CLINICAL ASSESSMENT OF A FOOD SUPPLEMENT INTAKE VERSUS PLACEBO ON SKIN PROPERTIES
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Outline of the study

The main objective of this study was the in vivo assessment of the effect of a daily intake for 8 weeks of a collagen peptides (SOLUGEL®) versus a placebo on skin properties in a mono-centered, double-blind, placebo-controlled randomized trial including 66 subjects . Assessments were done at the start of the study (before intake) and then after 56 days of daily intake. The study was carried out by Laboratoire COSDERMA, Bordeaux, France). It is done in corporation with the Dermatology Unit at St André Hospital. The skin properties that are studied are moisture related properties and biomechanical properties of the skin.

Introduction

The skin is an organ consisting of three layers: the epidermis, the dermis and the hypodermis. This envelope, which combines both flexibility and resistance, not only protects the body from the outside environment but also represents a place of exchange with inside of the body. The dermis, a compressible, stretchable and elastic tissue, provides support to the skin. Its properties are conferred by its particular architecture, resulting in the interactions between the extracellular matrix and the fibroblasts cells allowing both the synthesis and the degradation of the matrix components. The matrix is composed of a fibrous network of collagen, particularly of collagen I, and elastin fibres which are enclosed in a hydrated gel of proteoglycans and glucosaminoglycans. These macromolecules are strongly negatively charged, which makes them particularly hydrophilic.

During chronological ageing, all three layers of the skin undergo modifications. Within the dermis, the fibroblast density declines, leading to reduced synthesis of matrix elements. Moreover, fibres are damaged by free radical attack, lose their elasticity due to glycosylation, and lose their features due to decreased synthesis of enzymes involved in anabolic metabolism. As a result, the elastic network of the dermis is impaired. All these processes contribute to the loss of skin structure and firmness observed during ageing. The menopause accelerates skin ageing, leading to loss of collagen in the dermis and a decrease in skin thickness. In research protocols, it is thus pertinent to include post-menopausal women presenting clinical, biological and biophysics signs of loss of skin firmness that may have marked collagen.

Collagen is the most abundant protein in the human body and a major protein in the skin's structure. The native collagen is a triple helix alpha chain of amino acids forming solid fibres used through all the structural tissues of the human body; it is present throughout the body structural elements, such as the skin, the cartilage, the tendons, the ligaments and the connective tissues. The collagen represents up to 35% of the total proteins present in the body and insures the cohesion, the elasticity and the regeneration of all these tissues. Various types of collagen exist depending on the tissues involved. It is synthesized by particular cells called fibroblasts and is assembled in connective tissues. The biological function of the collagen is double. On the one hand, with elastin and glycoprotein, it is responsible for the cohesiveness between tissues and organs. On the other hand, the collagen provides hydration, resilience, elasticity and suppleness properties to these same tissues and organs. In the ageing process, the production of collagen by fibroblasts slows down and from the age of 25 its level diminishes within the body, leaving the first ageing signs appear: appearance of wrinkles, loss in skin elasticity and firmness.

Several scientific studies have allowed demonstrating the interest of ingesting hydrolyzed collagen in addition to a healthy and balanced diet in order to improve the skin properties. This supplementation

would stimulate the fibroblasts growth, responsible in the collagen synthesis, and in addition of the elastin and hyaluronic acid synthesis which would allow improving skin hydration, elasticity and appearance. The tested dosages within those several studies vary between 2.5 and 10g of daily intake during 1 to 3 months. Clinical tests have demonstrated that collagen peptides, administered orally at a dose of 5 to 10 g per day for approximately 8 weeks have a positive impact on the moisturization of the skin, skin elasticity and wrinkles. Another test indicated an increase in the soluble collagen content of women's skin after oral administration of 5 g per day for 5 or 10 weeks.

In the presented study, the effect of the daily intake of 10 g for 2 months of a collagen peptide, SOLUGEL®, was tested in the purpose of allowing an improvement of the collagen level in the skin. The increase of the collagen in the dermis would enable a better construction of the extra cellular (cutaneous thickening) and therefore an improvement of the firmness as well as epidermal functions such as skin moisture.

