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Original Article

Ultrasonographic assessment of the cutaneous changes induced by topical antiaging Interactive P63 complex

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ABSTRACT

Ultrasonography represents a noninvasive method which is able to assess the cutaneous structure by using several characteristic markers: the thickness of the epidermis and dermis, the dermal density, the presence of the subepidermal low echogenity band. In this study we assessed the cutaneous changes induced at facial level by an antiging active – Interactive P63, a metabolic dynamiser composed of alpha hydroxyacids, retinoids, a biomimetic peptidic complex and gluconolactone incapsulated in liposomes. The study was performed on fifty female volunteers, 30 being included in the study group who received applied the active product and 20 representing the control group who applied a placebo product, according to an established protocol. In the study group a significant increase of mean thickness of the epidermis, dermis and dermal density was observed as morphological expression of the changes induced by Interactive P63 complex at fibroblastic and extracellular matrix level. A more significant growth of the dermal density in subjects belonging to phototype class III, compared to phototype class II. The product was highly efficient especially in the 51-60 age interval. Only a slight increase of the epidermal and dermal thickness as well as dermal density was noticed in the control group, due to the skin hydration induced by the placebo product applied on skin.

KEYWORDS: antiaging therapy, Interactive P63, ultrasound diagnose

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INTRODUCTION

In the last 20 years, the ultrasonographic diagnosis was extended from the internal medicine field to the dermatological and dermatocosmetological field as well, having great use both in the noninvasive diagnosis of various cutaneous lesions, and the assessment of the efficacy of various topical or general therapies. There are numerous studies attesting the importance of ultrasound in the noninvasive, "histologically-leveled" assessment of the antiaging therapy [1,2]. High-frequency ultrasound applied in dermatology offers characteristic markers that quantify in a very accurate manner the cutanous structure: the thickness of the epidermis and dermis, the dermal density, the presence of the subepidermal low echogenity band [3,4].

Taking into consideration that nowadays we assist to an increase of life expectancy of the world population, the antiaging therapy became a priority and a continuous challenge for researchers. The senescence process involves complex, cumulative, intrinsic and extrinsic mechanisms that affect the entire organism and thus the integumentary system [5,6,7].

The identification of the mechanisms involved in the cutaneous aging process and their impact on certain age categories, correlated with the hormonal and neurogenetic constellation of the subject would be highly desirable since it is estimated that about 31% of the population is over the age of 60 (US Census Bureau, online database www.census.gov).

The increase of life expectancy, the psychosocial impact of the cutaneous aspect justifies the high amount of research studies of the aging mechanisms as well as of the efficacy of certain anti-aging therapies [8].
Interactive P63 is a metabolic dynamiser, able of interacting simultaneously at different cutaneous levels, both on anabolic and catabolic mechanisms. It contains 8 active principles, among which: alpha hydroxyacids, retinoids, G-factor P63 (a biomimetic peptidic complex derived from growth factors), gluconolactone incapsulated in liposomes, Dipeptide 3 (Biomimetic peptide).

The antiaging complex has a simultaneous action on three levels, epidermis, dermis and dermoepidermic junction. It has been tested in vitro on human fibroblasts cell culture (Line Hs 27) for cytotoxicity, apoptosis, proliferation index, collagen synthesis, matrix metaloproteinases activity. It has been shown that the product induces a significant increase in collagen synthesis, aspect that can be assessed in vivo by high frequency ultrasound.

The purpose of the actual study is to assess the cutaneous changes induced by topical use at facial level of an antiaging product, Interactive P63, for 12 weeks, using high-frequency ultrasound. The active complex, Interactive P63, was initially studied on cell cultures (cutaneous fibroblasts), proving a high tolerability, the absence of side effects as well as a significant increase of the collagen content, induced by fibroblastic stimulation.

MATERIALS AND METHODS

Patients

Fifty female subjects aged 40-75 years, who addressed themselves to the practice for antiaging therapy, were prospectively included in a controlled study. The study excluded patients with known allergies to topical products, cutaneous lesions at facial level, resurfacing or other antiaging therapies in the past months, or those who used phototherapy or oral contraception.

