

Efficacy Study of Skingain™ - A Product Containing Collagen 1 and a Marine Hydrolysate.

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Abstract

The present study was designed as an open phase study without placebo control in order to investigate if a novel product containing a combination of collagen 1 and a hydrolysate of marine source would have an effect on skin parameters.

In the study, collagen contents and skin elastic were measured prior to the intervention and at intervals of 4 weeks, for a total period of 12 weeks. In addition, close-up photos were taken prior to the intervention and after 8 and 12 weeks respectively, and 9 different parameters were analyzed by the investigator. 53 participants took part in the study, of which 48 completed all measurements.

The average collagen content increased by 25 % during the initial 4 weeks, via 35 % increase after 8 weeks to 45,6 % increase after 12 weeks (3 months). The improvement after 4 weeks were significant ($P < 0.01$) and after 8 and 12 weeks ($p < 0.001$) Skin elasticity measured as retention time improved after 4 weeks treatment ($p < 0.001$), but this part of the study was then discontinued due to heating during the cold season. Visual inspection of photos revealed significant improvements in wrinkle depth, plumping up, pigmentation, hydration and lifting up of eye lids and face, while changes in couperose and oil production were less pronounced.

Key words: Collagen, GAG, marine hydrolyzate, dermis, skin elasticity, skin aging, pigmentation

Introduction

The skin is the largest organ of the body, making up 16 % of the total body weight. Its main function is that of forming a physical barrier to the environment, allowing and limiting the passage of water electrolytes and other molecules both from the body and into the body, while providing protection against micro organisms, ultraviolet radiation, toxic agents and mechanical insults.

While keratinocytes are the main cells of the outer layer of the skin, the epidermis, the dermis is made up of collagen, elastin and proteoglycans that are all to a large extent produced by fibroblast cells of the dermis. In addition, the dermis contains mast cells and macrophages. Collagen, elastin, proteoglycans and glycosaminoglycans (GAGs) form the matrix that gives the dermis its properties. In addition the extracellular matrix of the dermis contain other glycoproteins, including fibronectin, laminin and entactin. Collagen fibers make up 70 % of the dermis, giving it strength and toughness, while elastin and the proteglycans, glycosaminoglycans and glycoproteins are involved in creating the structure of the dermis.

The skin constantly renews itself throughout life. This also applies to the hair and the nails. Both are appendices to the skin and many of the processes of this renewal are common for skin, hair and

nails. This renewal activity is high during childhood and adolescence, but is gradually reduced as we become older.

As we grow older, this process of renewing the skin, hair and nails is normally slowed down. This is a natural process, but the process may also be speeded up by external factors. Exposure to UV light, chemical contaminants, e.g. through smoking, etc may cause premature ageing of the skin. Protection against UV light exposure and against chemical contaminants should therefore form first line defense against such causes of premature ageing.

Nutrition may also affect the skin's ability to renew itself since all physiological processes are dependent on the sufficient presence of nutrients on the cellular level.

Skin aging is closely linked to decreased collagen production and increased production of collagen-degrading enzymes in the dermal fibroblasts and a breakdown in the structure of the matrix of collagen and other constituents of the connective tissue in the dermis. This breakdown is due to alterations in the chemical structure of the proteoglycans and GAGs of the extracellular matrix.

Clinically, skin aging, including premature skin aging, results in a general thinning of the skin, the appearance of wrinkles and fine lines, a reduction in the elasticity and drying out of the skin.

During the past 2-3 decades supplementation of collagen and GAGs has been proposed as effective prophylaxis against skin aging. Several smaller clinical studies have been published, indicating the both supplementation of GAGs and collagen may have a positive effect in delaying skin aging. If proven to be effective, such supplementation could potentially represent a better alternative to topical application of anti-aging products.

Materials and methods

This study was designed in order to evaluate if a novel supplement containing a combination of hydrolyzed collagen I and a marine hydrolyzate ingested orally could have a positive effect on skin parameters.

