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Supplementation with Ovoderm® Reduces the Clinical Signs of Skin Aging. A Double-Blind, Placebo-Controlled Study

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Abstract

Human skin physiology changes during the course of life involving age-related changes in skin appearance. It has been suggested that eggshell membrane, a natural ingredient containing collagen, hyaluronic acid, elastine, among others, can be used for improving skin. However, there are limited clinical studies using eggshell membrane as a dietary supplement to study skin health. The efficacy of Ovoderm®, an oral supplement from eggshell membrane, on skin biophysical parameters related to cutaneous aging was evaluated.

A double-blind, placebo controlled study with 50 healthy subjects randomized to intake 300 mg Ovoderm® or a placebo treatment during 60 days was performed. Skin elasticity, transepidermal waterloss, skin firmness and skin fatigue were measured before and after the treatment.

At the end of the study, the group intaking Ovoderm® improved the skins barrier function, showed by the tendency of transepidermal waterloss to decline. With regard to firmness and skin fatigue, a significant improvement was observed in Ovoderm® and placebo treatment, but the enhancement was 33% and 45% higher in Ovoderm® treatment for firmness and fatigue, respectively, than in the placebo treatment. After 60 days of Ovoderm® intake a significant improvement of skin elasticity was proven.

These results demonstrate that dietary Ovoderm® can improve the elasticity and the barrier function on human skin. Thus, Ovoderm® is effective to prevent and to reduce the gradual loss of skin elasticity, fatigue and firmness that are characteristic of aged skin. Ovoderm®, safe and well tolerated ingredient, helps to improve the appearance and health of the skin.

Introduction

In the human body, skin is the largest organ and is involved in several important functions. The two main layers that make up the skin are the outer layer or epidermis and the inner layer or dermis. The former is composed of a stratified squamous epithelium that overlay a basal layer composed of columnar cells arranged perpendicularly (proliferating and differentiated keratinocytes). The dermis is composed of connective tissue consisting of diverse extracellular matrix components, including collagen and elastin fibres and Glycosaminoglycans (GAGs), which are synthesized by dermal fibroblasts. Underlying these layers there is subcutaneous fat tissue [1].

Young skin is firm, smooth and of radiant appearance, nevertheless profound changes occur in the structure of the dermis and epidermis over time through processes of intrinsic (chronological aging) and extrinsic (photoaging) aging [2]. Skin changes associated with natural aging are generally characterized by fine wrinkling and laxity, which can be worsened by chronic ultraviolet (UV) light exposure. Clinical signs of photoaging include dryness, deep furrows, irregular pigmentation, elastosis, and a leathery appearance [2]. The dermis provides structure and support for the epidermis, as well as for the vasculature and appendages of the skin such as hair and nails. As a result, the structural integrity of the dermis is vital for the normal function and youthful appearance of the skin [1]. As the skin ages, the number of collagen and elastin fibres in the dermis decreases and total skin thickness decreases [2]. Collagen and elastin fibres become sparse and increasingly disordered, leading to formation of wrinkles and sagging of the skin [2]. The amount of Hyaluronic Acid (HA), which is abundant in both epidermis and dermis, also decreases with age. This is believed to contribute to the loss of skin hydration [3].

An important trend in skin care is the use of oral supplements to improve the skin's appearance and structure together with consumer demands for cosmetic products that contain natural ingredients. Therefore, the effects of nutritional factors on the skin have received increasing attention, and a number of clinical studies indicated that dietary supplementation can modulate skin functions [4]. These include, among others, collagen and HA, which provide building blocks of the skin. Collagen-based nutraceuticals have long been used for improving skin and cartilage tissues [5-7]. In vitro studies have shown the ability of collagen peptides to exert potent antioxidative activities [8]. Moreover, in experimental studies collagen has been reported to

have beneficial biological functions in skin [9-12]. Furthermore, previous studies reported that HA is closely involved in keratinocyte proliferation and differentiation [13] and may participate in maintaining the cell structure by utilizing its high water retention and viscosity [14].

