EVALUATION OF THE ANTI-AGING POTENTIAL OF A NUTRITIONAL SUPPLEMENTATION (NATICOL®) ON THE AGING SIGNS OF THE FACE OF ASIAN VOLUNTEERS

This clinical study was firstly intended to assess the anti-aging potential of a nutritional supplementation (NATICOL ®, fish collagen peptides) on the aging signs of the face of Asian volunteers. A second objective was to assess the overall tolerability of the nutritional complement (NATICOL®) on the base of adverse events reporting. The study was an interventional Biomedical Research, mono-centre, double-blind, randomized (a randomization list was prepared by the Biostatistics of the CPCAD using the SYSTAT software version 11.0 (SPSS, USA) by a person not participating to the study) and placebo-controlled study performed on two parallel groups.

I – MATERIAL AND METHODS

Treatment products

- Investigational product: NATICOL® (collagen peptides, Weishardt group)

Dose: one daily intake of a 10g bag in 20cl of liquid, before the breakfast for 12 weeks.

- Comparator product: maltodextrin, placebo of NATICOL®.

Dose: one daily intake of a 10g bag in 20cl of liquid, before the breakfast for 12 weeks.

Study population

Forty (40) healthy female subjects, aged 59±5 years old, with skin type II to V according to the Fitzpatrick scale, meeting the criteria of inclusion/non-inclusion were included in the study split in two homogeneous groups of 20 women each. Twenty-five subjects were from Asian origin (East and South-East Asia) and 15 of Caucasian origin.

Main Inclusion Criteria:

1. *Subject who has signed and dated informed consent before any trial related activity is carried out,*
2. *Healthy female subject of Asian origin, 50 to 70 years old with skin type II to V according to the Fitzpatrick scale, having periorbital lines of medium to moderate severity corresponding at least to the level 3 of the classification of Lemperle.*
3. *Female healthy subject of childbearing potential has to use a reliable contraceptive method (oral contraceptive pill, contraceptive implant, intrauterine device, bilateral tubal section/ligation, condoms) at least one month before the start of the study and has to accept to continue this method during the study; or of non-childbearing potential i.e., post-menopausal (one year without menstrual period, hysterectomy or bilateral ovariectomy),*
4. *Agreeing to take an oral supplementation during 12 consecutive weeks,*
5. *Healthy subject willing to follow the study restrictions and complete the study phase,*
6. *Healthy subjects registered with Social Security in agreement with the French law on study research.*

Main Exclusion Criteria:

1. *Female who are pregnant or breast feeding,*
2. *Female of child-bearing potential with positive urine pregnancy test at baseline (Day 1, before product application),*
3. *Subjects who had been treated with cosmetic or medical anti-aging treatments during the 8 weeks preceding the inclusion,*
4. *Subjects who had been treated with any physical treatment or procedure on*

the face during the last 6 months (laser, dermabrasion, peeling, …etc.),

5. *Subjects who had been treated by esthetic surgery or injected with an anti-wrinkle product on the face during the last 8 months (collagen, Botox, …etc.),*
6. *Subjects who had esthetic skin care or massage on the face during the two weeks preceding inclusion,*
7. *Subject with any active systemic or cutaneous disease that could in any way confound interpretation of the study results (e.g. atopic dermatitis or psoriasis),*
8. *Subjects who have a known allergy to fish or to fish extracted collagen,*
9. *Subjects who had been sunburned in the last month on the face or who plan to be exposed to sun or UV light on the face during the study,*
10. *Subject taking some dietary supplement which could interact with the study results (e.g. collagen supplementation) or which could modify the bioavailability of the tested product (e.g.: hypo-caloric diet, or high protein diet, etc…),*
11. *Subject with significant history of alcoholism or drug abuse,*
12. *Subjects hospitalized in a medical or social establishment for another reason than biomedical research or who have forfeited their freedom by administrative or legal award or who are under guardianship,*
13. *Subject unable to communicate or cooperate with the Investigator due to poor mental development, language problems or impaired cerebral function,*
14. *Subject who has received (or will receive) more than 4500 euros as indemnity for participating in clinical studies within the previous 12 months.*

Study duration

The whole study participation per subject (after a screening period of 2 weeks) was 84 days ±2 days (12 weeks).