There are little clinical tests on the effect of collagen peptides on the cutaneous parameters. Some studies have demonstrated an effect of collagen peptides on fibroblasts. One hypothesis of mechanical action could be as following: collagen peptides present within the growing media is recognized by the cells as a marker of the degradation of endogenous collagen. In response to this "degradation" signal, cells such as fibroblasts produce collagen. Such recognition mechanism is not clearly defined yet but some specific peptides resulting from collagen peptides would be recognized in preference by cell membranes. An important number of studies have been performed on other cell types, such as osteoblasts, bone osteoclasts and cartilage chondrocytes. These cells originate from the same stem cells and share certain characteristics, such as the capacity to produce endogenous collagen. It has been demonstrated in in-vitro studies that collagen peptides can stimulate collagen production by such cells.

Collagen peptide has been known since the 1980ies for its virtues on joint problems (reduction of arthritic and rheumatic pains), and is used today in a variety of food related products and supplements. Numerous scientific studies have proved the beneficial effects of collagen peptides on health, particularly on weight control, on reducing the risks of obesity and of type 2 diabetis and on the reduction of recovery time after a physical activity, on the health of joints, of bones as well as of the skin. Collagen peptide is acknowledged as being safe and healthy and, in addition, is considered as being a "natural" ingredient produced from a unique source, non-allergenic, without preservatives or additives and is not considered as a food additive (no E number as defined in the article 3.2 a (viii) of the EC regulation n°1333/2008 issued on December, 16th 2008 on food additives). In the United States, this it has « GRAS » stauts (Generally Recognized As Safe).

In this study, performed on 2 panels of 33 subjects (1 panels for the active tested product and 1 panel for the placebo) the effect of the daily intake for 8 weeks of 10 g collagen peptide on skin properties, the touch feel and visual aspect (in particular on skin sensitivity, moisture, elasticity, firmness and tonicity) as well as the effect on ageing and on the crow's foot wrinkles is assessed. The study also consists in a self-assessment questionnaire aiming to measure the skin improvement as felt by the subjects.

This EC regulation n°853/2004, last updated version, establishing the specific hygiene rules applicable to animal origin food related products, mentions the conditions of production of the collagen peptide as well as the specifications to which the product must comply.

Materials and methods

The study was performed with a powdered flavoured instant drink, principally consisting of sugars, acidifier, flavouring, natural colourings and placebo or active ingredient, i.e. SOLUGEL® collagen peptides. The hydrolysate has a molecular weight varying between 1500 and 5000 Daltons, and is manufactured by PB Leiner. SOLUGEL® is obtained from animals fit for human consumption and according to a process compliant with the European Regulation 853/2004, Appendix III. SOLUGEL®

contains mainly type 1 collagen, the same type of collagen present in the human bones and skin. As placebo maltodextrin (Cargill, France) is used. The drink contains 10 g bovine type SOLUGEL® or placebo for a duration of 56 days (8 weeks) with 15 days pre-inclusion period. During the inclusion period, subjects used a neutral cream. One sachet corresponds to the daily dose to be taken in one intake in the evening before bed time. The content of the sachet was to be diluted in a tall glass filled with cold water (150 mL).

Study setup is a mono-centered, double-blind, randomized, placebo-controlled trial with 66 subjects, assigned into three balanced groups (inclusion criteria are listed in Table 1):

- 33 subjects received the product containing 10 g SOLUGEL®

- 33 subjects received the product containing maltodextrin (placebo)

Table 1. Pre-Inclusion criteria (exhaustive list in annex)

● Caucasian woman, aged 35 – 55 years
● Skin phototype I –III and corneometric index UA 30 – 50 (dry skin); no use of own personal care products during the study
● wrinkles (crow's foot) with grade between ≥ 2 and < 5 (cfr. skin aging atlas)

Assessments are performed on Day 0 (D0, Inclusion) and Day 56 and the parameters assessed were:

- the assessment of the epidermis' upper layers moisture by capacitive measurements (Corneometer®),

- the assessment of the effect on the biomechanical properties of the skin by cutometric measurements (Cutometer®),

- the assessment of the cutaneous state by clinical scoring according to a scale in 10 points,

- the self-assessment of the cosmetic qualities and efficacy perceived by the subjects.

For a limited number of subject the assessment of the cutaneous surface moisture by illustrative measurements (Moisture Map®) was done. No statistics where done on the results.