Eggshell membrane, an organic substance, is known to increase cellular activity, increase collagen production, slow down skin aging and reduce the detrimental effects of damage from UV light and inflammation [15-17]. The objective of this research was to study the effectiveness of Ovoderm®, composed of eggshell membrane, on skin biophysical parameters related to cutaneous aging.

Materials and Methods

Food Supplement Under Investigation

The dietary supplement used in this study was Ovoderm® (Eggnovo, Spain), consisting of eggshell membrane separated from eggshells by a patented process (Patents: ES 2 181580 B1 and ES 2 327087 B2). Compositional analysis of eggshell membranes has identified a high content of protein (collagen types I-V-X, elastin, keratin) [18, 19] and moderate quantities of GAGs (Glucosamine, Chondroitin Sulfate, HA) [20].

Study Design

A randomized, double-blind and unicentric clinic-nutritional study was performed to evaluate the efficacy, acceptability and tolerability of the daily intake of an encapsulated food supplement containing 300mg of Ovoderm® (eggshell membrane). Volunteers had to intake one capsule a day of the food supplement with Ovoderm® or with placebo (microcrystalline cellulose) during 60 consecutive days.

Assessments were performed at the beginning (day 0) and at the end of the study (day 60). The safety and tolerability were evaluated by registering all the adverse events and evaluating the results. The adverse events, reported spontaneously by the participants or after the indication of the research team, were recorded.

The study was approved by the Research Ethic Committee of the Quirón hospital and it was conducted by IDERMA - Instituto Quirón Dexeus.

Subjects

A total of 52 healthy subjects, men and women, between 45 and 75 years old were enrolled in the study. Subjects were randomized to each of the two treatment groups to receive a daily dose of either Ovoderm® or placebo. Prior to the beginning of oral treatment and data acquisition a washout period of two weeks was stablished. Throughout the study volunteers could not use any cosmetic product on the test area. Volunteers fulfilling all the inclusion criteria were enrolled in the study. During the performance two participants abandoned voluntarily the study. The final analysis was conducted with 50 volunteers divided in two groups of 25.

Inclusion Criteria

The inclusion criteria were as follows: general good health, no hypersensitivity to any of the components, no digestive pathologies and no in pharmacological treatment; healthy and balanced living and dietary habits; ability to understand the clinical study, personal informed consent to participate in the study and willingness and capability to follow the study rules and a fixed schedule.

Exclusion Criteria

The exclusion criteria were as follows: allergy to eggs or allergies; eating disorders; gastrointestinal disorders or digestive surgeries the previous two years; systemic illnesses altering intestinal motility or changes in the intestinal motility by stress; diabetes, hipo or hyper thyroidism; medication or drugs changing the intestinal motility; changes in the diet habits in the previous 2 months; pharmacological treatment and/or food supplements that change the body weight or the appetite; alcohol, drugs, drug products or alcohol abuse; stop smoking the last 6 months or planning to stop during the study; pregnancy or period of breast feeding; intake of nutritional supplements; change in usual skin care routine; lack of compliance and intellectual or mental inability to follow the study instructions.

Measurements

Transepidermal Waterloss

Transepidermal Waterloss (TEWL) was measured using Tewameter® (Software del Tewameter® MPA 580) by applying a constant negative pressure. Prior to the measurement there was a 10 minute of acclimation. The scale is as follows: 0-10 (very healthy condition), 10-15 (healthy condition), 15-25 (normal condition), 25-30 (affected skin) and superior to 30 (critical condition) (Technical Guide).