Methodology

Selection visit, during the 2 weeks preceding Day 1 (Physical examination, medical history, assessments of the aging/photoaging signs of the face.

Day 1 : Baseline:

- Location and selection of the test-zones on the face.
- Clinical score of wrinkles of the selected periorbital zone.
- Clinical score of the skin aspect (homogeneity of skin radiance).
- Standardized photographs of the face (front and 45° left and right side) using the VISIA system (Canfield Scientific, USA) in white light, parallel polarized (for visualization of the defects of relief) and cross polarized (for visualization of pigmentation and microcirculation aspects).
- Hydration measurement on a selected zone.
- Skin biomechanics measurements.
- Realization of a skin replica (Silflo® elastomer rubber) of the selected periorbital zone.

The subjects received the test-product for a six-week period. They had to take 10 g /day, 15 min before the breakfast.

Week 6 :

- Location and selection of the test-zones on the face.
- Clinical score of wrinkles of the selected periorbital zone.
- Clinical score of the skin aspect (homogeneity of skin radiance).
- Standardized photographs of the face (front and 45° left and right side) using the VISIA system (Canfield Scientific, USA) in white light, parallel polarized (for visualization of the defects of relief) and cross polarized (for visualization of pigmentation and microcirculation aspects).
- Hydration measurement on a selected zone.
- Skin biomechanics measurements.
- Realization of a skin replica (Silflo® elastomer rubber) of the selected periorbital zone.
- Evaluation of the tolerance of the tested product.
- Recordings of adverse events and concomitant medications.
- Product recovery and new product dispensation for the next six-week period.

Week 12 : end of study :

- Location and selection of the test-zones on the face.
- Clinical score of wrinkles of the selected periorbital zone.
- Clinical score of the skin aspect (homogeneity of skin radiance).
- Standardized photographs of the face (front and 45° left and right side) using the VISIA system (Canfield Scientific, USA) in white light, parallel polarized (for visualization of the defects of relief) and cross polarized (for visualization of pigmentation and microcirculation aspects).
- Hydration measurement on a selected zone.
- Skin biomechanics measurements.
- Realization of a skin replica (Silflo® elastomer rubber) of the selected periorbital zone.
- Evaluation of the tolerance of the tested product.
- Collection of adverse events and concomitant medications.
- Product recovery.
- Global efficacy assessment by the dermatologist and by the subject.
- Satisfaction questionnaire by the subject.

Skin biomechanics measurements

- The cutometer® SEM 575 Skin Elasticity Meter® (Courage and KazhaKa, Köln, Germany) allowed to measure a deformation perpendicular to the skin surface using a suction method. A depression of 400 mbars was applied through the probe on the skin during 2 seconds, followed by a relaxation period of 2 seconds. This cycle was restarted 5 times. The deformation induced on the skin was measured by an optical system placed in the probe, whose aperture has a 2 mm diameter. The measured parameters were: Ue, the elastic deformation; Ur, the elastic back deformation; Uf the total extensibility of the skin; Ua, the total deformation recovery; Ur/Ue the pure elasticity. The variations of Ur/Uf and Ua/Uf between the 5th and the 1rst cycles were calculated as well. Three measurements of 5 cycles were performed on the test zone (bony part of the cheek). Each parameter was averaged for analysis. The skin deformation curve as a function of time is described in Figure 1 below.

Skin Hydration meaurements

- The corneometer CM 825® (Courage & Khazaka, Köln, Germany) allows to perform skin hydration measurements. This instrument assesses the hydration state of the first horny layers using electrical capacitance measurements. After the probe has been placed perpendicularly on the skin surface, measurements were taken in less than 1 second. Three measurements were taken on each site respectively, and were averaged for further evaluation.