The hydration level of the upper layers of the epidermis is assessed with the Corneometer CM 825® which runs at frequencies between 0.9 and 1.2 MHz. The surface of measurement for the probe is 49 mm². The capacitance of the system (electrodes and upper skin layers) being influenced by the changes of the dielectric constant of the skin's surface, when the hydration level of the stratum corneum varies, the dielectric properties of this medium are modified and consequently the capacitance of the system is modified. The capacitance values (corneometric indexes) are collected and expressed in arbitrary units ranging from 0 to 120. The instrumental measurements were performed in triplicate (M1, M2, M3) in a consecutive and shift way. The corneometric value given as a result is the mean of the M1, M2 and M3 measurements:

- Very dry skin was characterized with corneometer units below 30

- Dry skin between 30 and 50

- Normal skin higher than 50 a.u. (arbitrary units).

The hydration level of the upper layers of the epidermis is assessed using the Moisture Map®. The principle of measurement is based on a capacitance measurement of the dielectric environment. Any change of the level of hydration of the skin's surface engenders a modification of the dielectric constants and of the capacitance measurement. The capacitance measurements reflecting the level of hydration of the skin which is expressed in the software of acquisition in the form of a picture of grey levels of the studied area. The scale of grey level is expressed as follows:

- grey level: « 0 » = black picture (value of calibration with an aqueous gel),

- grey level: « 255 » = white picture (measurement in the air).

The biomechanical measure is based on the suction and the strain, using a Cutometer®. The device generates a negative pressure which can vary between 20 up to 500 mbar. The zone of skin to be measured is sucked up in the opening of the probe by this depression. The depth of penetration of the skin inside the opening of the probe is determined without contact, by an optical measurement system. This system is constituted by a transmitter and a receiver of light. Two opposite glass prisms transmit the light of the transmitter towards the receiver. The quantity of received light is proportional in the depth of penetration of the skin. The skin is sucked up inside the probe (diameter 2mm) for 2 seconds (phase of pursuit) with a constant negative pressure of 450 mbar. Then the depression stops and the skin returns to its initial state (phase of relaxation).
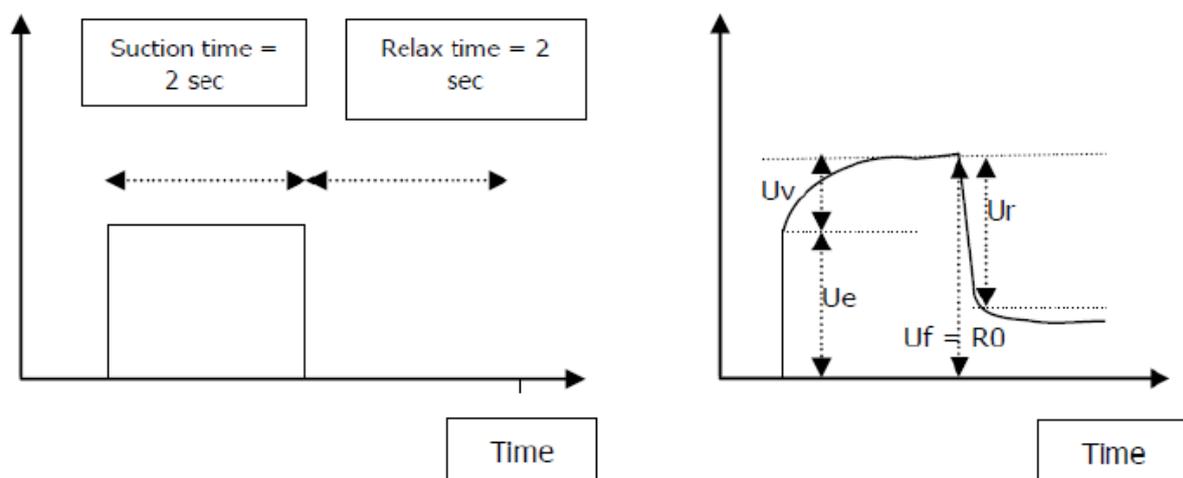
Because of individual intrinsic variation of the skin thickness between volunteers, intrinsic values cannot be directly compared. That is why different ratios are used, Ur/Ue, Ur/Uf and Uv/Ue, which minimize this thickness variability of the panel. So, the form of the curve obtained using the Cutometer device results in a description of the behaviour of the skin through the following parameters:
- Ue: elastic component (Uf-Uv): immediate elastic part of the curve. *Not to interpret alone.*
- Uv: visco-elastic component: visco-elastic curve part. *Not to interpret alone*
- Uf: maximal amplitude after 2 seconds of negative depression= Ue + Uv. The smaller Uf is the firmer the skin. *Possible to interpret alone because final result of Ue + Uv.*
- Ur: tonicity of the skin. *Not to interprete alone*
- Ur/Ue: net elasticity: The more the ratio tends to 1 (Ur near Ue), the more tonic is the skin. *Possible to interpret*
- Ur/Uf: idem. The higher the value of Ur and the ratio tends to 1, the more tonic the skin is. *Possible to interpret*
- Uv/Ue: The smaller the Uv value is and the ratio tends to 0, the more elastic the skin is. *Possible to interpret*

