Journal of Evidence-Based Complementary & Alternative Medicine
2017, Vol. 22(4) 816-823

DOI: 10.1177/2156587217743640
journals.sagepub.com/home/cam
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Ingestion of an Oral Hyaluronan Solution Improves Skin Hydration, Wrinkle Reduction, Elasticity, and Skin Roughness: Results of a Clinical Study

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Abstract

Intake of oral supplements with the aim of a cutaneous antiaging effect are increasingly common. Hyaluronic acid (HA) is a promising candidate, as it is the key factor for preserving tissue hydration. In our practice study, we evaluated the effect of an oral HA preparation diluted in a cascade-fermented organic whole food concentrate supplemented with biotin, vitamin C, copper, and zinc (Regulatpro Hyaluron) on skin moisture content, elasticity, skin roughness, and wrinkle depths. Twenty female subjects with healthy skin in the age group of 45 to 60 years took the product once daily for 40 days. Different skin parameters were objectively assessed before the first intake, after 20 and after 40 days. Intake of the HA solution led to a significant increase in skin elasticity, skin hydration, and to a significant decrease in skin roughness and wrinkle depths. The supplement was well tolerated; no side effects were noted throughout the study.

Keywords

hyaluronic acid, hydration, wrinkle reduction, elasticity, skin roughness

Received September 1, 2017. Accepted for publication October 12, 2017.

The skin is the largest organ of the body, which acts as a barrier protecting against exogenous factors. Because of the synergistic effects of chronological, extrinsic, and intrinsic aging, skin appearance and integrity worsen over the course of time. Aging is a multifactorial process defined as the accumulation of damage.[1] Skin changes associated with aging are a loss of hydration, elasticity, and turgor.[2,3] Wrinkles in the face are the most prominent recognized signs of skin aging, which often appear in the periorbital area and include fine wrinkles.

Hyaluronic acid (HA) is a high-molecular-weight polysaccharide composed of repeated polymeric disaccharides of D-glucuronic acid and *N*-acetyl-D-glucosamine.[4] It is a major component of the extracellular matrix of the skin and plays a key role in the metabolism of the dermis. It is one of the most hydrophilic molecules in nature and can be described as naturés moisturizer.[4,5] With aging, the epidermal HA content decreases from 0.03% in women aged 19 to 47 years down to 0.015% in women aged 60 years and halves to 0.007% in women aged 70 years.[6] In senile skin, HA is still present in the dermis, while HA of the epidermis has disappeared entirely.[7] In addition, HA polymers in senescent skin have a diminished ability to take on water of hydration with the consequence of a loss in skin moisture, commonly seen in aging skin.[8]

An important trend in skin care is the use of diet and oral supplements to improve the appearance of skin, as healthy skin largely reflects the general health status.[9] Nutritional factors exert promising actions on the skin, but information on the effects of low-to-moderate doses of nutrients consumed long term by healthy individuals is lacking, as are data on direct effects on basal skin properties, including hydration, sebum production, and elasticity.[10]

Structural proteins and glycosaminoglycans are particularly promising agents for oral skin rejuvenation: Oral supplementation of specific collagen peptides has been shown to increase skin elasticity and reduce eye wrinkle volume,[11-13] Another trial showed that supplementation with an oral collagen peptide improves density and skin hydration.[14] Other micronutrients add to the positive effect on aging skin. In a trial including

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53 female volunteers, an oral supplement containing glucosamine, amino acids, minerals, and antioxidants led to a significant reduction in the number of visible wrinkles and fine lines, but did not affect epidermal hydration.[15]

HA is increasingly used as a dietary supplement. In a Japanese trial, intake of HAs with 2 different molecular weights improved the skin condition by increasing the moisture content in Japanese women aged 35 to 60 years, who complained about dry and sagging skin or wrinkles around the outer canthus.[16] However, data regarding influence of oral HA on the skin of Caucasians are scarce. In our study, we assessed objectively and subjectively the influence of HA of biotechnological origin with a molecular weight of $\geq$1 MDa, designed for nutritional use, diluted in a cascade-fermented organic whole food concentrate (Regulatpro Hyaluron) on skin hydration, elasticity, roughness, and wrinkle depths. The complex method of cascade fermentation is a natural biochemical extraction that contains essential amino acids, di-, tri-, and oligopeptides, polyphenols, flavonoids (phytochemicals), all obtained from organically grown fruits, nuts, and vegetables and probiotic components from the lactic acid bacteria.