The subjects were divided in 2 groups: the study group with 30 patients and the placebo group with 20 patients. In the study group, 16 subjects belonged to phototype class II and 14 subjects to phototype class III, wheareas from the placebo goup, 10 subjects were phototype II and 10 subjects were classified in phototype III. The subjects were divided into 3 age categories: 40-50 years, 50-60 years and over 60 years.

The subjects from the study group underwent topical therapy with Interactive P63 product, whereas the rest of 20 subjects from the control group used a product without active ingredient.

The subjects taken into the study followed the proposed antiaging therapy for 12 weeks, according to a standard protocol. During this period, no other treatments apart from moisturizing cream were applied.

The Interactive P63 Product/placebo was applied twice a week for 30 minutes at facial level for 12 weeks. For every subject, ultrasonographic images were taken from zygomatic area, initially and after 12 weeks of topical use of Interactive P63/placebo product at facial level. The study was approved by the Ethical Committee of UMF Cluj-Napoca, Romania. Every subject was informed about the nature and purpose of the study, and signed a written consent before enrolling into the study.

Ultrasonographic evaluation

The epidermal and dermal thickness of the integument represent imagistic markers that can quantify the cutaneous aging process [5,9,10]. The equipment used for the ultrasonographic assessment of the efficacy of the topically applied products was a 20 MHz Dermascan device (Cortex Technologies, Denmark), that allows the “in vivo” acquirement of sectional cutaneous images up to a depth of 2.5 cm [2,11,12].

The images were obtained using the transducer, placed perpendicularly on the site of interest (left zygomatic area), and then analyzed, using a special software (Dermavision), that allowed the segmentation of each image in different regions of interest, by selecting one or more homogenous amplitude bands. After having defined an area of interest, by selecting a certain amplitude interval, we assessed quantitatively the areas where the amplitude belonged to the chosen intervals, and obtained its extension both in mm and number of pixels [13]. The following parameters were assessed: the thickness of the epidermis and dermis, the

number of: low echogenic pixels (LEP 0-30), medium echogenic pixels (MEP 50-150), high echogenic pixels (HEP 200-255), LEPs/LEPi ratio (low echogenic pixels in the upper dermis/lower dermis) [14].

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.

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. These ratios 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, general or topical therapy.

Statistical analysis

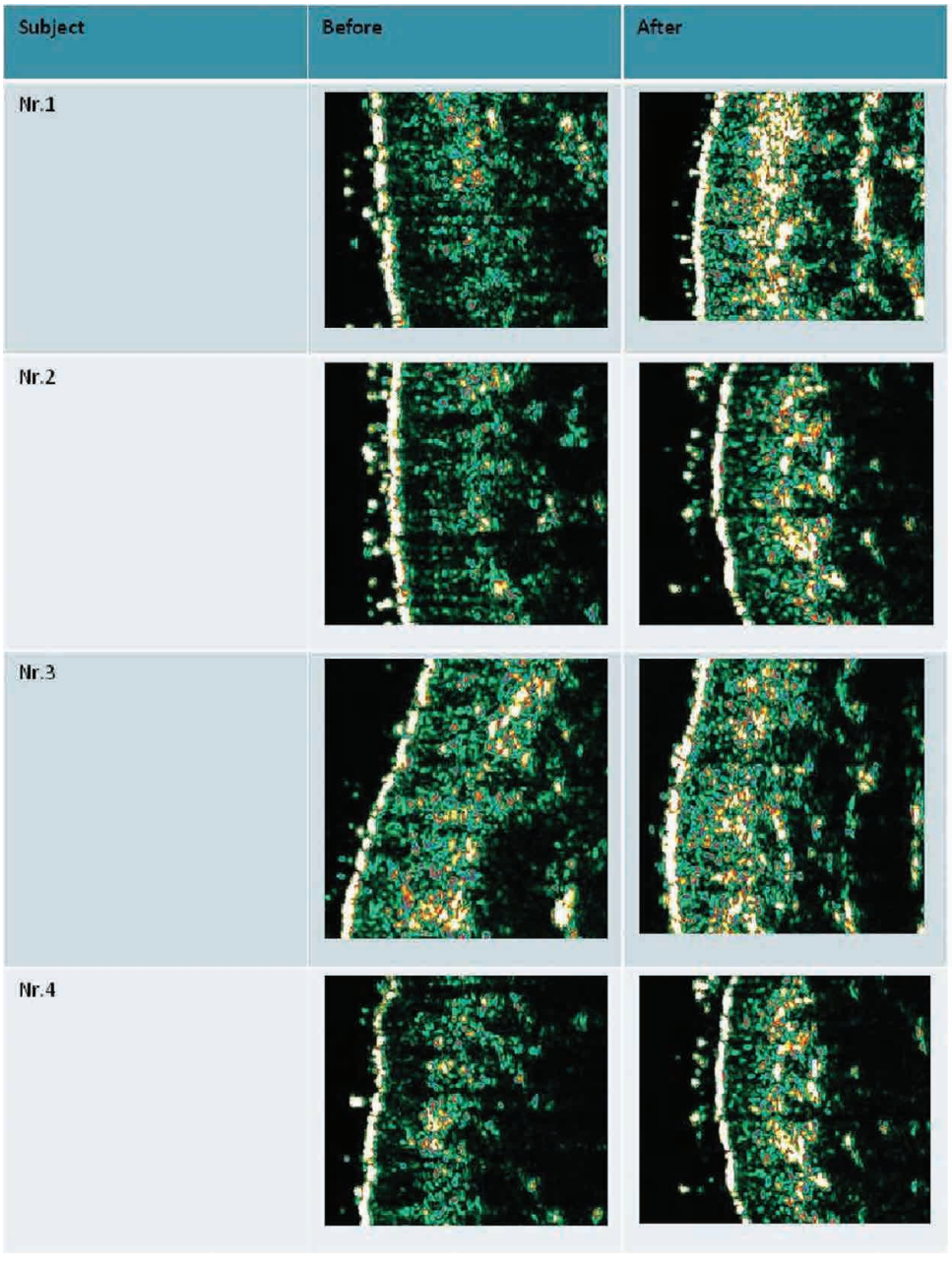
The statistical analysis was performed using ANOVA and T Student test. The obtained data were analyzed by calculating the mean and standard deviation for all quantitative variables. The difference of means before and after treatment was tested using T test for paired samples. A p-value <0.05 was considered significant.