The product (Skigain™) used in the intervention was composed of 5,000 mg hydrolyzed collagen 1 from bovine sources, 300 mg hydrolyzed marine compound and 50 mg of vitamin C two times per day (morning and evening). The marine hydrolysate contains a combination of collagen II and proteoglycans and glycosaminoglycans. In addition, participants were recommended to take a colon cleanser for better digestion. The test product was supplied by Med-Eq AS, a Norwegian supplier of health and beauty products.

The Study was designed as an open phase study without placebo control. Objective parameters were: Measurements of collagen contents in the dermis, skin elasticity as the time needed for the skin to resume its normal shape after being pinched out in a standardized way as well as measurements of moisture contents in the dermis. Control points were at baseline, and then after 4,

8 and 12 weeks. Skin hydration was initially included in the study, but this part was discontinued due to changes in the conditions participants were exposed to during the study.

In addition visual inspection of before and after photos (at baseline and after 8 and 12 weeks respectively) was performed by the investigator. After the conclusion of the study, the participants were asked to fill out a self evaluation questionnaire.

The study was implemented during the autumn of 2011 over a period of 12 weeks, and was completed during December 2011. During the latter part of the study period humidity levels dropped significantly as a result of dropping outdoor temperatures and indoor heating of houses. As a result of this the skin became less elastic – especially towards the end of the study period. Consequently, the study focused on collagen contents of the skin, which is not affected by these factors. The development in skin elasticity during the first 4 weeks is also included since the temperature was relatively stable during September and October.

53 participants took part in the study, of which 48 completed all objective measurements. 45 of these had pictures taken at inclusion and after 8 and 12 weeks. The participants were all women from the county of Vestfold, Norway, in the age group 30 – 75 years, and participants were recruited through ads in a local newspaper.

Measurements of the collagen contents of the skin and skin elasticity were carried out using DermaLab® Combo Skinlab supplied by the Danish company Cortex. The instrument involves the use of ultra sound scans for real time evaluation of the skin (collagen contents) in combination with traditional parameters as elasticity. Validation of the methods used has been performed by Cortex.

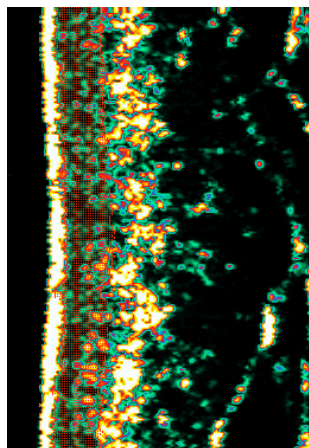
Visual evaluation was carried out by Ms Audrey Wong. The visual evaluations were made in connection with measurements as well as afterwards based on the before and after pictures. Pictures were taken before the study, and after 8 and 12 weeks respectively. Only pictures before the study and after 12 weeks have been analyzed.

Dr Roald Strand has acted as medical advisor during the study. He has overseen measurements in order to secure the quality and consistency of these.

Results

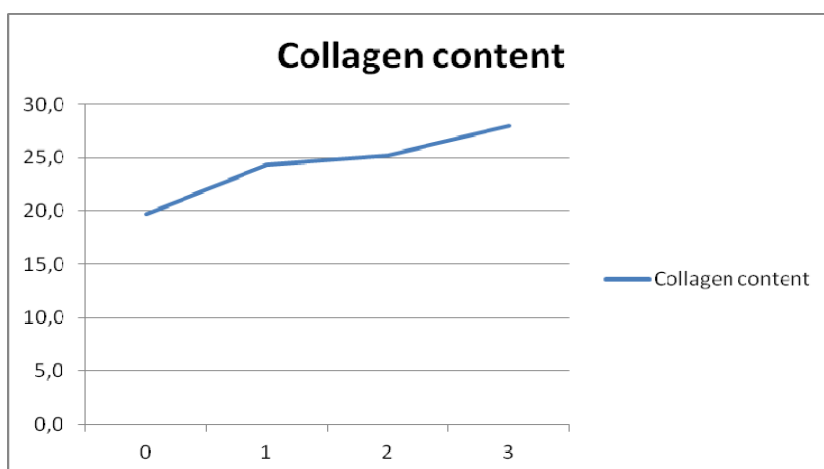
Collagen Contents in the Skin

The collagen contents of the skin were measured by the use of DermaLab® Combo SkinLab ultra sound sonde. The acoustic response of electric pulses that are directed into the skin is measured. When the pulse hits varying structures in the skin, it will be partially reflected, while the remaining part will penetrate further into the skin. As a result a picture, as demonstrated below, will appear