To the best of our knowledge, this is the first study demonstrating that an oral solution of hyaluronic acid in a cascade-fermented organic whole food concentrate is effective in moisturizing the skin, reducing wrinkles, and improving elasticity and skin roughness in Caucasian women.

Materials and Methods

Test Product

The test product used in this study was a HA of biotechnological origin (NUTRIHYL) with a molecular weight of $\geq$1 MDa, designed for nutritional use, diluted in in a cascade-fermented organic whole food concentrate supplemented with biotin, vitamin C, copper, zinc, and natural silica.

The product was provided by Dr Niedermaier Pharma GmbH (Hohenbrunn, Germany), commercially available as Regulatpro Hyaluron.

Study Design

The study was carried out as an open, uncontrolled monocentric study on the effects of this specific HA preparation on 20 female healthy participants after 40 days of daily intake.

All test subjects received detailed information listing every single parameter relevant to the study. All subjects gave signed, informed consent after receipt of the written information and having had a possibility for further questioning.

The study was approved by the "Freiburger Ethic-Kommission International," Freiburg, Germany.

Subjects

A total of 20 female subjects with healthy skin in the age group of 45 to 60 years were enrolled in the trial. The product was drunk by the participants at home once daily in the morning. Participants were advised to drink one glass of still water following the consumption of 20 mL Regulatpro Hyaluron, to enable the HA to hydrate as soon as possible. Before study entry, all participants were examined by a dermatologist.

Inclusion Criteria

The inclusion criteria were as follows. Females with healthy skin, without pathologic skin changes in the test area, ranging in age from 45 to 60 years, personal informed consent to participate in the study, personal presence on the predefined days at the institute. Persons had to be able to communicate with the investigator, and to understand and follow the requirements of this clinical trial.

Exclusion Criteria

Exclusion criteria were any deviation from the aforementioned inclusion criteria, severe or chronic skin diseases (eg, psoriasis, atopic eczema), severe internal or chronic diseases, systemic medications that are able to diminish skin reaction (glucocorticoids, antiallergics, topical immune modulators), application of drug containing preparations or cosmetic preparations 7 to 10 days prior to the start, severe allergies or severe side effects toward cosmetic products in the past, tanning, or use of sunbeds during the trial. Pregnant, breastfeeding, or cancer patients were also excluded from the trial.

During the study, participants were advised not to change their lifestyle or usual habits (eg, regarding smoking or alcohol consumption) and not to change or stop intake of estrogen or progesterone.

Assessments

The test sites were the wrinkle area around both eyes (latheral canthus). Skin hydration was assessed at the forearm.

Measurement Times

All measurements were performed before the first intake of the product, after 20 and 40 days.

Measurement of Skin Hydration

Assessment of skin surface hydration by electrical capacitance was carried out using the Corneometer CM 825 (Courage + Khazaka electronic GmbH, Cologne, Germany) as recommended by the manufacturer and described in earlier publications.[17,18]

Measurement of Skin Elasticity

Skin elasticity was assessed with the Cutometer MPA 580 (Courage + Khazaka electronic GmbH). The Cutometer measures elasticity of the upper skin layer using negative pressure, which deforms the skin mechanically. The measuring principle is based on the suction method. Negative pressure is created in the device and the skin is drawn into the aperture of the probe and after a defined time released again. Inside the probe, the penetration depth is determined by a noncontact optical measuring system. This optical measuring system consists of a light source and a light receptor, as well as 2 prisms facing each other, which project the light from transmitter to receptor. The light intensity varies due to the penetration depth of the skin. The resistance of the skin to the negative pressure (firmness) and its ability to return into its original position (elasticity) are displayed as curves (penetration depth in mm/time) in real time during the measurement. This measurement principle allows getting information about the

elastic and mechanical properties of skin surface and enables to objectively quantify skin aging.