RESULTS AND DISCUSSION

The study group included 50 female subjects, aged 40-75. The first group (30 subjects), using the INTERACTIVE P63 product was divided into 3 age categories: 10 subjects belonged to the 40-50 age interval, 10 subjects were aged 50-60 and 10 subjects were over the age of 60. Sixteen subjects belonged to phototype class II (53%) and fourteen to phototype class III (47%) The placebo group (20 subjects) was composed of 7 subjects aged 40-50, 6 subjects 50-60 and 7 subjects over 60 years, 12 of these being included in phototype class II, the rest of 8 were included in phototype class III. The therapy was well tolerated by all subjects involved in the study, no adverse effects (erithema, pruritus, ocular disturbance) being reported after 30 minutes of contact.
After therapy, in the Interactive P63 group an increase of the mean thickness of the epi-

dermis (0.117 ± 0.021 mm vs 0.135 ± 0.023 mm, p=0.0024), and dermis (1.537 ± 0.23 mm vs 1.710 ± 0.244 mm, p=0.0076) was observed. At dermal level, the number of low echogenity pixels decreased in a significant manner after topical therapy (18484.4 ± 4666.5 mm vs. 14138.97 ± 3779.5 mm, p=0.00021) whereas the number of the medium (3118.63 ± 974.4 mm vs. 4608.93 ± 1105.6 mm, p=0.001) and high echogenic pixels (379.6 ± 274.17 mm vs. 1004.9 ± 458.78 mm, p<0.0001) increased significantly (Fig. 1).

FIGURE 1. Ultrasonographic evaluation of the zygomatic area, before and after topical P63 therapy



The LEPs/LEPi ratio increased significantly after therapy (1.149 ± 0.251 mm vs 1.574 ± 0.317 mm) especially due to the significant decrease of the number of low echogenic pixels (LEPi) in the lower dermis (8740.4±2711.01 vs 4921±2373.6, p=0.016).

The general variation pattern of the quantifiable ultrasonographic parameters after therapy is shown in Table 1. The topical therapy using Interactive P63 complex induces a significant growth of the dermal and epidermal thickness. Several studies have shown that various cutaneous antiaging therapies induce a significant increase of the skin thickness [15], counteracting the cutaneous atrophy associated with aging and post-menopausal period.

