

## EVALUATION OF THE EFFICACY OF ANTI-AGEING AND HYDRATING PRODUCTS ON THE CUTANEOUS SENESENCE PROCESS. PRELIMINARY DATA

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### Abstract

During the last years, skin ageing and anti-ageing therapies have become an area of increasing research interest. High frequency ultrasound allows the *in vivo* appreciation of certain histological parameters and offers new characteristic markers, which may quantify the severity of the cutaneous senescence process and efficacy of anti-ageing therapies. The study presents some preliminary data regarding the effects of certain hydration and anti-ageing products containing Viniferol, at cutaneous level. The changes in the dermal echogenicity confirm the efficacy and direct action of Viniferol upon the cutaneous fibroblasts. The anti-ageing therapy can be correlated with the age of the subjects and the cutaneous phototype. The "critical age interval" involves specific changes in dermal echogenicity that quantify intense molecular, biochemical and structural changes, being thus mostly and highly responsive to the anti-ageing therapy.

Enlarged studies, on greater number of subjects are still necessary for the standardization of the study method (high frequency ultrasound), regarding the efficacy of the topically applied products.

### Rezumat

În ultimii ani, cercetările în domeniul îmbătrânirii pielii și al terapiilor *antiageing* au devenit un subiect de cercetare de mare interes. Ultrasunetele cu frecvență înaltă permit aprecierea *in vivo* a unor parametrii histologici și oferă noi markeri caracteristici, care pot cuantifica severitatea procesului de senescență cutanată și eficiența terapiilor *antiageing*. Studiul prezintă date preliminare legate de efectul preparatelor hidratante și antirid pe baza de Viniferol la nivel cutanat. Modificările ecogenității dermice confirmă efectul țintit și eficient al Viniferolului asupra fibroblastului cutanat. Terapia *antiageing* se corelează cu vârsta și cu fototipul cutanat. Sunt necesare studii extinse pe loturi suficient de mari pentru standardizarea metodei de studiu a eficienței preparatului prin ultrasonografie de înaltă frecvență.

**Keywords:** cutaneous ageing, ultrasound, hydration, flavonoids.

## Introduction

High-frequency ultrasound is a non-invasive diagnosis method with a large application area in the field of internal medicine. In the last years, ultrasonographic investigations have also been successfully used in the fields of dermatology and dermatocosmetics [2, 3, 8]. High frequency ultrasound can provide very useful information regarding the lateral and axial extension of cutaneous tumors or various inflammatory processes. It also provides characteristic ultrasonographic markers that may quantify the complex cutaneous senescence process and allows an appreciation of the efficacy of various anti-ageing therapies. Ultrasonography applied in the dermatological field facilitates the noninvasive appreciation at histological level both of the epidermis and dermis thickness and of the dermal echogenicity. These parameters are all influenced both by the normal senescence process, and also by the locally or generally applied therapies [1, 2, 4, 5, 6, 9, 18]. The objective of our study was the ultrasonographic evaluation of the cutaneous changes (at facial level) after the topical use of certain hydrating, anti-ageing and dermatocosmetic products containing Viniferol.

Many studies in medical literature confirm the important role of the flavonoids in the anti-ageing process. Flavonoids contained in Viniferol act at fibroblastic level, activating the synthesis of the extracellular matrix, due to “hormone-like” receptors present on the cutaneous fibroblasts [1, 5, 6, 18]. Fibroblasts are young, metabolically active cells, which are responsible for the synthesis of collagen, elastic fibers, and also polysaccharides. The adult cells, the fibrocytes, play an important role in the maintenance of the extracellular matrix. In specific conditions, fibrocytes reactivate from the metabolic point of view and are able to synthesize new fibrillar components.

Ultrasonographically, it is possible to quantify the proteic structures, the newly synthesized collagen, that appears as medium or highly echogenic. The new synthesized sulfurated or nonsulfurated glycosaminoglycans (hyaluronic acid) bind to proteins, form proteoglycans that retain water, playing thus a major role in the cutaneous hydration process [4, 7, 9, 18].

## Material and Methods

The topically applied products may significantly influence the cutaneous characteristics [12, 13]. The products used in our study consisted of an emollient, hydrating cream, based on occlusive hydrating agents [14]

and an anti-ageing product with Viniferol, extracted from grapevine, which contains flavonoids, active antioxidants with a proven anti-ageing role.