In this trial factor R2 parameters were used (R2 = Ua/Uf, the relation between final deformation (Uf) and total deformation recovery at the end of the stress of period (Ua). R2 is a good parameter of gross elasticity, defined as resistance versus the ability of returning of the skin. It is also known as the overall elasticity of the skin, including creep and creep recovery.[19]

Measurement of Skin Roughness

The skin roughness was assessed using the PRIMOS Compact (GFMesstechnik, Teltow, Germany) device for analysis with a measuring field size of 10 × 8 mm. PRIMOS compact is an optical 3-dimensional in vivo skin measuring system based on the stripe projection technique. A parallel stripe pattern is projected onto the measuring area via projection optics and displayed on the chip of a charge-coupled device matrix camera via recording optics. Ra value is calculated from height profile that is measured from the reflection of the parallel stripes. Reduction of Ra means reduction of total area of roughness.

Measurement of Wrinkle Depth

Assessment of wrinkle depth was performed with PRIMOS compact handheld, the same 3-dimensional optical system described above, but with a handheld device that allows flexibility in the target area. With this device, wrinkles in the periorbital area were assessed in an area of 40 × 30 mm.

Dermatological Assessment

Before intake of the HA preparation and after 20 and 40 days, a dermatologist assessed the skin regarding redness, scaling, and dryness.

Subjective Assessments of Treatment Effects

All patients filled in a questionnaire after 20 and 40 days consisting of 25 questions regarding the effect of the preparation on skin parameters, hair and nails, and their satisfaction with the product and its taste.

Statistical Analysis

All parameters (skin hydration, elasticity, skin roughness, wrinkle depth) were assessed at the 3 observation times at baseline, after 20 and after 40 days. The obtained values are descriptively presented as mean with standard deviation, minimum, maximum, and quartiles. The Kolmogorov-Smirnov test was performed to test for normal distribution. As no significant deviations from normal distribution were detected (Kolmogorov-Smirnov test, $P \geq .05$), further statistical analyses were carried out using parametric tests.

Measurements of corneometry, elasticity, skin roughness, and wrinkle depths at baseline, after 20 and after 40 days were compared using repeated-measures analysis of variance (ANOVA). In case of significant global effects, post hoc comparisons between 2 time points were carried out using Bonferroni corrected matched samples *t* tests.

Statistical tests were performed 2 sided with significance level of 5%. IBM SPSS Statistics 24 (SPSS Inc, an IBM Company, Chicago, IL) was used for the statistical calculations.

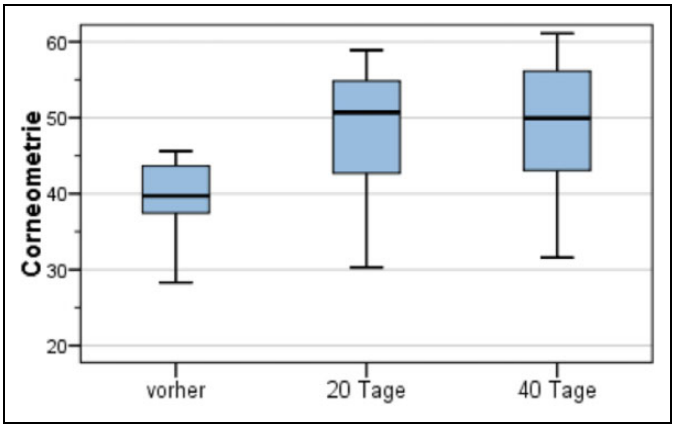

Figure 1. Skin hydration over the course of the trial. At both assessments (after 20 and 40 days), skin hydration was significantly improved versus baseline ($P < .001$).

Results

Skin Hydration

Skin hydration at baseline was 39.32 ± 5.17 and increased significantly to 47.79 ± 8.19 after 20 days and to 48.87 ± 8.41 after 40 days (repeated-measures ANOVA, $P < .001$) (Figure 1). This corresponds to an increase in skin hydration by 21.63% at the first assessment at 20 days and by 24.43% after 40 days. At both timepoints, skin hydration was significantly higher than before intake. Maximum increase in skin hydration was 37.18%.

