cytokine and growth factor receptors in dermal cells and keratinocytes. These are known to play a role in skin aging, but the exact nature of their significance has not yet been clarified.² Presently, this process is thought to be induced by UV exposure, which affects growth factor and cytokine receptors, contributing to downstream signal transduction by spurring mitogen-activated protein (MAP) kinase pathways and triggering the matrix-metalloproteinase-1 (MMP-1) and MMP-2.¹⁸ Therefore, reducing inflammation may very well be another rewarding approach to preventing wrinkle formation. In previous studies, high-molecular-weight HA (HMWHA) has been reported to act in an anti-angiogenic manner, whereas low-molecular-weight HA

dermatitis. The parameter of elasticity did not change in the same way for all HA formulations. After two months of treatment, the maximum skin deformation (R_0) was improved only for 800 kDa, the overall elasticity values (R_2) for all HA-based creams, and viscoelastic ratio (R_6) for 50 and 2000 kDa. The measurements of wrinkle depth also revealed differences between the creams with HA of different molecular weights. After 60 days, mean roughness values (R_a) showed an improvement for 130, 300 and 800 kDa HA. The values of maximum roughness (corresponding to the deepest wrinkle) were improved even after one month of treatment with cream-based formulation with 50, 130 and 800 kDa.

FIGURE 4. The effects of topical HA formulations containing **A)** 130 and **B)** 50 kDa HA on roughness values (R_a , **a**) and maximum roughness values (R_z , **B**) reported as percentage of variation) in comparison to a placebo-treated area.

Bars represent means; error bars correspond to S.E.M. Asterisks denote statistical significance: $P<0.05$ for every comparison between groups.

TABLE 4a.The Effects of Topical HA Formulations on Skin Elasticity Parameter Viscoelastic Ratio (R_6) in Comparison to Untreated Area: Mean Values

	T_0	T_{30}	T_{60}	Variation ($T_{30}-T_0$)	Variation ($T_{60}-T_0$)
50 kDa HA	0.243 ± 0.054	0.194 ± 0.046	0.155 ± 0.030	$-0.049^*(-20.2\%)$	$-0.088^{**}(-36.2\%)$
130 kDa HA	0.141 ± 0.057	0.162 ± 0.058	0.141 ± 0.026	$+0.021(14.9\%)$	0
300 kDa HA	0.156 ± 0.047	0.174 ± 0.089	0.136 ± 0.023	$+0.018(11.5\%)$	$-0.020(-12.8\%)$
800 kDa HA	0.251 ± 0.040	0.242 ± 0.036	0.237 ± 0.040	$-0.009(-3.6\%)$	$-0.014(-5.6\%)$
2000 kDa HA	0.213 ± 0.099	0.190 ± 0.102	0.173 ± 0.089	$-0.023(-10.8\%)$	$-0.040^{**}(-18.8\%)$

* $P \leq 0.05$; ** $P \leq 0.01$.

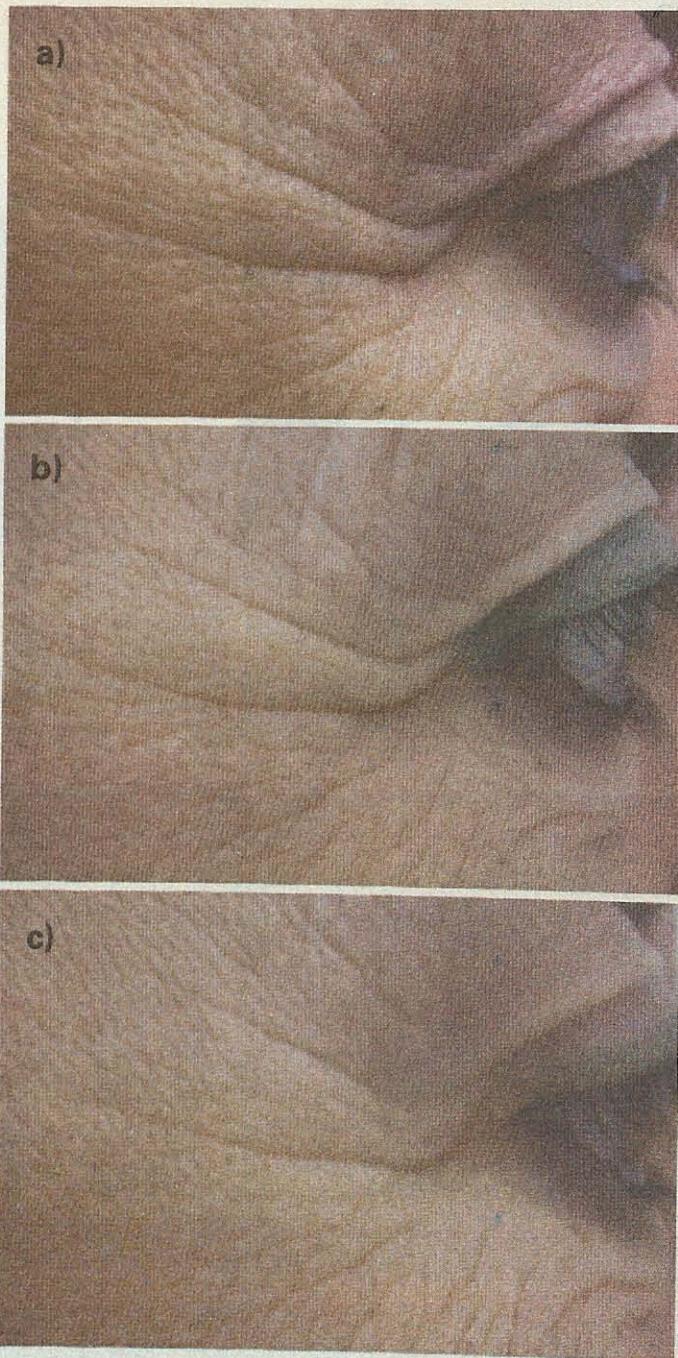
TABLE 4B.The Effects of Topical HA Formulations on Skin Elasticity Parameter Viscoelastic Ratio (R_6) in Comparison to Placebo-Treated Area