Skin Elasticity, Firmness and Fatigue or Tiring Effect

Skin elasticity was measured with the Cutometer® (Software del Cutometer® MPA 580) by applying a constant negative pressure. Prior to the measurement there was a 10 minute of acclimation. The resistance of the skin to be sucked up by negative pressure (firmness) and its ability to return into its original position (elasticity) are displayed as curves. To measure the firmness R0 value was recorded. This parameter looks at the maximum amplitude and represents the passive behaviour of the skin to force (firmness). To analyse skin elasticity, R6 value was recorded (Portion of the visco-elasticity on the elastic part of the curve) which decreases with increasing skin elasticity. R9 parameter represents tiring effects of the skin after repeated suction and release of the skin. The lower the R9 value the smaller the fatigue or tiring effect (Technical Guide).

Eye Contour Appearance

A picture of the eye contour was taken before and after the treatments to each volunteer. The picture of three volunteers (volunteer 29, volunteer 20 and volunteer 43) intaking Ovoderm® is included in Figure 6.

Statistical Analysis

Statistical analysis of skin parameters was performed. Differences between baseline (day 0) and final data (day 60) were analysed by paired t-test. Statistical significance was considered when $P < 0.05$. Results are shown as mean ± standard error.

Results

Fifty out of fifty-two subjects completed the full course of treatment and follow-up. None of the dropouts were related to product intake or study procedure in general. There were no discomfort or adverse reactions reported.

Participants' Characteristics

The mean age of the volunteers was 52.46 ± 6.33 years being the minimum age 45 years old and the maximum age 73 years old. Regarding age and gender distribution 40% were under 50 years old and 90% of the participants were women. The predominant phototypes were II described as "White" (40%) and III defined as "Medium" (44%) Table 1.

Table1: Descriptive data of the participants. Age, gender, skin phototype and skin type are shown as percentage of the participants in each category.

Variables	Subcategories	Percentage (%)
Age	< 50 Years	40
	>50 Years	60
Mean ±SD: 52.46±6.33 years old		
Gender	Women	90
	Men	10
Skin phototype	Type II (White)	40
	Type III (Medium)	44
	Type IV (olive)	16
Skin type	Normal	86
	Dry	14

Water loss

The transepidermal waterloss (TEWL), known as the skin's barrier function, was categorised at the beginning of the study as "healthy condition" (values between 10 to 15 g/m^{2}h) in both groups, being 14.74 ± 1.22 g/m^{2}h and 13.91 ± 1.46 g/m2h the values of the placebo and Ovoderm® group respectively. After 60 days of treatment with Ovoderm® there was a 18.75% of decrease in the water loss (Figure 5) reaching a value of 11.30 ± 1.03 g/m^{2}h (Figure 1), which is close to the definition of very healthy condition (< 10 g/m^{2}h). In the placebo group the TEWL values kept stable (a slight decrease of 1.18% was measured) (Figure 5) staying the parameters in the "healthy condition".

Firmness

The starting level of skin firmness (R0 parameter) in the Ovoderm® group was 0.22 ± 0.03 mm (Figure 2A) and a significant decrease ($p=0.0003$) of 51.06% after 60 days was observed (Figure 5) reaching values of 0.11 ± 0.01 mm (Figure 2A). In the placebo group a decrease of 37.4% was also measured in the R0 parameter until values of 0.13 ± 0.01 mm at day 60. However, subgroup analysis focusing on age in either participants under or above 50 years old revealed a significant decrease in the Ovoderm® group that was not assessed in the placebo group (Figures 2B, 2C).