The study was performed on 10 female subjects aged 26-86, belonging to phototype II and III, according to Fitzpatrick's classification (Table I). All the subjects included in the study have previously signed a consent form. During the study, no other dermatocosmetic products were applied to the skin, or alimentary supplements taken, that could have influenced the cutaneous structure.

The ultrasonographic images were taken at facial level (zygomatic area) at three different stages: initially (after facial cleansing), at 4 weeks interval, after topic application of the emollient and hydrating cream, three times a day, and after other 4 weeks, during which the subjects applied hydrating and emollient cream in the morning, and Viniferol containing anti-ageing cream in the evening.

**Table I**  
Subjects involved in the study.

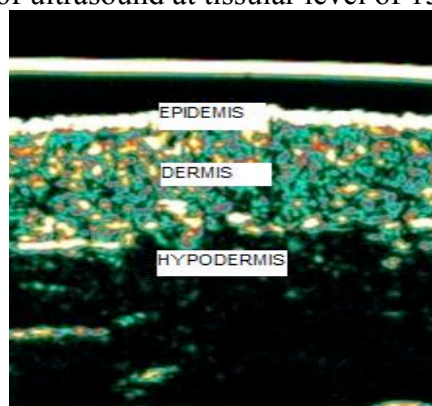
| Subject | Age | Phototype (Fitzpatrick) | Smoker |
|---------|-----|-------------------------|--------|
| 1.      | 76  | III                     | No     |
| 2.      | 50  | III                     | No     |
| 3.      | 57  | II                      | No     |
| 4.      | 61  | III                     | No     |
| 5.      | 57  | II                      | No     |
| 6.      | 32  | III                     | No     |
| 7.      | 86  | II                      | Yes    |
| 8.      | 52  | II                      | No     |
| 9.      | 26  | III                     | No     |
| 10.     | 52  | III                     | No     |

The ultrasound investigation was performed using a high frequency cutaneous ultrasound device 20 MHz (Dermascan C, Cortex Technology, Denmark) which allowed us to obtain *in vivo* sectional images of the tissue up to 2.5 cm in depth.

The ultrasonographic wave is partially reflected at the interface of two adjacent structures, generating echoes of different amplitudes. The intensity of the echogenicity is evaluated by a microprocessor and visualized as a colored bidimensional image. The color scale of the echogenicity is as follows: white>yellow>green>blue>black.

The Dermascan Soft allows the selection of pixels with different amplitudes on a scale varying from 0-250, the qualitative and quantitative evaluation of the number of pixels with certain amplitudes and the measurement of various parameters (mm).

The epidermis, stratified squamous keratinized epithelia, appears on the ultrasonographic image as a white, hyperechogenic band. The dermis, a dense, collagen-rich structure, shows different echogenicity degrees, according to age, anatomical site, the presence of different pathological processes, and the senescence degree. The dermis is expressed as a 2 color composition: yellow and/or red (Figure 1), and the subcutaneous layer appears black with green bands that quantify the conjunctive-vascular septa between the adipose lobules [7, 15, 16]. The curve was adjusted at a value of 20 dB, at a speed of ultrasound at tissular level of 1580m/s (Figure 1).



**Figure 1**

High-frequency ultrasound: ultrasound image of the skin: epidermis, dermis, hypodermis (Dermascan, Cortex Technologies.)

For each image, by using a special analysis software (Dermavision, Cortex Technologies) we measured the following parameters: the thickness of the epidermis, the thickness of the dermis, the number of low echogenic pixels (LEP: 0-30), and the LEPs/LEPi ratio (number of low echogenic pixels in the upper dermis/number of low echogenic pixels in the lower dermis). The thickness of the epidermis was obtained by establishing the mean of three measurements performed at three different sites of each image (the 2 extremities of the analyzed image and the center of the image).

The thickness of the dermis was obtained by measuring the distance between the dermo-epidermal and the dermo-hypodermic junction at the same three different sites and by establishing the mean of the three values.

The analysis software used, has the pixel amplitude corresponding to a numerical scale set between 0-255. By selecting a certain interval from the 0-255 scale, we obtained values corresponding to a certain pixel type, present in the analyzed image. Thus, the 0-30 interval corresponds to low echogenic pixels (LEP), the 50-150 interval to medium echogenicity pixels, and the 200-255 interval to high echogenicity pixels.