($T_{30}-T_0$) verum-treated area versus ($T_{30}-T_0$) placebo-treated area
 ($T_{60}-T_0$) verum-treated area versus ($T_{60}-T_0$) placebo-treated area

	T_{30}	T_{60}
50 kDa HA vs. placebo	$P > 0.05$	$P > 0.05$
130 kDa HA vs. placebo	$P > 0.05$	$P > 0.05$
300 kDa HA vs. placebo	$P > 0.05$	$P > 0.05$
800 kDa HA vs. placebo	$P > 0.05$	$P > 0.05$
2000 kDa HA vs. placebo	$P > 0.05$	$P > 0.05$

* $P \leq 0.05$; ** $P \leq 0.01$.

FIGURE 5. Photographs of right periorbital area a) before, b) after 4 and c) after 8 weeks of treatment with 50 kDa HA cream 0.1%.



(LMWHA) is highly angiogenic, attracting inflammatory cells and also inducing expression of inflammatory cytokines.³

UV light, the main causal factor for the extrinsic component of skin aging, significantly upregulates the synthesis of several types of collagen-degrading enzymes known as MMPs, specifically collagenase MMP-1 and gelatinase MMP-2.¹⁹ By characterizing the wide-ranging effects of UV in activating cell surface growth factor and cytokine receptors, research-

ers have been able to ascertain that skin ageing (extrinsic, but also intrinsic) is marked by elevated AP-1 activity and MMP expression, inhibited transforming growth factor β (TGF β) signalling, as well as reduced collagen synthesis and greater collagen degradation.²⁰

In several studies, HA has been shown to be able to reduce the inflammatory reaction in different tissues.⁴ In synovium, expression of MMP-3 and interleukin-1 β (IL-1 β) was suppressed in the mild grades of osteoarthritis after the intra-articular injection of HA.²¹ The inhibition of IL-1 β action by HA has been shown to be mediated by CD44, the principal HA receptor.²² Furthermore, it has been demonstrated that HA suppresses IL-1 β -enhanced MMP-1 and MMP-3 synthesis in rheumatoid synovial fibroblasts via intercellular adhesion molecule-1 (ICAM-1) through downregulation of NF- κ B and p38.

In this in vivo study examining the anti-aging and anti-wrinkle properties of HA-based creams of different HA molecular sizes, all HA-based creams showed an improvement of skin hydration and some in skin elasticity and wrinkle depth in the periorbital region as compared to the placebo cream, some of them as early as after one month of application.

In this in vivo study examining the anti-aging and anti-wrinkle properties of HA-based creams of different HA molecular sizes, all HA-based creams showed an improvement of skin hydration and some in skin elasticity and wrinkle depth in the periorbital region as compared to the placebo cream, some of them as early as after one month of application. Furthermore, objective measurement methods were used to document the effects—and not only a photo documentation and subjective assessment by the investigator and the volunteer. Although the epidermal and dermal concentration of HA were not examined before or after the treatment, the reported data suggest that these novel HA molecules are able to penetrate into the skin after topical application and reduce the ageing process, contrary to the claims of Bauman and Rieger.^{2,17}

One possible explanation for this might be a novel source of HA; the structural identity of *B. subtilis*-generated HA with the natural HA might well be of note.