Skin Elasticity

Before intake, overall elasticity values (R2 = Ua/Uf ratio) were 0.616 ± 0.056. After 20 days of intake, elasticity significantly increased to 0.668 ± 0.057, and after 40 days of intake once more significantly higher values of 0.697 ± 0.051 were assessed (repeated-measures ANOVA, $P < .001$). This corresponds to an overall increase of 8.58% at day 20 in comparison with the start of the study. After 40 days of intake, an increase by 13.25% was noticed. In the course of the study, there was a continuous significant increase of elasticity from baseline to day 20 to day 40 (Figure 2). The maximum gain of elasticity was 26.16%.

Skin Roughness

Before intake, skin roughness was 151.48 ± 17.92. Skin roughness decreased to 132.34 ± 18.47 after 20 days and 125.94 ± 19.18 after 40 days (Figure 3). The overall change and also differences between each assessment were significant (repeated-measures ANOVA and post hoc comparisons, $P < .001$ for each comparison). Skin roughness decreased from baseline to the assessment after 20 days by 12.67% and after 40 days by 16.9% The maximum decrease seen in this trial was 30.40%.

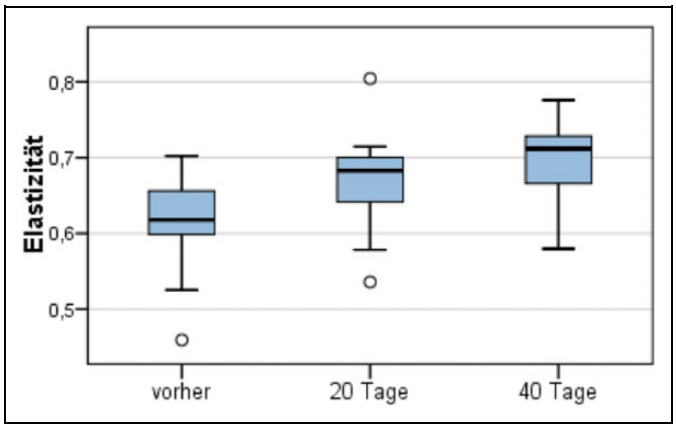

Figure 2. Increase of elasticity throughout the trial. Skin elasticity increased steadily to the end of the trial. The gain of elasticity was significant for each comparison ($P < .001$).

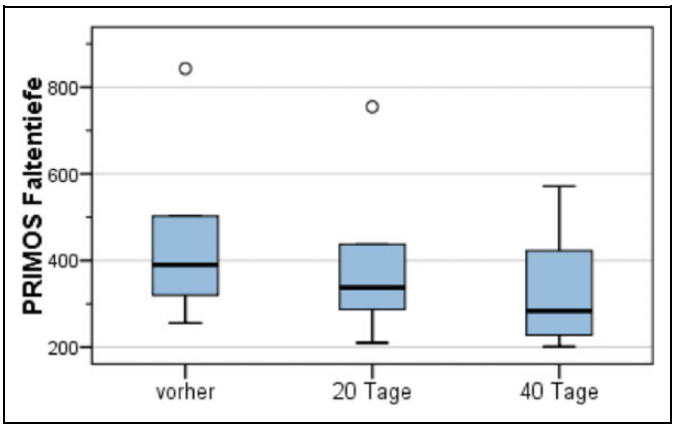

Figure 4. Wrinkle depth was reduced till the end of the trial. Wrinkle depths were significantly lower than baseline at both assessments ($P < .001$).

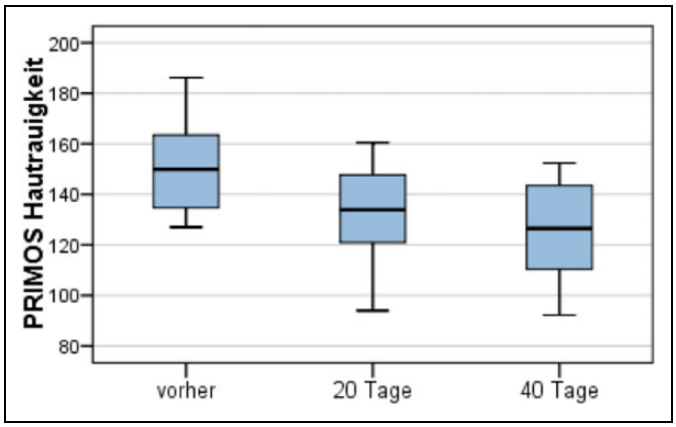

Figure 3. A continuous decrease of skin roughness was seen throughout the trial. The differences were significantl for each comparison ($P < .001$).