The number of low echogenicity pixels (LEP 0-30), quantifies the cutaneous hydration degree and may be correlated to age, cutaneous oedema or degenerative processes of the extracellular matrix, such as photoinduced elastosis or collagen degeneration.

The 50-100 and 100-150 intervals correspond to the medium echogenicity pixels (MEP) whereas the 200-255 interval represents the high echogenicity pixels (HEP). The high echogenicity pixels appear in a greater amount in the deep dermis in elderly subjects and after phototherapy, quantifying structural, degenerative, biochemical changes that are typical for the intrinsic cutaneous senescence process [10, 11, 15, 17, 18].

LEP was determined at the dermal level, between the epidermis and the hypodermis. Additionally, the LEP area was divided into two other regions, differently quantified: superior LEP (LEPs), and inferior LEP (LEPi). The limit between the two regions was obtained by dividing the dermis into two equal parts, by drawing a parallel line to the epidermis echogenicity line. The LEPs/LEPi ratio was established. This ratio allows an appreciation of the density and integrity of the extracellular matrix, both from the papillary and lower dermis, which may vary according to age, cutaneous affections, UV exposure, and therapy. It is a specific ultrasonographic marker that allows the quantification of the dermal density and of the fibrillary structure. [10]

### Results and Discussion

The thickness of the epidermis (mm) did not show significant changes after the application of the hydrating and anti-ageing products. Significant changes appeared at dermal level, where the thickness (mm) increased in 6 out of 10 subjects. Similar results were published in other previously published studies [10].

The LEPs /LEPi ratio shows interesting aspects at facial level after the application of the product, which may be correlated to age, cutaneous phototype and smoking (Figure 2 and figure 3).

The graph in figure 3 shows that after the use of the emollient and hydrating cream, the LEPs/LEPi ratio decreases because of an increase of LEPi, which stands for a profound and efficient hydration at reticular dermis level. Ultrasonographically, an increase of LEP (0-30) was noticed in the lower dermis.

After the application of Viniferol-based anti-ageing cream, the LEPs/LEPi ratio increased due to a decrease of LEPi, correlated to an accumulation of proteins secreted by fibroblasts (key cells in the anti-ageing process) at dermal level.

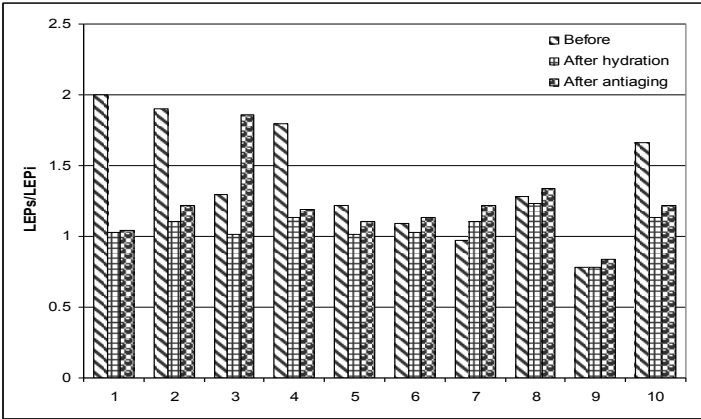


Figure 2

LEPs/ LEPi ratio during the three stages of the study: before therapy, after hydration and after anti-ageing product application, according to age and phototype in the 10 subjects included in the study

Ultrasonographic images of the skin taken from the subjects involved in the study are shown in figure 3.