The clinical scoring by a dermatologist consists in a visual and tactile assessment of the cutaneous state at the level of the face, before then after the intake of investigational product, in order to determine its cosmetic efficacy. In order to be as accurate as possible and always under the same intensity of light, some landmarks were set in the examination room so that the subject would be

installed in the same position than on D0. The clinical scoring was performed using a scale scored in 10 points (from 0 to 9) according to the following items:
- Elasticity of the skin (0 = Not elastic, 9 = very elastic), tactile, right hand side cheek
- Moisturized aspect of the skin (0 = Not moisturized, 9 = Very moisturized), visual, whole face

After 56 days on conditions of normal use, the subjects answered self-assessment questionnaires about the investigational product (Table 2). The answering modalities were the following: "very good", "good", "neutral", "bad", "very bad" / "yes", "no". For each item, the percentage of satisfaction (= percentage of "very good" + percentage of "good", or percentage of "yes") was calculated.

N°	Question	D56
1.	Overall improvement of the state and aspect of the skin, compared to day 0	X
2.	Improvement of the firmness of the skin, compared to day 0	X
3.	Improvement of the appearance of the skin, compared to day 0	X
4.	Improvement of the skin hydration, compared to day 0	X

The statistical analyses were performed by the COSDERMA laboratory using the software SPSS®. The homogeneity of the groups on day 0 and the differences between groups were done with a Tukey test ($\alpha = 0,05$); the evolution through time for each group was done using a Dunnett test ($\alpha = 0.05$).

Results
Subjects
54 subject completed the study : 8 subjects were not included cfr. the inclusion criteria; 4 subjects were withdrawn from the study:
- 2 subjects showed non-related adverse effects
- 1 subject voluntarily withdrew from the study
- 1 subject did not respect study's constraints

This resulted in the following number of valid cases:
- 26 for the group getting 10 g SOLUGEL®
- 28 for the placebo group

Clinical scoring by a dermatologist
Result for the clinical scoring of elasticity of the skin and moisturized aspect of the skin show a clear effect of collagen peptides during 8 weeks ($p < 0,001$) with an improvement of the skin elasticity by more than 17% (percent of variation) (Figure 1). A smaller trend was observed for the placebo confirming the benefit of SOLUGEL®. A similar observation can be made for the moisturized aspect of the skin with an increase of nearly 21% (percent of variation).
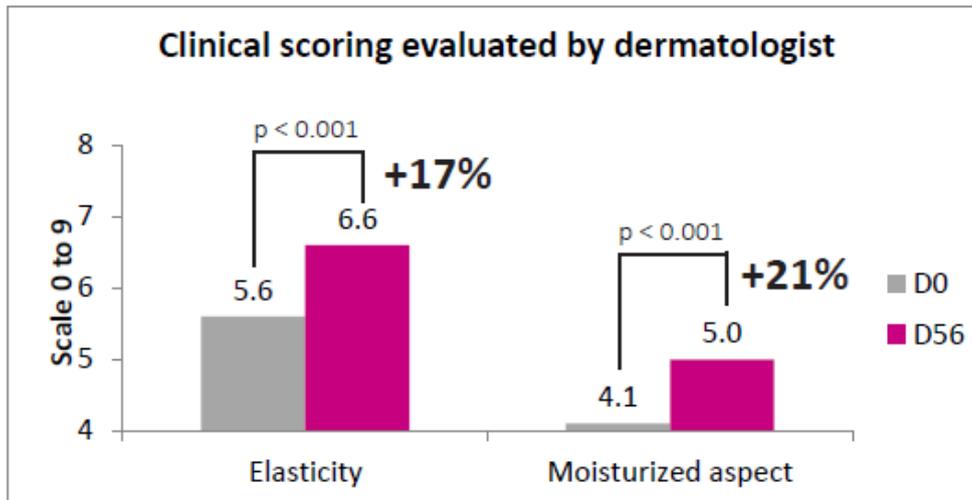
Figure 1. Clinical scoring of the effect on elasticity and moisturized aspect of the skin (face) (0 = not hydrated, 9 = very hydrated)