The collagen content can be seen as the intensity of an area between two vertical lines in the ultrasound picture. These lines are placed in the same manner through the study, 711 μ m apart.

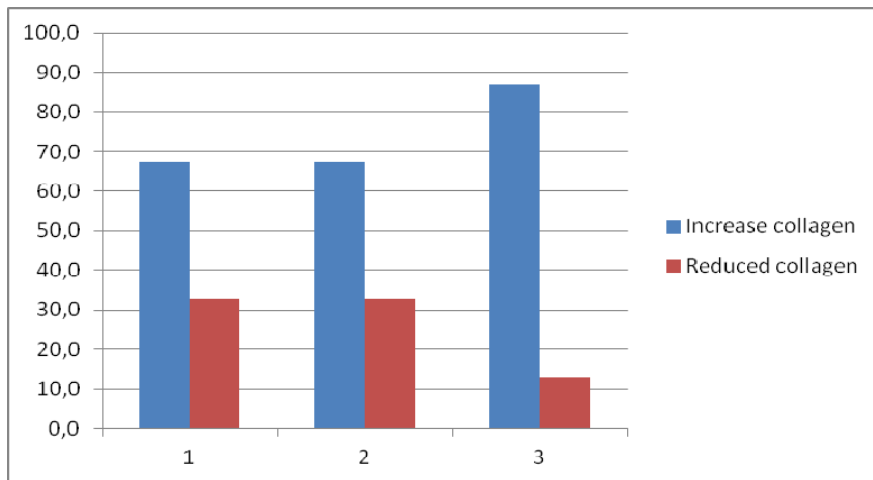
A great majority of the participants demonstrated increased collagen contents in dermis from baseline to the 3 following measurements (after 4, 8 and 12 weeks). The average collagen content increased by 25 % during the initial 4 weeks, via 35 % increase after 8 weeks to 45,6 % increase after 12 weeks (3 months).



The increase in collagen contents in the skin is statistically significant after 4 weeks ($p < 0.01$) and later measurements ($p < 0.001$).

67 % of the participants had increase collagen contents in the skin after 4 weeks – a number that was stable after 8 weeks and that increased to 87 % after 12 weeks. It can therefore seem as if users may experience a quick and noticeable increase in the collagen contents of the skin, while a minority will need longer time to experience an effect. Those who demonstrated increased collagen contents

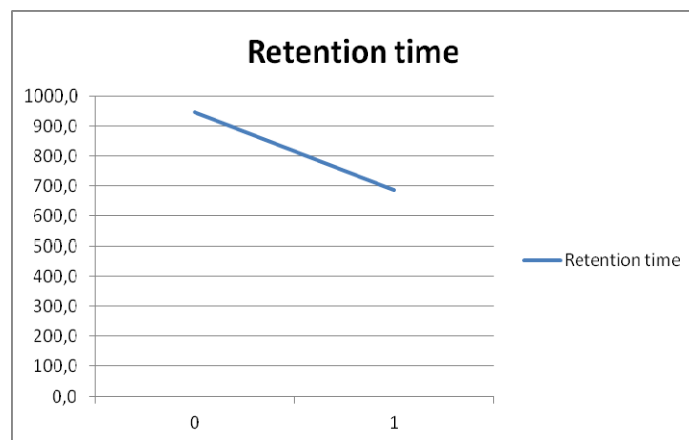
produced generally higher increases in the measurements than the reduction in collagen contents among those who did not respond positively to the treatment.