Figure 5. Participant before intake of the hyaluronic acid solution.

Wrinkle Depth

Measurements of wrinkle depth were performed in 6 patients. Wrinkle depth before intake was 450.28 $\pm$ 209.12. After 20 days of intake, wrinkle depth significantly decreased to 394.02 $\pm$ 191.74 and after 40 days of intake a further significant reduction of wrinkle depth to 331.35 $\pm$ 142.18 was assessed (repeated-measures ANOVA, $P = .020$ and post hoc comparisons with baseline, $P = .002$ at day 20 and $P = .048$ at day 40) (Figure 4). This corresponds to a reduction of wrinkle depth by 12.5% after 20 days and by 26.36% after 40 days. The maximum reduction of wrinkle depths was −37.57%. Figures 5 and 6, respectively, show one of the participants before and after 40 days of intake.

Dermatologic Skin Evaluation

Before the intake of the HA solution, all subjects had healthy skin. At this time, 1 subject had normal skin, 7 dry skin, 11 dry/sensitive skin, and 1 very dry skin.

According to the self-assessment, 6 out of 20 participants had stronger hair after 20 days of intake. The percentage of patients who believed to have stronger hair compared with baseline increased to 50% (n = 10) after 40 days. Likewise, nails became firmer: After 20 days of intake, 30% of participants had firmer nails (n = 6), and after 40 days of intake 45% (n = 9) had firmer nails.

Participants' Satisfaction

At the end of the study, 70% of patients (n = 14) would recommend the HA supplementation, 60% (n = 12) were willing to buy the product.

Figure 6. Participant after intake of the hyaluronic acid solution.

Tolerability

Tolerability of the oral HA preparation was very good in all participants according to the clinical assessment of 2 dermatologists. There were no side effects or unwanted skin changes throughout the study. The dermatologist confirmed an excellent tolerability of the product regarding skin parameters during the whole study.

Discussion

Aging is a multifactorial and complex process. Loss of hydration, elasticity, and turgor are hallmarks of senescent skin.[2] HA is the prototype of a glycosaminoglycan, which is found in all tissues and body fluids of vertebrates as well as in some bacteria.[4] It is widely distributed in body tissues and intracellular fluids, and is present at high concentration in the synovial fluid, vitreous fluid of the eye, and umbilical cord.[5] Its function in the body is, among other things, to bind water and to lubricate movable parts of the body, such as joints and muscles.

More than 50% of the total body content of HA is present in the skin.[20] It plays a key role in maintaining extracellular spaces, preserving tissue hydration, and facilitating the transport of ion solutes and nutrients to cells in the upper layer of the skin because of its water-retaining capacity.[21] HA can bind up to 6000 times their volume in water, thus controlling tissue hydration. In addition, it protects fibroblasts against cell damage mediated by hydroxyl radicals, which play a key role in skin aging.[22] Recent studies suggest that HA, via the CD44 receptor, is also capable of increasing cell differentiation and cell motility.[23]

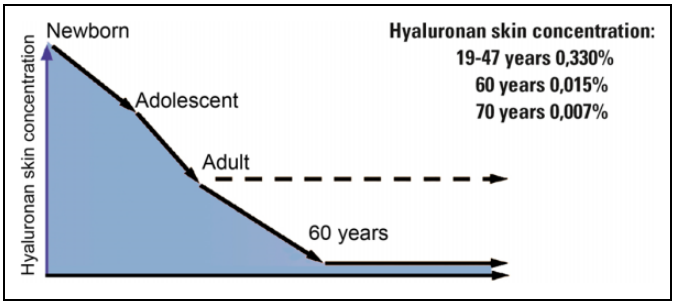

Figure 7. The decrease of epidermal hyaluronic acid content is a hallmark of skin aging on the molecular basis (adapted from Necas et al[6]).