Measurements of hydration and biomechanical properties

The moisturizing effect was also assessed by capacitance measurements, using a Corneometer®. The results confirm the observations assessed with the clinical scoring : after 56 days of oral intake of SOLUGEL® there is statistically significant moisturizing effect (Figure. 2). And a small improvement was also noticed with the placebo but to a smaller extent than obtained with the collagen peptides.
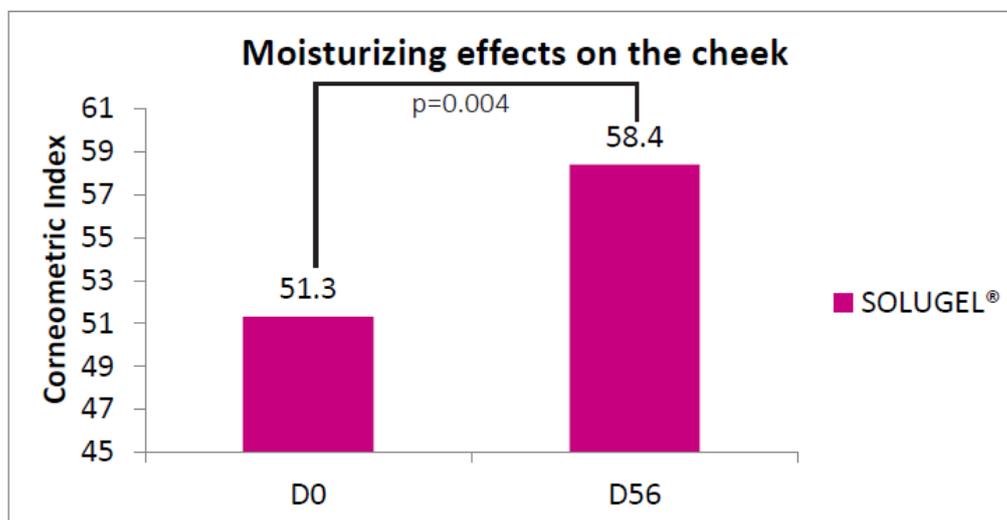

Figure 2. Effect of oral intake of a collagen peptides, SOLUGEL®, on skin hydration (capacitance measurement with a Corneometer®)

A scan was taken from a fixed area of the skin of the forearm and processed to illustrate the moisture level, expressed in a Moisture Map®. The effect on the skin hydration of the daily intake of 10 g SOLUGEL® and its moisturizing effect could also clearly be seen as illustrated in Fig. 3 for one particular subject that showed a very good increase in the corneometric measurements.
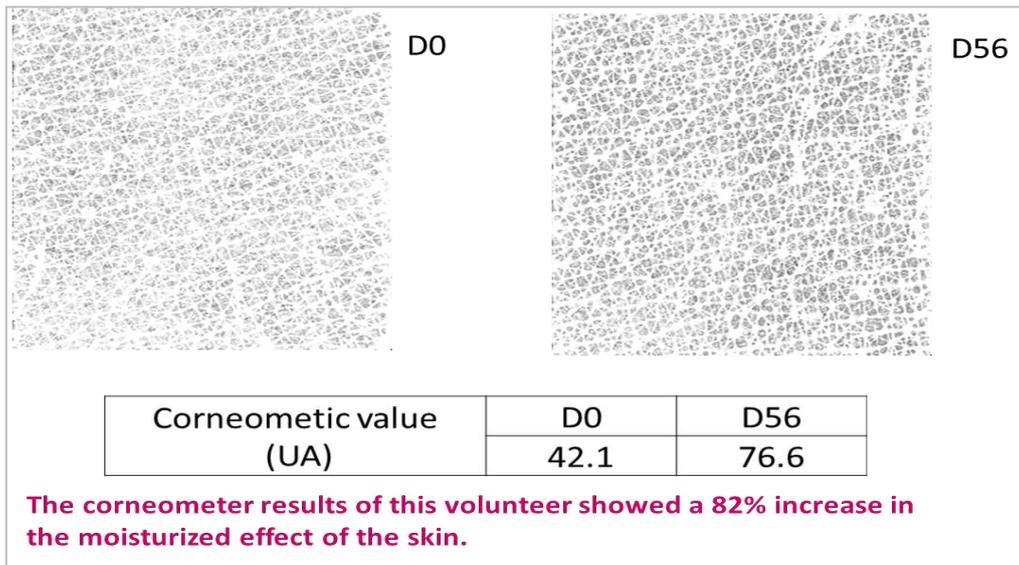
Corneometic value	D0	D56
(UA)	42.1	76.6

The corneometer results of this volunteer showed a 82% increase in the moisturized effect of the skin.