Skin Elasticity

The skin elasticity was measured using DermaLab® Combo Skinlab. A probe was placed against the chin of the participants. A vacuum was created, lifting the skin surface by 2.5 mm. Two integrated sensors in the probe are placed 1.5 mm apart, registering the contraction of the skin and measuring the time needed for the skin to retract by 1.5 mm.

This part of the study was discontinued after 4 weeks due to the change of weather conditions; exposure to dry conditions as a result of indoor heating of houses and work places, coupled with lower humidity and cold weather.



After 1 month the average retention time (the time needed for the skin to retract by 1.5 mm -after having been sucked - by vacuum by 2.5 mm) was reduced by 23 %. The improvement in elasticity is statistically significant ($p < 0,001$). During the subsequent period, elasticity was still improved compared to baseline, but not statistically significant.

In order to measure long-term efficacy, it shall be necessary to repeat the study under controlled climatic conditions throughout the study period.

Due to the same reason, measurements of moisture contents of the skin were abandoned from the study as these measurements would only be valid if participants would stay in controlled environment with the same humidity prior to measurements in order to obtain reliable data. In addition, control of the intake of liquids would have to be controlled. Measurements of skin humidity taken showed an increase compared to baseline, but the change was not statistically significant.

Visual inspection of photos

Visual inspection included evaluation of depth of wrinkles, plumping up, couperose, pigmentation, naso labial fold depth, hydration, eye lid lift, face lift and oil production.

Parameter	No change		Very moderate		Moderate		Good		Very good		Excellent	
	#	%	#	%	#	%	#	%	#	%	#	%
Depth of wrinkles	0	0,0	6	13,3	13	28,9	14	31,1	8	17,8	4	8,9
Plumped up	0	0,0	3	6,7	13	28,9	17	37,8	6	13,3	6	13,3
Couperose	15	23,1	10	25,6	5	12,8	5	12,8	6	15,4	4	10,3
Pigmentation	5	7,0	11	25,6	12	27,9	5	11,6	3	7,0	9	20,9
Naso labial fold	3	6,7	6	13,3	14	31,1	12	26,7	7	15,6	3	6,7
Hydration	0	0,0	6	13,3	7	15,6	18	40,0	7	15,6	8	17,8
Eye lids lift	0	0,0	11	24,4	13	28,9	13	28,9	7	15,6	1	2,2
Face lift	0	0,0	2	4,4	17	37,8	17	37,8	3	6,7	6	13,3
Oil production	14	20,5	4	10,3	8	20,5	8	20,5	5	12,8	6	15,4

45 participants were photographed at the planned occasions, As can be seen from the table above, The majority of visual inspection showed a moderate to good effect in the various parameters, however with less improvement in couperose and oil production, and the most important improvements in hydration of the skin and - pumping up of the skin closely followed by depth of wrinkles and face lift. Also moderate to good effect was seen for reductions in hyperpigmentation.

Self Evaluation

Only 18 participants returned questionnaires after the end of the study. Due to this, results from the self evaluation can only be taken as an indication of the perceived effect.

Of the 18 who filled in and returned the questionnaire, 11 reported smoother skin (61 %), while 4 (22 %) did not judge that the skin had become smoother. 10 of 18 (56 %) reported a reduction in fine lines and wrinkles, while 6 (33 %) did not see any difference. 11 of 18 (61 %) would continue to use and recommend that their friends also should use the product, while 4 (22 %) would not continue and 2 (11 %) would not recommend their friends to use the product.

Although these results are positive, one would need to secure that a higher number of participants completed the questionnaire in order to obtain reliable results.

Discussion

Skin aging in the face is a visual sign of physiological changes in the dermis. This is what is seen as dryness, lack of elasticity, wrinkles and fine lines and general thinning of the skin. The results from this study involving 48 Norwegian women showed that daily supplementation of a combination of hydrolyzed collagen I and a marine hydrolyzate with the addition of vitamin C, the test product marketed as Skingain™, may offer improvement in parameters of skin aging.