The most dramatic histochemical change observed in senescent skin is the marked decrease in epidermal HA. With aging the epidermal HA content decreases from 0,03% in women aged 19 to 47 years down to 0.007% in 70-year-old women (Figure 7).[6] In senile skin, HA is still present in the dermis, while HA of the epidermis has disappeared entirely.[7] In addition, HA polymers in senescent skin become progressively more tissue associated with the concomitant loss of HA extractability.[8,24] Such tissue-bound HA may have diminished ability to take on water of hydration with the consequence of a loss in skin moisture, commonly seen in aging skin. The increased binding of HA with tissue as a function of age parallels the progressive cross-linking of collagen and the steady loss of collagen extractability with age. Each of these phenomena contribute to the apparent dehydration, atrophy, and loss of elasticity that characterizes aged skin.[7] Thus, changes in glycosaminoglycans and proteoglycans are important contributors to skin aging.[25] Reductions in epidermal HA are consistently found both in intrinsically aged skin and in photoaged skin.[25,26] In photoaged skin, abnormal accumulation of HA in regions of solar elastosis are observed, caused by cumulative damage from chronic sun exposure.[27]

Free radicals play a key role in skin aging. Free radicals directly act on cytokine and growth factor receptors in dermal cells and keratinocytes and cause chronic inflammation. This process is known to play a role in skin aging, but the exact nature of its significance has not yet been clarified.[2] HA counteracts aging processes by its anti-inflammatory and radical scavenging properties. It can both be protective as a free radical scavenger and at the same time be a target for free radial stress. because it is itself harmed by the more toxic free radicals. The result of the destruction of HA by free radicals are HA fragments that are themselves highly angiogenic and inflammatory. This is the rationale to protect HA by antioxidants to prevent degradation.[7] Thus, the combination of antioxidants and HA, as evident in the HA product we tested, may help maintain HA integrity, and may have a major effect against natural aging and photoaging alike.

As the demand for interventions to ameliorate visible signs of aging is continuously growing, interest in the development

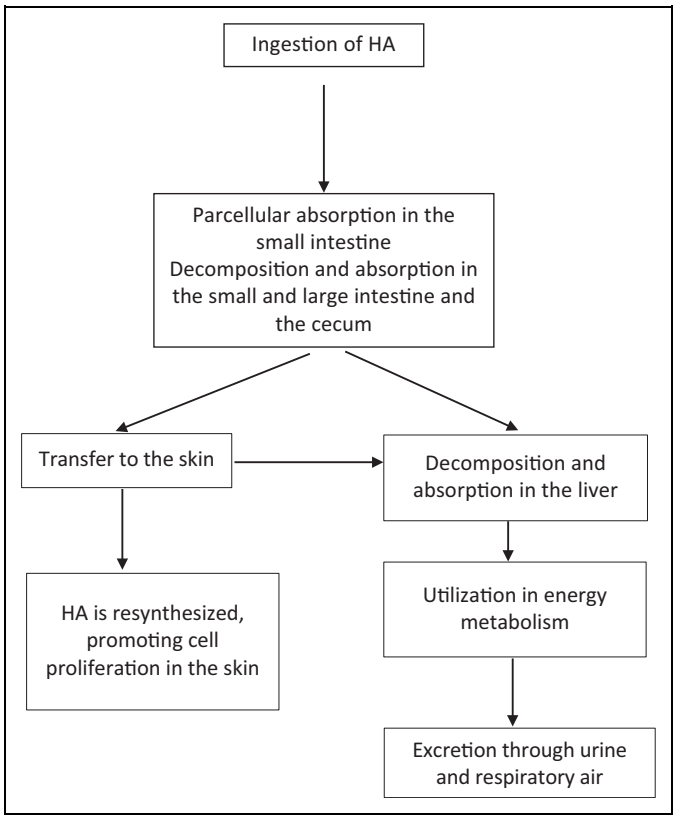

Figure 8. Pharmacokinetics of ingested hyaluronan (adapted from Balogh et al[31]).