Interactive P63 complex contains active principles that act on the main mechanisms of the cutaneous aging process: the genetic factor, cumulative sun exposure, hormonal status, conferring in terms of imagistic, a certain display of the pixels at cutaneous level. The increase of the epidermal/dermal thickness represents the morphological expression of the changes induced by Interactive P63 complex at fibroblastic and extracellular matrix level. The activation of the fibroblasts as well as the inductive effect upon stem cells, associated with the inhibition of the mechanisms responsible for the destruction of the fibrillary structures, induce an increase of the dermal density.

Thus, we noticed a general, significant decrease of the mean number of low echogenic pixels (LEP) at dermal level, more pronounced in the lower dermis (LEPi) than the upper one (LEPs), suggesting important structural, biochemical, mollecular and architectural changes that vary according to certain particular properties of the upper

TABLE 1.

Variation of the imagistic parameters in the P63 study group, before and after 12 weeks of therapy			
Parameters	Before treatment	MTL and LATS	*P*
Thickness of epidermis (mm)	0.117 ± 0.021	0.135 ± 0.022	0.0024
Thickness of dermis (mm)	1.537 ± 0.23	1.719 ± 0.244	0.0076
LEP Table	18484.4 ± 4666.5	14138.97 ± 3778.5	0.00021
MEP	3118.63 ± 974.4	4608.93 ± 1105.61	0.001
HEP	379.6 ± 274.17	1004.9 ± 458.78	<0.0001
LEPs	9743.9 ± 2515.75	9217.96 ± 2772.79	0.444
LEPi	8740.43 ± 2711	4921 ± 2373.6	0.016
LEPs/LEPi	1.149 ± 0.25	1.574 ± 0.31	<0.0001

and lower dermis. Parallel to the decrease of LEP after therapy, a statistically significant increase of the mean number of medium (MEP) and high ecogenic pixels (HEP) was noticed, quantifying the increase of dermal density and thus, collagen neosynthesis. If we consider the variation of the ultrasonographic parameters after topical therapy according to the phototype class of the subjects, it can be noticed that after therapy, there is a significant increase of the LEPs/LEPi ratio in the subjects belonging to phototype class III, not II (Fig. 2).

FIGURE 2. Variation of the LEPs/LEPi ratio before and after topical therapy, according to phototype

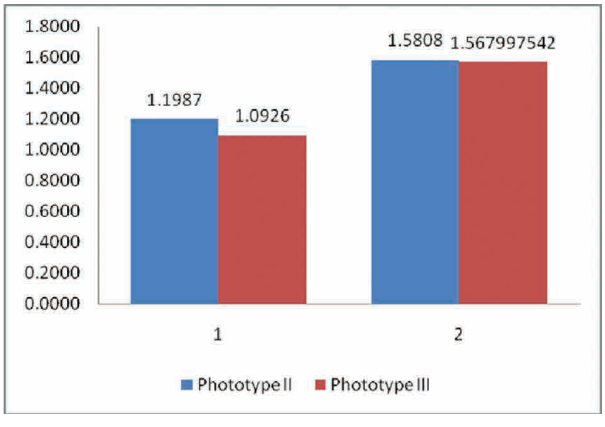
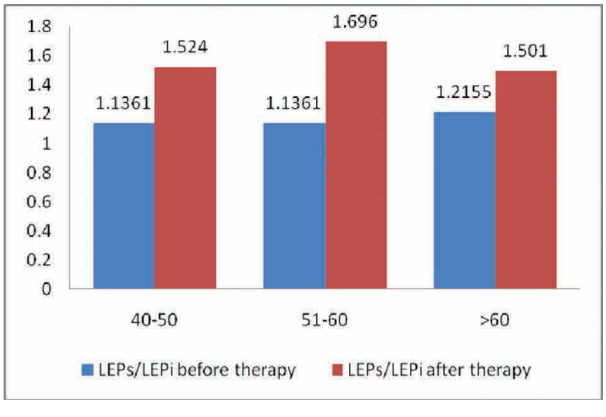


FIGURE 3. The variation of the mean of the LEPs/LEPi ratio on different age interval before and after therapy