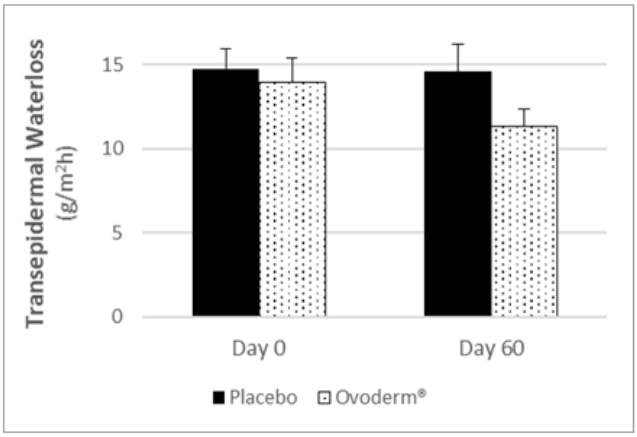

Figure 1: Change in the transepidermal waterloss after 60 days of treatment. Black bars represent the placebo treatment and dotted bars the treatment with Ovoderm®. Values are mean ± SE (n = 25 for Ovoderm®; n = 25 for placebo).

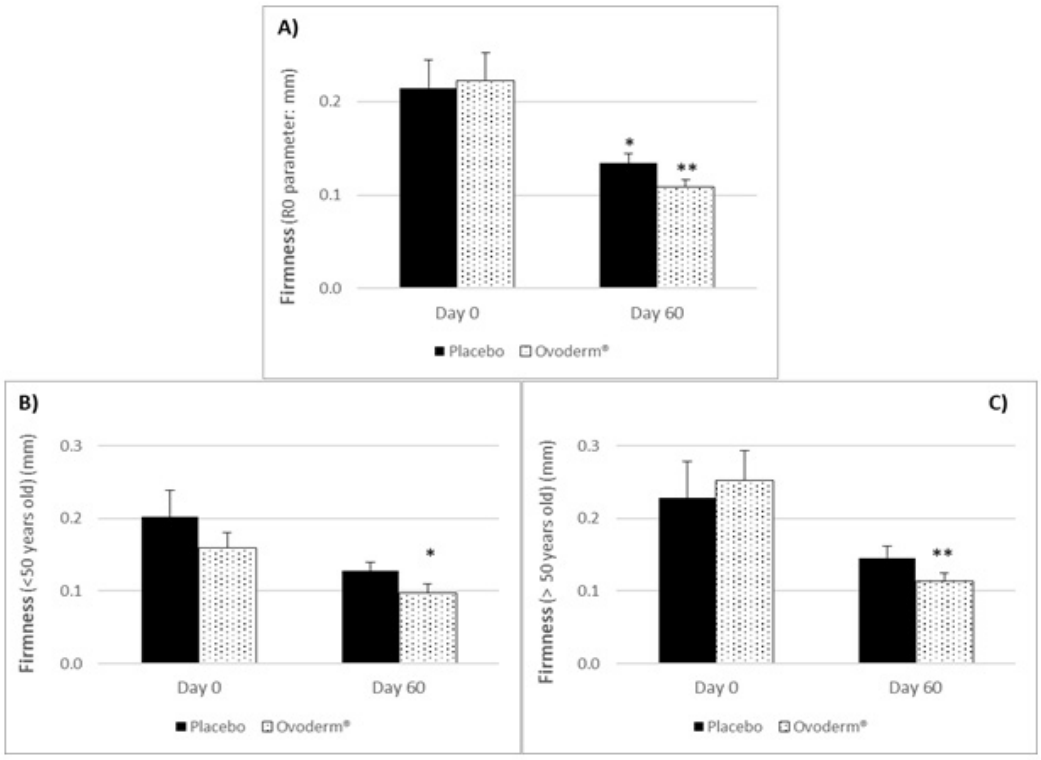

Figure 2: Change in firmness after 60 days of treatment. **A)** Firmness values of all the volunteers; **B)** Firmness values of the age subcategory under 50 years old; **C)** Firmness values of the age subcategory over 50 years old. Black bars represent the placebo treatment and dotted bars the treatment with Ovoderm®. Asterisks indicate significant differences (Student's paired t-test, $P \leq 0.05$ (*) or $P \leq 0.01$ (**)) between the beginning and the end of the study. Values are mean ± SE (n = 25 for Ovoderm®; n = 25 for placebo). The smaller the value, the higher the firmness.