of dietary supplements and functional food products for skin health has increased as well.[28]

Recently, HA has been used in cosmetics and foods with the aim of improving skin condition. The topical application of HA led to significant improvement in skin hydration and elasticity. In addition, topical application of low-molecular-weight HA was associated with a significant reduction of wrinkle depth.[29]

As HA is a big molecule, there have been some speculations whether it can be absorbed. However, in the literature there are a couple of experimental and clinical trials demonstrating that this is indeed the case. A Japanese group showed that HA with high molecular weight can be absorbed and migrates into the skin of rats.[30] Another trial documented the absorption of a high-molecular-weight hyaluronan in rats and dogs.[31] According to Kawada et al,[32] HA can be absorbed by humans and is distributed, in part, to the skin (Figure 8). Ingested HA contributes to the increased synthesis of HA and promotes cell proliferation in fibroblasts.[32] Another trial confirmed the uptake of HA in connective tissue.[31]

A number of trials have demonstrated that oral HA intake improves symptoms of osteoarthritis.[33-35] A review highlighted the role of oral HA supplementation for the treatment of pain due to osteoarthritis.[34] According to this review, oral HA supplementation is effective in relieving osteoarthritis pain, synovial effusion, or inflammation, and improving muscular knee strength. The mechanism by which HA exerts its effects has gradually been clarified: HA is absorbed via the paracellular pathway in the small intestine. In addition, HA is decomposed into 2- to 6-membered polysaccharides by enteric bacteria, and these polysaccharides are partially absorbed into the body by the small intestine. Following the decomposition of HA, free polysaccharides are known to migrate into the joints and other tissues.[34] In another trial, a HA preparation inhibited bone resorption and cartilage degradation in athletes.[36]

In an experimental trial, the HA used in our study has been shown to be absorbed in rats and was able to inhibit bone resorption and provide a protective effect on bone density in ovariectomized rats.[37]

In our open, uncontrolled monocentric clinical trial the oral intake of the before mentioned HA dissolved in a cascade-fermented organic whole food concentrate (Regulatpro Hyaluron) led to an increase in skin hydration, gain of skin elasticity, decrease of skin roughness, and decrease in wrinkle depths. The fact that elasticity was continuously increasing and skin roughness decreasing up to the last assessment hints to the fact that a longer duration of this trial might have led to an even more pronounced result regarding these parameters.

The increase of skin hydration is in line with other trials that assessed the effect of oral glycosaminoglycans. In a Japanese trial, the ingestion of HA (molecular weight 800 and 300 kDa) by subjects with dry skin increased their skin moisture content and improved skin aging symptoms such as the luster and suppleness of facial skin.[16] In an experimental study of the same author group, HA was able to prevent skin dryness and epidermal thickening in hairless mice after ultraviolet radiation.[38] In a recent trial, a combined supplement, which consists of hydrolyzed collagen, hyaluronic acid, vitamins, and minerals was investigated.[13] In this trial, intake of this supplement for 60 days led to a noticeable reduction in skin dryness, wrinkles, and nasolabial fold. In another trial, an oral supplement containing glucosamine, amino acids, minerals, and antioxidants was able to improve significantly appearance of skin (assessed in a visual analogue scale photoaging score), skin hydration, sebum, and tonicity 2 weeks after the end of a 4-week treatment period in patients affected by moderate to severe facial photoaging.[39]

Three trials have assessed the oral intake of collagen.[11,12,40] In a double-blind placebo-controlled trial, supplement with collagen peptides for 8 weeks was compared with placebo in 69 women aged 35 to 55 years.[11] At the end of the trial and after a follow-up period of 4 weeks, skin elasticity showed a mean 7% increase (assessed with a cutometer), which was statistically significant compared with placebo ($P < .05$). However, objective measurements regarding skin moisture and skin roughness showed that this supplement failed to show a significant effect on these parameters. In a second double-blind, placebo-controlled trial by the same authors, the collagen peptides led to a significant reduction of eye wrinkle volume after a treatment period of 4 and 8 weeks ($P < .05$).[12] Additionally, after 8 weeks of intake, a statistically significantly higher contents of procollagen type I and elastin were detected.[12] In another trial, therapy with collagen peptides led to an improvement in the degree of cellulite and a reduced skin waviness on