According to our not yet published data, the LMWHA with a MW of 50 kDa has been demonstrated to have the best penetration and anti-inflammatory potential of all examined HA molecules (130, 300, 800 and 2000 kDa) in vitro.

TABLE 5a.**The Effects of Topical HA Formulations on the Mean Roughness (Ra) Values in Comparison to Untreated Area: Mean Values**

	T ₀	T ₃₀	T ₆₀	Variation (T ₃₀ -T ₀)	Variation (T ₆₀ -T ₀)
50 kDa HA	29.6±2.6	28.4±5.3	27.1±5.6	-1.2 (-4.1%)	-2.5 (-8.4%)
130 kDa HA	27.9±3.3	26.1±2.3	24.9±1.8	-1.8 (-6.5%)	-3.0** (-10.8%)
300 kDa HA	27.6±5.1	24.8±4.1	22.7±4.4	-2.8 (-10.1%)	-4.9* (-17.7%)
800 kDa HA	35.80±7.45	32.81±7.53	32.24±6.86	-2.99** (-8.4%)	-3.56* (-9.9%)
2000 kDa HA	24.66±3.32	24.64±2.60	24.55±3.19	-0.02 (-0.1%)	-0.11 (-0.4%)

*P≤0.05; **P≤0.01.

TABLE 5b.**The Effects of the Topical HA Formulations on the Maximum Roughness (Rz) Values in Comparison to an Untreated Area: Mean Values**

	T ₀	T ₃₀	T ₆₀	Variation (T ₃₀ -T ₀)	Variation (T ₆₀ -T ₀)
50 kDa HA	156.7±13.6	140.7±25.0	139.4±13.0	-16.0* (-10.2%)	-17.3* (-11.0%)
130 kDa HA	105.1±9.8	99.5±8.4	96.6±12.2	-5.6* (-5.3%)	-8.5* (-8.1%)
300 kDa HA	161.30±23.91	157.98±19.26	162.22±20.06	-3.32 (-2.1%)	+0.92 (0.6%)
800 kDa HA	177.4±17.2	168.7±16.3	166.4±17.9	-8.7* (-4.9%)	-11.0* (-6.2%)
2000 kDa HA	115.98±17.28	115.67±18.21	114.98±16.74	-0.31 (-0.3%)	-1.00 (-0.9%)

*P≤0.05; **P≤0.01.

TABLE 6a.**The Effects of Cream-Based HA Formulations of Different Molecular Weights on Different Parameters After One Month in Comparison to Placebo**

	Corneometry	R0	R2	R6	Ra	Rz
50 kDa	Ø	Ø	↑	Ø	Ø	↑
130 kDa	↑	Ø	↑↑	Ø	Ø	Ø
300 kDa	Ø	Ø	↑	Ø	Ø	Ø
800 kDa	Ø	↑	Ø	Ø	Ø	Ø
2000kDa	Ø	Ø	↑	Ø	Ø	Ø

Ø: P>0.05; ↑: P<0.05; ↑↑: P<0.01

TABLE 6b.**The Effects of Cream-Based HA Formulations With Different Molecular Weights on Different Skin Parameters After Two Months in Comparison to Placebo**

	Corneometry	R0	R2	R6	Ra	Rz
50 kDa	Ø	Ø	↑↑	Ø	Ø	↑
130 kDa	↑↑	Ø	↑↑	Ø	↑	Ø
300 kDa	↑	Ø	↑↑	Ø	Ø	Ø
800 kDa	↑	↑	↑↑	Ø	Ø	Ø
2000kDa	↑↑	Ø	↑↑	Ø	Ø	Ø

Ø: P>0.05; ↑: P<0.05; ↑↑: P<0.01

Although in this study the relation between the molecular weight of HA molecules in the formulation and its anti-aging and anti-wrinkle potential in the periorbital region did not show a generally close correlation (Table 6a and b), the molecular weight might nevertheless play an important role, especially in the UV-induced inflammatory changes in the aging process. Future clinical intra-individual comparative studies of HA formulations with HA molecules of different molecular weights as well as in vivo examination of HA-induced changes addressing molecular parameters, like MMPs, different cytokines and growth factors and their receptors, are needed to bring more light into this complex field.

DISCLOSURES

This study was funded by Evonik Goldschmidt, Essen, Germany and by Novozymes Biopharma DK A/S, Bagsvaerd, Denmark. Mike Farwick and Peter Lersch are employees of Evonik Goldschmidt. Khadija Schwach-Abdellaoui and Birgitte Malle are employees of Novozymes Biopharma. Hans Christian Korting, Gerd G. Gauglitz and Tatjana Pavicic do not have any relevant conflicts of interest to disclose besides the already mentioned.