Skin Elasticity

There was a significant improvement in the elasticity in the group intaking Ovoderm® that was not measured in the placebo group (Figure 3A). The elasticity parameter, R6 (unitless), declined significantly from 0.79 ± 0.09 to 0.59 ± 0.07 (Figure 3A) which meant a significant decrease of 25.08% in the R6 measurement (Figure 5) while no significant changes were assessed in the placebo group. Similarly, in the age subcategory of participants above 50 years old a significant decrease in the R6 value was recorded from 0.85 ± 0.10 to 0.60 ± 0.10 that was not observed in the placebo group (Figure 3C). In volunteers under 50 years old no significant changes were viewed in none of the groups but a tendency to decline (20.6%) in Ovoderm® treatment was viewed (Figure 3B).

Skin Fatigue

The skin tiring or fatigue, R9 parameter, decreased significantly in both groups after 60 days of treatment (Figure 4A), being the decline of R9 a 33.12% in the group intaking Ovoderm® and a 17.58% in the placebo group (Figure 5). In volunteers under 50 years old intaking Ovoderm® the R9 starting level was about 0.024 ± 0.004 mm and significantly declined to 0.016 ± 0.004 mm after 60 days of treatment while no significant changes were observed in the placebo group (Figure 4B). In participants over 50 years old there was a significant decrease of R9 after 60 days of treatment in both groups, 21.5% in the placebo treatment and 33.8% in the treatment with Ovoderm® after 60 days (Figure 4C).

Eye contour appearance

The pictures before and after the treatment with Ovoderm® shown in Figure 6 show the visual difference of the skin from the eye contour.

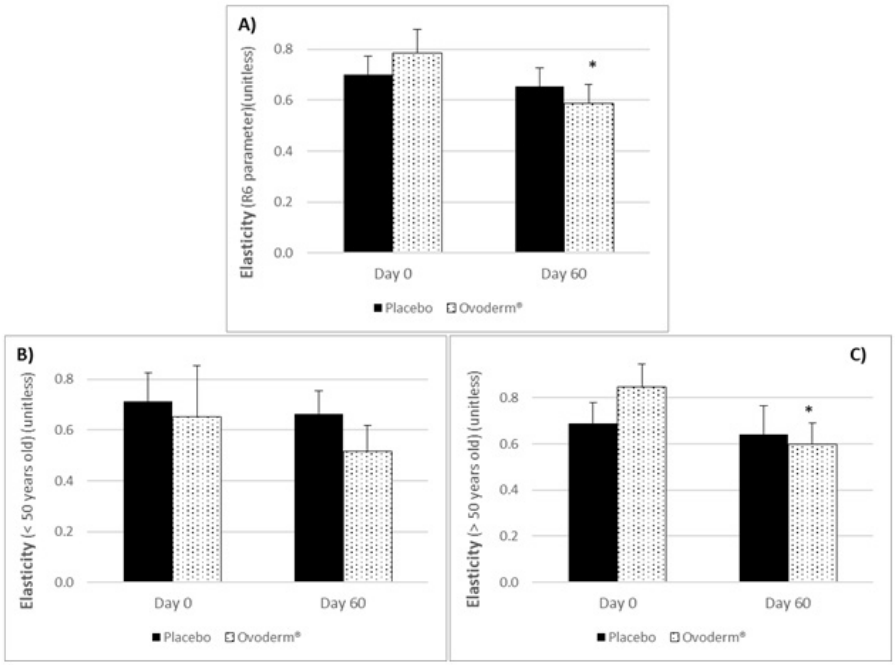

Figure 3: Skin elasticity change after 60 days of treatment. A) Firmness values of all the volunteers; B) Firmness values of the age subcategory under 50 years old; C) Firmness values of the age subcategory over 50 years old. Black bars represent the placebo treatment and dotted bars the treatment with Ovoderm®. Asterisks indicate significant differences (Student's paired t-test, $P \leq 0.05$ (*) or $P \leq 0.01$ (**)) between the beginning and the end of the study. Values are mean ± SE (n = 25 for Ovoderm®; n = 25 for placebo). The smaller the value, the higher the elasticity.