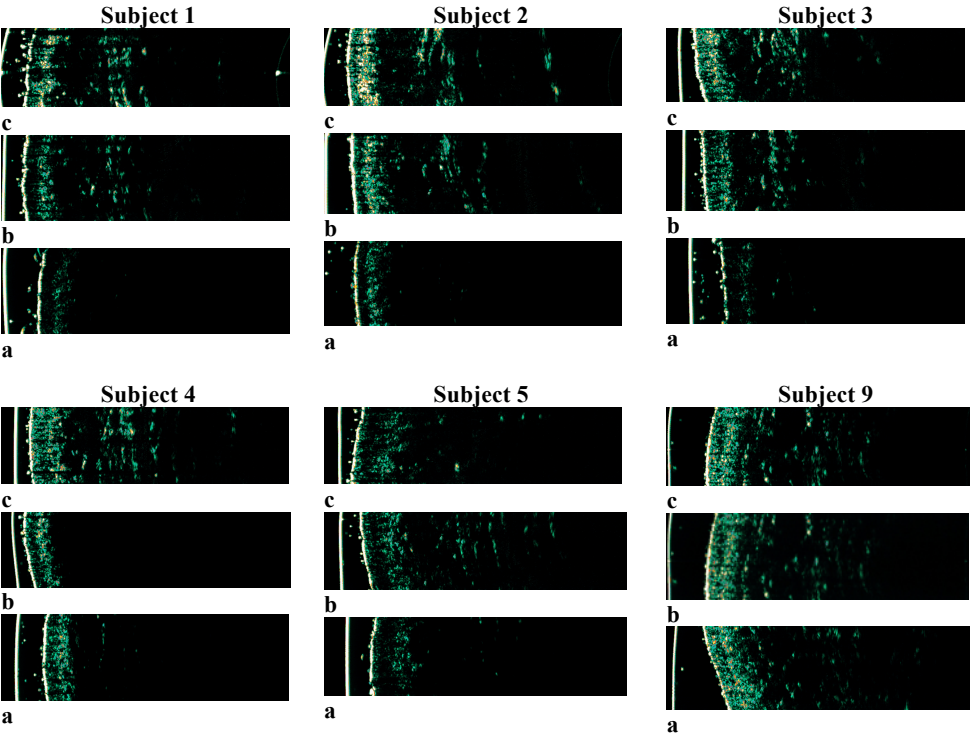


Figure 3

Ultrasonographic images taken from the subjects who participated in the study, in the three stages: initially (a), after emollient and hydrating cream application (b) and after anti-ageing cream application (c).

An intense hydration process was noticed (decrease of the LEPs/LEPi ratio) in subject 1, aged 76, that obviously presented a dehydrated, aged integument, due to age and UV-exposure, in comparison to subject 9, aged 26, where the hydration degree did not change.

The hydration process was present, in different degrees, in all subjects who participated in the study. It may also be noticed that the proteic synthesis process appears in all subjects, with certain peculiarities according to age and cutaneous phototype. Thus, it may be observed that the collagen synthesis may also be activated at extreme ages, after 70 years (subject 1) and before 30 (subject 9).

The analysis of the ultrasonographic images showed that subjects belonging to the cutaneous phototype II (subjects 3 and 8), are more receptive to the flavonoid-therapy, compared to the subjects belonging to phototype III.

More extended studies are still needed in order to better understand how the cutaneous phototype, that is genetically determined, can influence the anti-ageing therapy (evaluated through the changes in the LEPs/LEPi ratio).

Even though we present preliminary data, our study did conclude that cutaneous hydration is necessary, compulsory and efficient for all age categories. The collagen synthesis may be amplified before the age of 30 and reactivated after 70 years by the use of products that have a specific effect on the dermal fibroblasts. According to published data and to our actual results, we consider that there is a “critical age interval” between 20-40 years during which the anti-ageing prophylactic therapy should begin.

The “critical age interval” involves specific changes in dermal echogenicity that involve intense molecular, biochemical and structural changes, being thus mostly and highly responsive to the anti-ageing therapy [3].

Also, the different reactivity of the subjects regarding the neosynthesis of the dermal matrix opens new study perspectives in the field of specific therapies according to the cutaneous phototype.

### Conclusions

High-frequency ultrasound allows the obtaining of *in vivo* histological images at cutaneous level and offers characteristic noninvasive markers (LEPs/LEPi ratio) that quantify the efficacy of active substances from the topically applied products.

The degree of cutaneous hydration, collagen and proteoglycans neosynthesis are correlated to age and cutaneous phototype. We consider

“the 20-40 critical age interval” as the highly efficient and responsive period for the initiation of the anti-ageing prophylaxis. The emollient/hydrating and anti-ageing products containing Viniferol extracted flavonoids act specifically at the level of cutaneous fibroblasts and interfere with the ageing process.

A continuation of our study on a greater number of subjects is necessary in order to reach a consensus on method standardization.

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