The measuring principle of the corneometer CM 825® is based on the world-wide acknowledged capacitance method. Measuring of capacitance is based on the completely different dielectric constant of water and other substances. The measuring capacitor shows changes of capacitance according to the moisture content of the samples. An electric scatter field penetrates the skin during the measurement and the dielectricity is determined. There exists no physical unit.

Skin replica procedure and analysis (3D- analysis of wrinkles)

Replicas (prints) were taken using the following procedure:

The subject was installed in a comfortable position (e.g. reclined in a chair). She had to remain immobile and try to keep the jaw lightly closed during the taking of the impression (about 3 minutes). Three (3) ml of SILFLO^{R} rubber were put into a Petri dish and mixed, using a spatula, with 3 drops of catalyst. The whole was mixed for about 10 seconds until an homogenous mixture was achieved, then spread over a diameter of about 3cm on the area to be analysed. The thickness of the SILFLO^{R} film had to be about 2mm and as even as possible so that the external face of the print be as flat (or smooth) as possible.

During the hardening of the print (≈3 minutes), the subject had to remain immobile. When set, the print was delicately removed from the skin of the patient, identified with the patient number and time, an arrow aligned along the body axis is drawn as well on the replica. Then the replica was placed in a small storage box.

The replicas or prints were analyzed using an image analysis system. This system was composed of a camera (4912-5000/0000, Cohu Inc, San Diego, CA, USA) which was fitted with a Computar macro-objective. The replica was lit with incident light at 35° which produces shadowing behind each wrinkle or hollow. Digitized resultant images were recorded by a CCD camera and then analyzed by computer dedicated software (Quantirides software, Monaderm, Monaco).

Satisfaction questionnaire by the Subject and investigator

The Subject and investigator had to answer using the categories of responses described as following:

Completely agree
Partially agree
Non opinion
Partially disagree
Completely disagree

The question for the subject was the following: “Would you say that the treatment you have tested has reduced the wrinkles on your face?”

The Investigator and the Subject had to answer separately at the following question:

“Would you say that the treatment you have tested is globally efficient for the treatment of aging signs of the face?”

Evaluation criteria

Primary criteria:

- Skin relief parameters from the skin replica of the periorbital zone (crow’s foot).
    - Form factor

Secondary criteria:

- Clinical score of wrinkles
- Hydration value
- Skin elasticity parameters
- Score of improvement on Standardized photographs
- Global efficacy assessment by the dermatologist and by the subject.

- Satisfaction questionnaire by the subject.

Safety
Safety was assessed at each assessment time.
General safety: General adverse events were to be reported descriptively on an on-going basis.

Statistical methods
Descriptive statistics (N, mean, standard error, min, max,) were calculated by tested treatment for each parameter. The variation from Day 1 (Δ value) was calculated as well.

Prior to any statistical analysis, the normality of each variable was tested using the Shapiro Wilks test of normality.

For the primary criteria, for the clinical score of wrinkles and for the hydration parameter, the variation from Day 1 (Δ value) was calculated as well.
Prior to any statistical analysis, the normality of each variable was tested using the Shapiro Wilks test of normality.
For each normal variable an analysis of variance (ANOVA) using factors "Subject" and "Treatment" was performed. In order to take into account the factor "Subject" in the analysis, the variables were adjusted by subject from the global Treatment mean of the subject prior to each analyse.
In case of the significance of the "Treatment" factor, both treatments were compared using the multiple comparison test of Tukey (or equivalent), using the residual variance from the ANOVA, and significance determined using the 0.05 probability level.
For each variable with non normal distribution, treatments were compared using the Kruskal Wallis test with "Treatment" as grouping factor.

The frequency of responses to questions (questionnaire, score of improvement) was analysed by the Khi-2 test. Due to the fact that the number of answers by category was often lower than 5, and therefore leading to invalid the statistics of the Khi-2 test, categories of answers were grouped to form only two categories:

Positive = « Totally agree » and « Partially agree »

Negative = « Without opinion », « Partially disagree » and « Totally disagree »

The Δ values were analyzed in the same manner as that used for the absolute values.
The significance level of the comparison tests was fixed at 0.05.