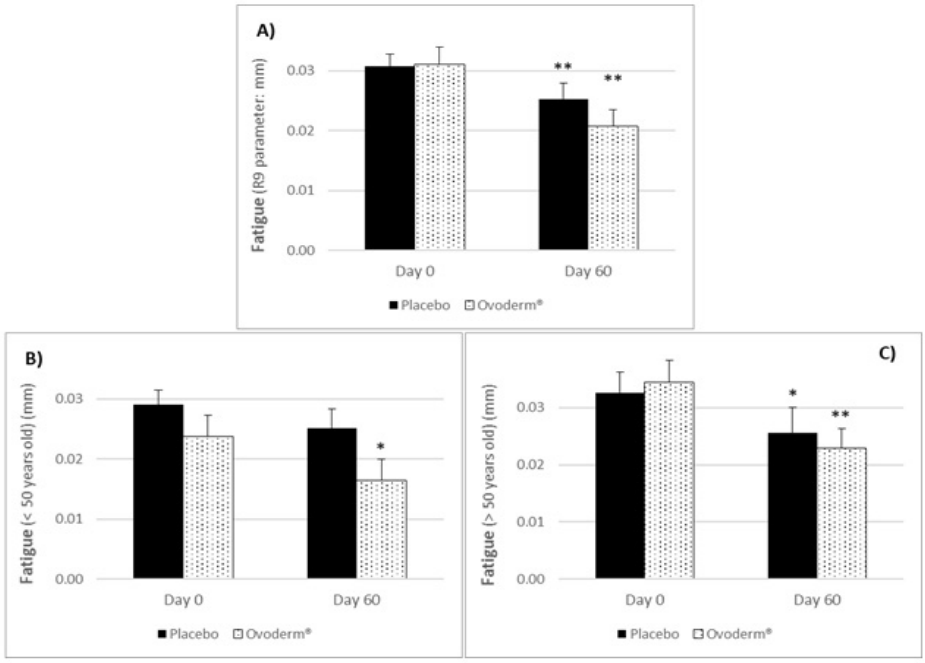

Figure 4: Skin fatigue change after 60 days of treatment. A) Firmness values of all the volunteers; B) Firmness values of the age subcategory under 50 years old; C) Firmness values of the age subcategory over 50 years old. Black bars represent the placebo treatment and dotted bars the treatment with Ovoderm®. Asterisks indicate significant differences (Student's paired t-test, $P \leq 0.05$ (*) or $P \leq 0.01$ (**)) between the beginning and the end of the study. Values are mean ± SE (n = 25 for Ovoderm®; n = 25 for placebo). The smaller the value, the lower the fatigue.

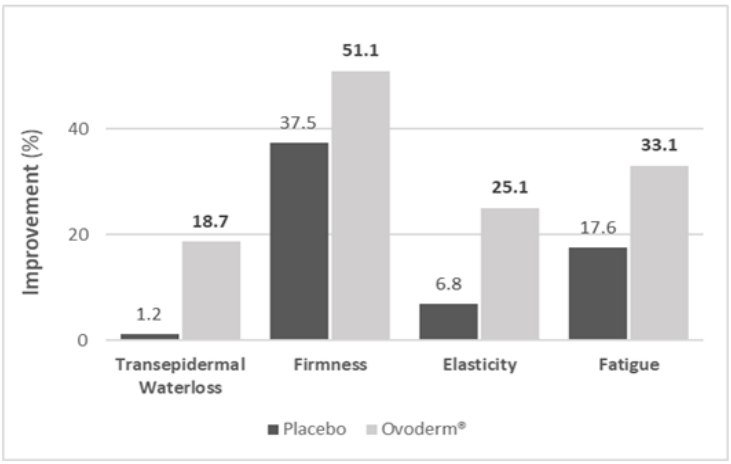

Figure 5: Percentage of improvement after 60 days of treatment in TEWL, firmness, elasticity and fatigue. Dark bars represent the placebo treatment and light bars the treatment with Ovoderm®.