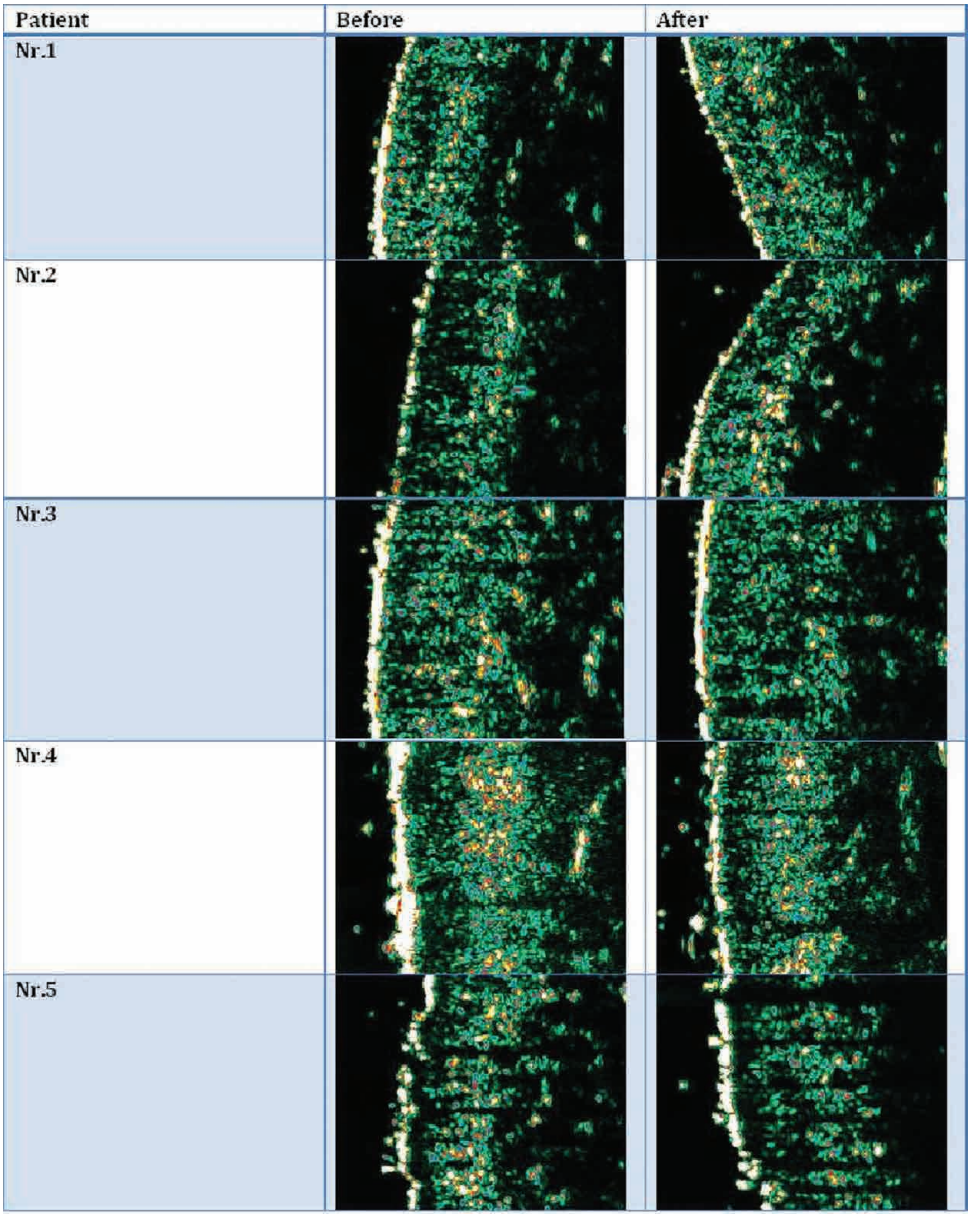


The LEPs/ LEPi ratio, an essential imagistic marker that quantifies the dermal density, increased in a significant manner, due to the important decrease of the number of low echogenity pixels from the lower dermis (LEPi). Considering the LEPs/LEPi ratio on the three age categories: 40-50, 51-60 and over 60 years, a significant increase was noticed in all three age intervals (Fig.3) [13].