II – RESULTS AND DISCUSSIONS

In the conditions of this study, it was observed that:

Concerning the parameters of the relief of the periorbital zone (crow's-foot), the evolution compared to Day1, indicated a significant decrease of the form factor for the product NATICOL®, which expressed a significant smoothing of the relief of the skin (Fig. 2) No effect was observed in the Placebo group. On the other hand, the clinical score of wrinkles, assessed by the Investigator, decreased significantly under NATICOL® at Week6 and Week12, while it remained unchanged for the Placebo.

The skin hydration increased significantly during the study for Naticol group (Fig.3). Concerning the skin biomechanics parameters, the skin extensibility was found significantly improved (+24% at Week12, fig. 4) for the group NATICOL® at both Week6 and Week12 visits whereas no significant effect was shown in the Placebo group. Due to the increase of the skin extensibility, the ability to return to the initial state after deformation was also increase for the group NATICOL®. (Fig.4)
Concerning the answers to the questionnaire on the efficiency of products, the number of positive answers was almost always higher for NATICOL® than for Placebo. The difference between products was significant for the question: " Would you say that the treatment, you have tested, has reduced the wrinkles on your face?" where 79 % of the subjects under NATICOL® answered positively whereas 58 % of the subjects under Placebo gave positive answers (Fig. 5).

The tolerance to the tested products was globally excellent.

These results indicate that NATICOL® expressed significant improvements of the skin status compared to baseline whereas Placebo had no effect. Since 3-month treatment duration represents a minimum for the anti-aging topical actives such as retinoic acid to produce significant effects on fine wrinkling, it could be expected that an expanded treatment duration for NATICOL® of 4 to 6 months would have produced better significant results with more significant difference vs. Placebo.
In conclusion, this study has shown that NATICOL® improves the biomechanical properties of the skin with regard to baseline, in particular for the skin extensibility. An effect of smoothing observed on the skin imprints of the peri-orbital zone was confirmed by the clinical observation of the wrinkles aspect. Concerning the questionnaire of appreciation, the subjects found NATICOL® significantly better than Placebo for reducing the wrinkles on their face.

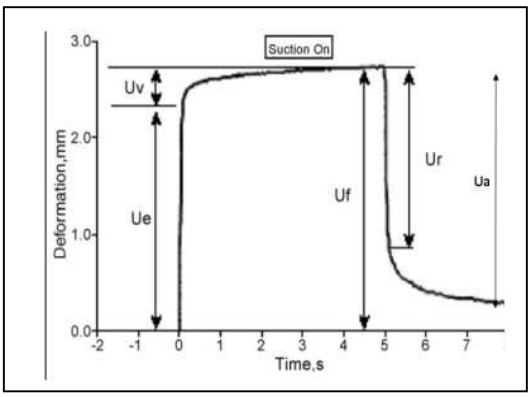


Figure 1

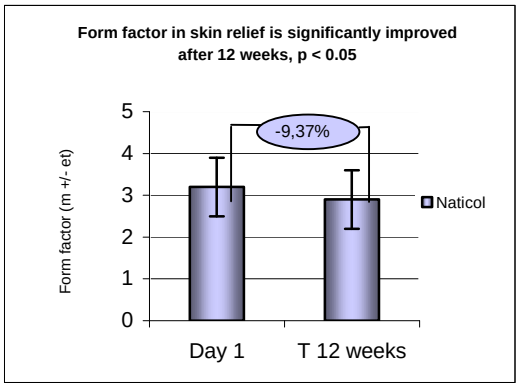
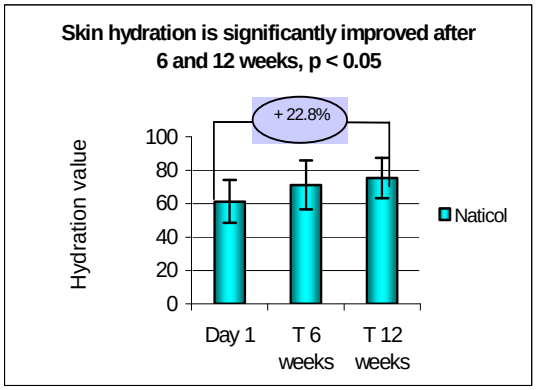