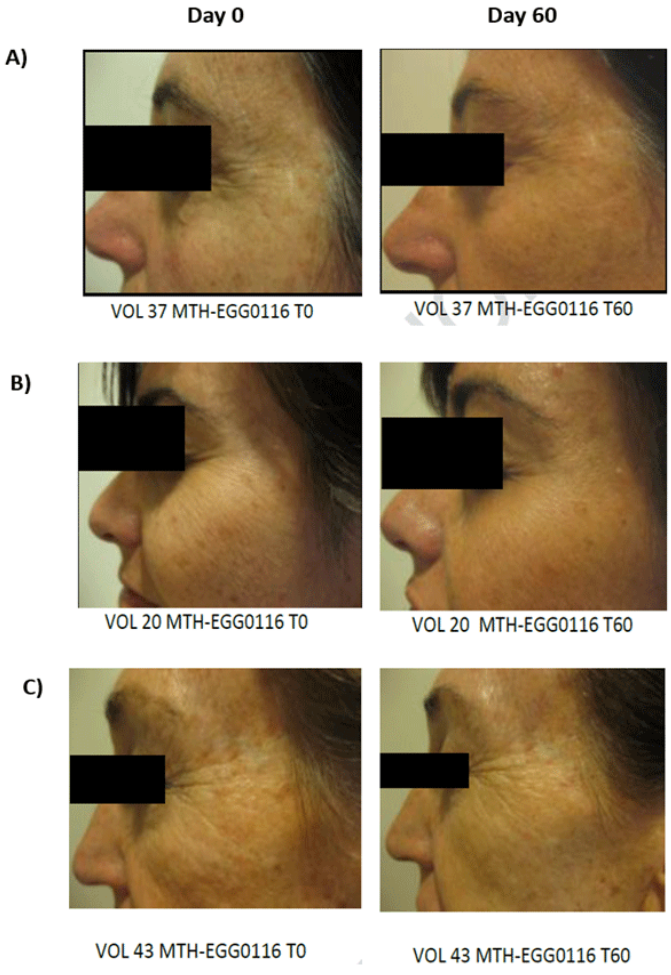

Figure 6: Pictures of the eye contour before and after the treatment with Ovoderm® in three different volunteers (A, B and C).

Discussion

The mechanical function of the skin is related to the biomechanical properties, the quality of the extracellular matrix and the structural organization of its components, being the most significant the epidermal stratum corneum, dermal collagen, elastic fibres and viscosity of the interstitial fluid. The dermis contains predominantly type I collagen (85%-90%) with lesser amounts of type III collagen (10%-15%). Collagen fibrils are part of the extracellular matrix macromolecules, where proteoglycans are also an important part [21].

Human face skin changes during the course of life and it is also a part of the human body that is exposed to UV radiation. Skin aging and photoaging are characterised by a reduction in skin thickness, loss of skin elasticity, collagen fibre degeneration and wrinkle formation [17, 22]. Some studies hypothesised that skin wrinkling that appears progressively as a function of age, could be due to the gradual alteration of collagen bundles that weakens the mechanical supporting tissue of stratum corneum [22]. The alterations in collagen, the major structural component of skin, have been suggested as a cause of the clinical changes observed in photoaged and naturally aged skin.