Figure 3. Moisture Map® from one volunteer (on the forearm) as well as the corresponding corneometric measurements.

The biomechanical properties of the skin are assessed by Cutometer® measurements. After 56 days intake of 10 g SOLUGEL®, the effect on firmness or elasticity is quite different (Figure. 4). Whereas there is little difference between the intake of placebo for 56 days on firmness and net elasticity of the skin, the effect of 10 g SOLUGEL® is significant with a clear improvement of both aspects. Hydration and biomechanical properties are quite different aspects of the skin : while for hydration there may be a kind of dynamic equilibrium at a certain plateau value as the skin may show a kind of maximum moisture retention capacity and which may be a rather quick effect. The underlying mechanisms for build-up or restoration of the collagen network in de skin, related to firmness and elasticity, are different. Higher dosages of collagen may be required or the effect is only clear after a longer period at lower dosages. Studies over a longer period of time, including different dosages are required to confirm this hypothesis.
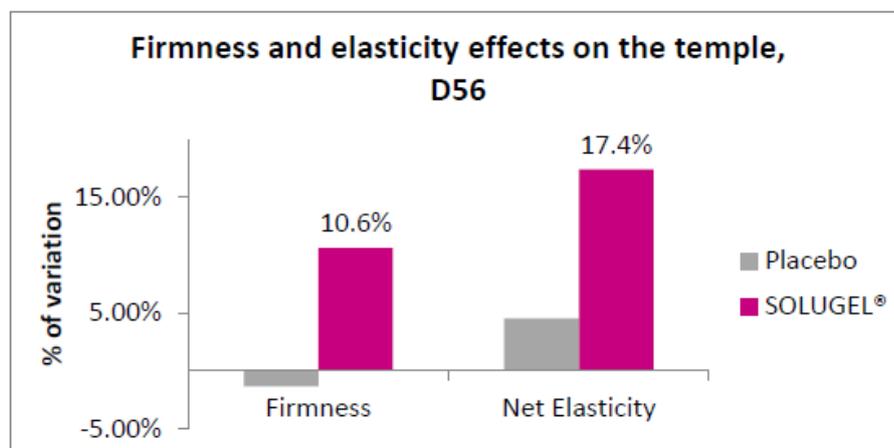

Figure 4. Effect of oral intake of a collagen peptides, SOLUGEL®, on biomechanical properties (capacitance measurement with a Cutometer ®)

At the end of the study (56 days) the subjects had to complete a self-assessment of the appreciation of the instant drink and the cosmetic qualities and efficacy. Overall, all products were well accepted with no differences in appreciation of the drinks with either maltodextrin as placebo or 10 g SOLUGEL®. Volunteers taking SOLUGEL® demonstrated a better perception of the product efficacy compared to the placebo group in terms of skin hydration, firmness of the skin, appearance of the skin, as well as the overall improvement of the state and aspect of the skin. People taking 10 g SOLUGEL® per day gave a higher score on overall improvement of the skin, improved firmness and smooth aspect than those receiving placebo. This further confirms the results of the measurements as described above.

Conclusion

The aim of the study was to evaluate the effect of the daily intake for 8 weeks of a collagen peptides, SOLUGEL®, on aspects related to healthy skin like skin hydration, and elasticity and firmness of the skin (face). Although the studied period is relatively short, it proves that the oral intake of 10 g SOLUGEL® has a significant beneficial moisturizing effect on the skin after 56 days. The benefits of taken a daily dosage of 10 g Solugel for 56 days are seen in the significant boost of the skin performance and aspects like firmness and elasticity. The study also suggest that the intake of SOLUGEL® for a longer period like 3 to 4 months will have an even more beneficial effect. SOLUGEL® bioactive collagen peptides offer the natural solution to skin aging.

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