Figure 2



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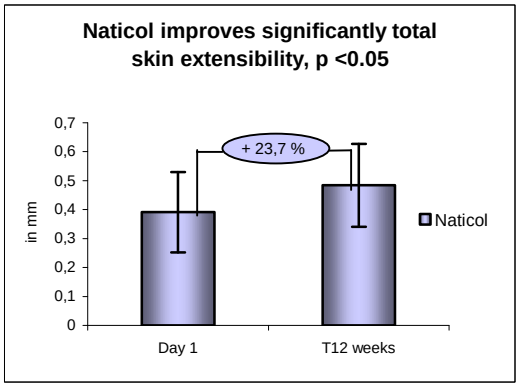

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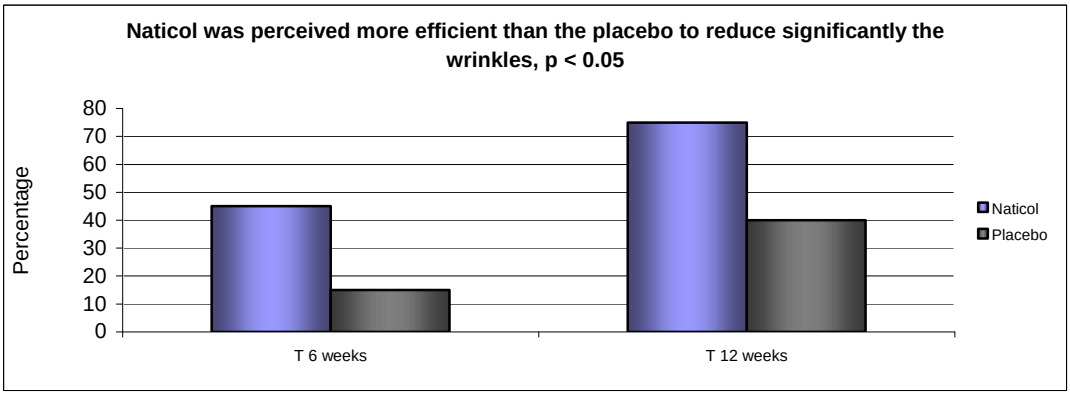

Figure 5

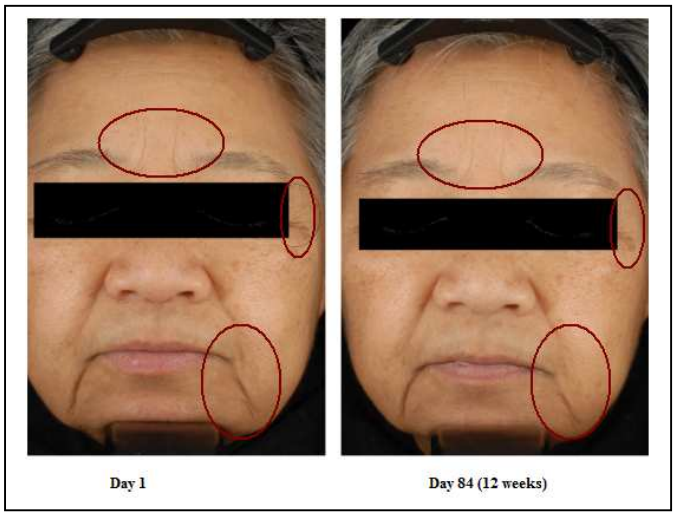

Figure 6 – VISIA CR Standardized photographies of a volunteer face