Eggshell membrane has been shown *in vitro* to be an ingredient that increases the cellular activity, collagen production and reduces the damage caused by UV light [16, 17, 23]. So far, few studies have been performed to investigate the effect of orally administered eggshell membrane on various skin parameters [24]. To our knowledge, this is the first clinical study set out to explore the efficacy of Ovoderm®, eggshell membrane, on skin physiology and appearance related to cutaneous aging. In this study the oral intake of 300mg of Ovoderm® was investigated in healthy humans demonstrating a significant improvement on skin physiology. The results revealed that only the volunteers intaking Ovoderm® improved their skin's barrier function as indicated by the 18.7% decline of TEWL after 60 days consumption. Moreover, the skin of volunteers taking Ovoderm® at the beginning of the study was catalogued as "healthy condition" (TEWL values of 10-15 g/m2h) and this condition improved to close to "very healthy condition" (TEWL values 0-10 g/m2h) after 60 days intake. Low TEWL is a characteristic feature of an intact skin protective function where the stratum corneum acts as a fully functional barrier. In damaged or certain cases of diseased skin water loss rates increase [25].

Skin aging has been related to the decrease in elasticity and greater fatigue compared to young skin. The present results showed a significant improvement of 25% in elasticity in volunteers intaking Ovoderm® at day 60. Moreover, focusing in the results obtained by age group, a greater improvement was observed in the group above 50 years old (29.67%) than in the group under 50 years old (20.60%). The improved elasticity values mean higher distensibility of the elastin fibres. Our results are in agreement with previous investigations where oral administration of collagen-derived products have shown an improvement on skin elasticity [7, 26]. Regarding skin fatigue, a significant decrease was observed in both treatments Ovoderm® and placebo, nevertheless, the decline in skin fatigue was greater in Ovoderm® group (33%) than in the placebo group (18%). As mentioned, fatigue decreased significantly in both treatments at the end of the study so as the firmness. Once again, the volunteers intaking Ovoderm® experienced a bigger improvement in firmness, 51%, than the placebo volunteers, 37%. It is worth noting that separating participants by age, only participants intaking Ovoderm® improved significantly in firmness both, over and under 50 years old whereas no significant changes were observed in placebo group. Sagging (the contrary of firmness) and wrinkling of skin during aging are physiologically associated with diminished elasticity. Here, the improvement in elasticity and firmness are shown together with the decline of skin fatigue and the visual diminution of wrinkles showed in the eye contour pictures. The improvement observed in skin elasticity suggests a long lasting positive physiological effect, opposite to those of topical skin care products, which increase skin elasticity predominantly by enhancing epidermal hydration [27]. The ability of eggshell membrane to inhibit the collagen and elastin degradation shows its potential for application in anti-wrinkle cosmetics [16]. Furthermore, eggshell membrane reduces the matrix metalloproteinases, enzymes that are capable of degrading extracellular matrix proteins in human skin, which are induced by photoaging and natural skin aging with the concomitant reduction in collagen synthesis [28, 29, 30]. Eggshell membrane thus, prevents the decline of collagen synthesis and the collagen degradation and accordingly, the loss of hydration and elasticity in skin resulting in wrinkle formation [16, 31]. Moreover, treatment with eggshell membrane has previously shown a positive effect on diverse skin parameters such as pigmentation, wrinkles and hydration [24]. These effects seem to be mediated by an increase of collagen and HA levels, a reduction of matrix metalloproteinases activity, inhibition of collagenase and elastase, as well as inhibition of skin inflammation [16, 17].

Conclusions

Taking all the results together we can conclude that the oral intake of Ovoderm®, in a short period of time and with a low dosage, ameliorates skins' protection function by improving the skin barrier function. The study clearly demonstrates a significant increase in skin elasticity. Moreover, a reduction in skin fatigue and firmness of the skin was also observed. The obtained data suggest that Ovoderm® is beneficial for both, people under and above 50 years old, but the improvement in people above 50 years old is superior.

It has to be stated that the demonstrated efficacy refers to the specific composition of Ovoderm® and could not be extrapolated to eggshell membrane in general. Ovoderm® appears as an effective, safe and well tolerated dietary supplement that counteracts the clinical signs of skin aging.

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