Daily ingestion of the test product led to significant increase in collagen contents in the skin after 4, 8 and 12 weeks. In addition elasticity improved significantly during the first 4 weeks. This part of the study as well as skin humidity measurements were, however, thereafter compromised by changing temperature and humidity levels in connection with the onset of winter and heating of houses.

The increase in collagen content in the dermis was confirmed also through visual inspection showing more plumped up skin and reduced depth of wrinkles and fine lines. Reduction in the naso labial fold may be due to the same. The visual inspection also revealed improved skin elasticity seen as a general face lift and eye lid lift.

The tendency seen in the visual inspection of increased humidity in the skin cannot be verified by objective measurements of increased humidity in the skin.

Changes in climatic conditions, changes in exposure to UV radiation and other variations of external factors may influence the results of such a study. In order to obtain optimal study conditions such environmental factors should therefore be kept as stable as possible. This is, however, difficult in a study in volunteers. Without a placebo control group and in lack of information about changes in factors that may affect the individual measurements in the study, the findings in the present study should be read with caution. Seen in connection with other studies that all point in the direction that supplementation of collagen I and II may have a positive effect in skin aging,

The most common treatment against skin aging is that of using anti-aging cosmetics. Some agents have a proven effect of removing the outer layers of the skin, exposing the healthier, underlying skin cells. Other cosmetic treatments claim to supplement the skin with important nutrients that are

claimed to pass through the skin barrier and enter the underlying cells or otherwise prevent or reverse aging

Therapeutically, effective treatment of skin aging has centered around topical application of retinoic acid and its derivatives, laser treatment and injection of hyaluronic acid. These treatments are well documented, but undesired side effects have been seen in retinoic acid treatment and laser treatment (1)

Already in 1988 the first experimental studies provided indications that hydrolyzed collagen could increase firmness and hydration of the skin. (2, 3).

Later, several preclinical studies have demonstrated that ingestion of collagen hydrolyzates led to a significant increase in fibroblast density and diameter and density of collagen fibrils. In addition a clinical study demonstrated that ingestion of collagen hydrolyzate increased skin hydration (4, 5).

Today, it is well established that skin aging is closely related to collagen fragmentation in the extracellular matrix of the dermis, leading to a reduction in the fibroblast production of collagen, and possibly other extracellular matrix constituents. Topical application of retinoic acid, as well as laser treatment, has been shown to stimulate production of new, undamaged collagen. The same applies for injection of hyaluronic acid (1).

Since collagen fragmentation itself seems to be the cause of reduced fibroblast activity, a possible explanation for the positive effect of supplementation of collagen through oral intake may therefore be that such supplementation stimulates the production of new, undamaged collagen that is necessary for continued fibroblast activity, as well as supplementation of the peptides and amino acids that are building blocks of collagen to the dermis directly.

Fibroblasts, in addition to producing collagen, is also responsible for the production of other main components of the extracellular matrix, proteoglycans, glycosaminoglycans and elastin; reduced fibroblast activity may lead to reduced levels of these structural components, further progressing the breakdown of the structural matrix of the dermis. It has been demonstrated that the glycosaminoglycans of adult skin have a different structure to those of younger individuals (6)

Several studies on commercial products containing various marine substances containing proteoglycans have demonstrated an effect in skin conditions - of the marine substance alone and in combination with antioxidants. We have, however, not been able find extensive review of research performed in this field. Although proteoglycans only represent a minor part of the weight of human dermis, its water binding capacity and its function in the matrix structure of the extracellular matrix make this group of substances important for the preservation of a beautiful, healthy skin.

Deterioration of the proteoglycans, mainly hyaluronic acid and chondroitin sulfate in the skin, seem to be central in skin aging. Supplementation of proteoglycans may therefore represent an important contribution in preventing aging of the skin (7)

As for the potential improvements in hyperpigmentation, some studies have indicated that the administration of vitamin C and multivitamins may have a positive effect in treating hyperpigmentation. A recent review article has in addition pointed out that bioactive oligopeptides,

in addition to their effect in improving collagen synthesis, may also help regulate the melanin synthesis implicated in causing hyperpigmentation of the skin (8)

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