REFERENCES

- Goldberg RA, McCann JD, Fiaschetti D, Ben Simon GJ. What causes eyelid bags? Analysis of 114 consecutive patients. *Plast Reconstr Surg.* 2005;115:1395-1402.
- Baumann L. Skin ageing and its treatment. *J Path.* 2007;211:241-251.
- Stern R, Maibach HI. Hyaluronan in skin: Aspects of ageing and its pharmacological modulation. *Clin Dermatol.* 2008;26:106-122.
- Weindl G, Schaller M, Schäfer-Korting M, Korting HC. *Skin Pharmacol Physiol.* 2004;17:207-213.
- Alkraib JA, Mrestani Y, Strochl D, Wartewig RJ, Chen WY. Comparison of the antioxidant properties of wound dressing materials, carboxymethylcellulose, hyaluronan benzyl ester and hyaluronan, towards polymorphonuclear leukocyte-derived reactive oxygen species. *Biomaterials.* 2003;24:1549-1557.
- Nehls V, Hayen W. Are hyaluronan receptors involved in three-dimensional cell migration? *Histol Histopathol.* 2000;15:629-636.
- Chen WY, Abatangelo G. Functions of hyaluronan in wound repair. *Wound Repair Regen.* 1999;7:79-89.
- Wiest L, Kerscher M. Native hyaluronic acid in dermatology – Results of an expert meeting. *J Dtsch Dermatol Ges.* 2008;6:176-180.
- Andre P. Hyaluronic acid and its use as a "rejuvenation" agent in cosmetic dermatology. *Semin Cutan Med Surg.* 2004;23:218-222.
- Huang HC, Chang TM. Ceramide 1 and ceramide 3 act synergistically on skin hydration and the transepidermal water loss of sodium lauryl sulfate-irritated skin. *Int J Dermatol.* 2008;47:812-819.
- André T, De Wan M, Lefèvre P, Thonnard JL. Moisture Evaluator: A direct measure of fingertip skin hydration during ob-ject manipulation. *Skin Res Technol.* 2008;14:385-389.
- Giacomoni PU. Advancement in skin aging: The future cosmeceuticals. *Clin Dermatol.* 2008;26:364-366.
- Koblenzer CS. Psychosocial aspects of beauty: How and why to look good. *Clin Dermatol.* 2003;21:473-475.
- Bernstein EF, Underhill CB, Hahn PJ, Brown DB, Utto J. Chronic sun exposure alters both the content and distribution of dermal glycosaminoglycans. *Br J Dermatol.* 1996;135:255-262.
- Meyer LJM, Stern R. Age-dependent changes of hyaluronan in human skin. *J Invest Dermatol.* 1994;102:385-389.
- Waller JM, Maibach HI. Age and skin structure and function, a quantitative approach (II): Protein, glycosaminoglycan, water, and lipid content and structure. *Skin Res Technol.* 2005;12:145-154.
- Rieger M. Hyaluronic acid in cosmetics. *Cosm Toil.* 1998;113:35-42.
- Fisher GJ, Voorhees JJ. Molecular mechanisms of photoaging and its prevention by retinoic acid: Ultraviolet irradiation induces MAP kinase signal transduction cascades that induce Ap-1-regulated matrix metalloproteinases that degrade human skin in vivo. *J Invest Dermatol Symp Proc.* 1998;3:61-68.
- Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin ageing induced by ultraviolet light. *N Eng J Med.* 1997;337:1419-1428.
- Rittie L, Fisher GJ. UV-light-induced signal cascades and skin aging. *Ageing Res Rev.* 2002;1:705-720.
- Takahashi K, Goomer RS, Hatwood F, Kubo T, Hirsawa Y, Amiel D. The effects of hyaluronan on matrix metalloproteinase-3 (MMP-3), interleukin-1 β (IL-1 β), and tissue inhibitor of metalloproteinase-1 (TIMP-1) gene expression during the development of osteoarthritis. *Osteoarthritis Cartilage.* 1999;7:182-190.
- Hiramatsu T, Yasuda T, Ito H, Shimizu M, Julovi SM, Kakinuma T, Akiyoshi M, Yoshida M, Nakamura T. Intercellular adhesion molecule-1 mediates the inhibitory effects of hyaluronan on interleukin-1 β -induced matrix metalloproteinase production in rheumatoid synovial fibroblasts via down-regulation of NF- κ B and p38. *Rheumatology.* 2006;45:824-832.

ADDRESS FOR CORRESPONDENCE

Dr. Tatjana Pavicic, MD

Department of Dermatology and Allergology

Ludwig Maximilians University, Munich

Frauenlobstraße 9-11

D-80337 Munich, Germany

Phone: +49(0)89/5160-6010

Fax: +49(0)89/5160-6110

E-mail: tatjana.pavicic@med.uni-muenchen.de