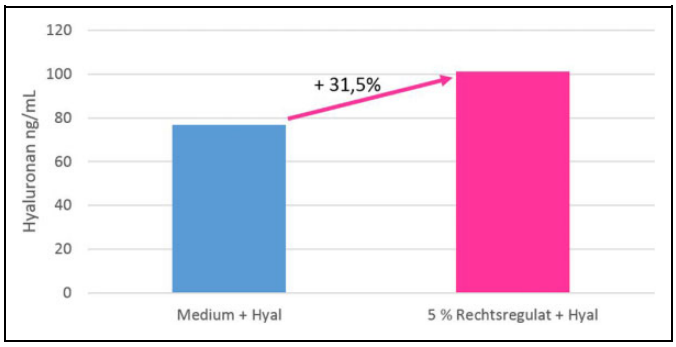

Figure 9. Increase of hyaluronan absorption (mean value) by the addition of Regulat in vitro (adapted from Niedermaier Pharma GmbH[44]).

thighs ($P < .05$) in normal weight women. Moreover, dermal density was significantly improved ($P < .05$) compared with placebo.[40] These trials clearly demonstrate that collagen peptides lead to an increase of elasticity, probably by increasing dermal matrix macromolecule biosynthesis or synthesis of procollagen type I and elastin.[11,12] However, an influence on skin hydration and roughness could not be demonstrated. This is in line with the physiologic effect of elastin and collagen, because these fiber components are mainly responsible for skin viscoelasticity. In contrast, skin hydration is highly related to the content and distribution of dermal glycosaminoglycans, especially HA. The amount of HA in the skin is one of the main factors that determines the skin moisture content.[26] Increases in cell numbers elevate the amount of HA synthesis in the skin, thereby suppressing skin water loss by filling the gaps between skin cells.[16] The supplementation of intact HA is obviously able to improve water binding capacity and thus, increasing hydration. In addition, the supplementation with HA also increased elasticity in our trial. This 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] In addition, HA stimulates human fibroblast proliferation within a collagen matrix.[41] These multiple effects of HA could explain the superior results seen in our trial compared to the supplementation with collagen peptides that influence first and foremost skin elasticity.

The superior results demonstrated in our study might have been due to the solution of HA in a cascade-fermented organic whole food concentrate, which acts as carrier and leads to improved absorption of HA. The Caco-2 cell system, a well-characterized intestinal in vitro model, makes it possible to evaluate the ability of chemicals to cross the intestinal barrier, as well as to study their transport mechanisms.[42] In vitro studies with this model show that the cascade-fermented organic whole food concentrate Regulatessenz displays a carrier effect for micronutrients, particularly for iron.[43] Another in vitr -study with this cell line demonstrated that uptake of HA is significantly enhanced, if it is applied together with Regulatessenz (5% or 15%) compared with HA and medium alone (Figure 9).[44]

The HA solution in our trial also contains other compounds such as zinc, copper, biotin, and vitamin C, which may have further enhanced the effect on skin parameters.

In conclusion, the results of the study clearly demonstrated that the oral intake of a specific HA dissolved in a cascade-fermented organic whole food concentrate led to a statistically significant increase in skin hydration and elasticity. Moreover, a significant decrease in skin roughness and wrinkle depth could be demonstrated. Overall, intake of HA over a longer period of time seems to have a positive impact on skin health. It has to be emphasized that the demonstrated efficacy refers to the specific HA composition (Regulatpro Hyaluron) used in this study and because of the unique preparation results cannot be extrapolated to HA in general.

After these encouraging results, more research is needed. Our results should be confirmed in a larger scale double-blind placebo controlled setting over a longer period of time.

Author Contributions

IG was involved with study planning and performance. WV was involved with study planning. UvH performed the statistical analysis. SK planned the study and reported findings.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The project was funded by Dr Niedermaier Pharma GmbH, Georg-Knorr-Straße, 85662 Hohenbrunn, Germany.

Ethical Approval

This study was approved by the "Freiburger Ethic-Kommission International", Freiburg.

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