Considering the LEPs/LEPi, parameter we noticed a significant increase of the ratio in all subjects part of the study, especially in the 51-60 age interval (40-50 age interval: p=0.01, 51-60 age interval: p=0.0009, >60 age interval: p=0.02). The fact that the most significant increase of the dermal density occurred in the 51-60 age interval, may be correlated with the post-menopausal status as well as with the estrogen-like activity of INTERACTIVE P63 complex. During menopause, due to a decrease of estrogen and cutaneous estrogen receptors, a progressive decrease of dermal collagen occurs, with a loss of collagen content of 1-2% every menopausal year [16,17,18]. Several studies certify the fact that topical estrogen therapy in menopausal women induces an increase of almost 5.1% of dermal collagen [19]. It is also a fact that the efficacy of hormone therapy is dependent on the basal collagen status at the beginning of the therapy. The initiation of a precautious therapy in menopause has a prophylactic role, while a delayed therapy has a therapeutic purpose.

In the placebo group, we noticed a slight increase of the dermis (1.496 ± 0.14 mm vs.

FIGURE 4. Ultrasonographic aspect of the zygomatic area in the placebo group



1.571 ± 0.174, p=0.07) and of the dermal low echogenic pixels (13812 ± 2070 vs. 14787 ±2218, p=0.08) due to an optimal hydration of the skin and a discreet tendency of the high echogenity pixels to decrease (379,8 ± 137.18 vs 316.3 ±163.43, p=0.11). The LEPs/LEPi ratio showed no particular display according to the age or phototype of the subjects (Fig.4).

G factor P63 is a biomimetic peptide complex derived from growth factors. Recent studies have shown P63's capacity for the activation of the stem cells [20,21,22]. In addi-

tion, other functions have been described: stimulation of fibers synthesis by fibroblasts and glycozaminoglycans, counteract of protein glycation, inhibition of MMPs, inhibition of IL-1, inhibition of oxidative iron release from ferritin [23].

INTERACTIVE P63 complex acts at cellular level, interfering the cutaneous estrogenic receptors (α and β) that, even though with structural and functional similarities, have different expression conditions and act differently in menopause, explaining the changes regarding dermal density on age categories.

INTERACTIVE P63 has a complex action, interacts with the mechanisms of anabolic and catabolic, interfering both the intrinsic and extrinsic mechanisms of cutaneous aging: it increases protein synthesis, activates stem cells, increases fibroblasts activity, active restructuring of the DEJ (G factor P63). It has a strong anti-glycation action, inhibits the metalloproteinases and has an estrogen-like effect (retinal and G factor P63), decreases reactive species of oxygen and, amplifies the cutaneous energetic source, renewing epidermis cell turnover.

Regarding the phototype of the subjects, a certain particular reactivity is to be mentioned: a more significant growth of the dermal density in subjects belonging to phototype class III, compared to phototype class II. The cutaneous phototype is genetically determined, while the cutaneous reactivity is multifactorial. INTERACTIVE P63 complex interacts at the same time genetical, metabolical, hormonal, oxidative mechanisms, that are involved in the cutaneous senescence process.

Regarding the placebo group of study, we noticed a slight increase of the dermal thickness and of the dermal low echogenic pixels, due to the optimal hydration of the skin and a tendency of the high echogenity pixels to decrease. The LEPs/LEPi ratio showed no significant variation neither with the age or the phototype of the subjects.

High-frequency ultrasound is a noninvasive method that detects the changes of the cutaneous connective tissue as well as of collagen content in a particular manner, being yet comparable to the assessment of total collagen by using the colorimetric method, the determination of the “de novo” synthesis of collagen by measuring procollagen propeptides in blister fluid, and immunohistochemistry [24].

CONCLUSIONS

1. High frequency ultrasound is a noninvasive research method that allows the assessment of both physiological and pathological aspects of the skin, offering new perspectives for the study of the efficacy of topical products.

2. Interactive P 63 product acts on specific sensitive receptors, may interfere with estrogen-like receptors, activating the fibroblast “key cell” and increases the synthesis of collagen. It has real and important anti-

ageing properties on large age intervals, since it interferes concomitantly the oxidative, genetic, immunologic, hormonal and metabolic mechanisms. It is highly efficient especially in the 51-60 age interval.

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