



**PROFESSOR NATALIA LINKOVA**

**PEPTIDE BIOREGULATORS  
APPLICATION IN  
COSMETOLOGY: CLINICAL AND  
MOLECULAR ASPECTS**

Saint Petersburg, 2023



Saint Petersburg Institute of Bioregulation and Gerontology

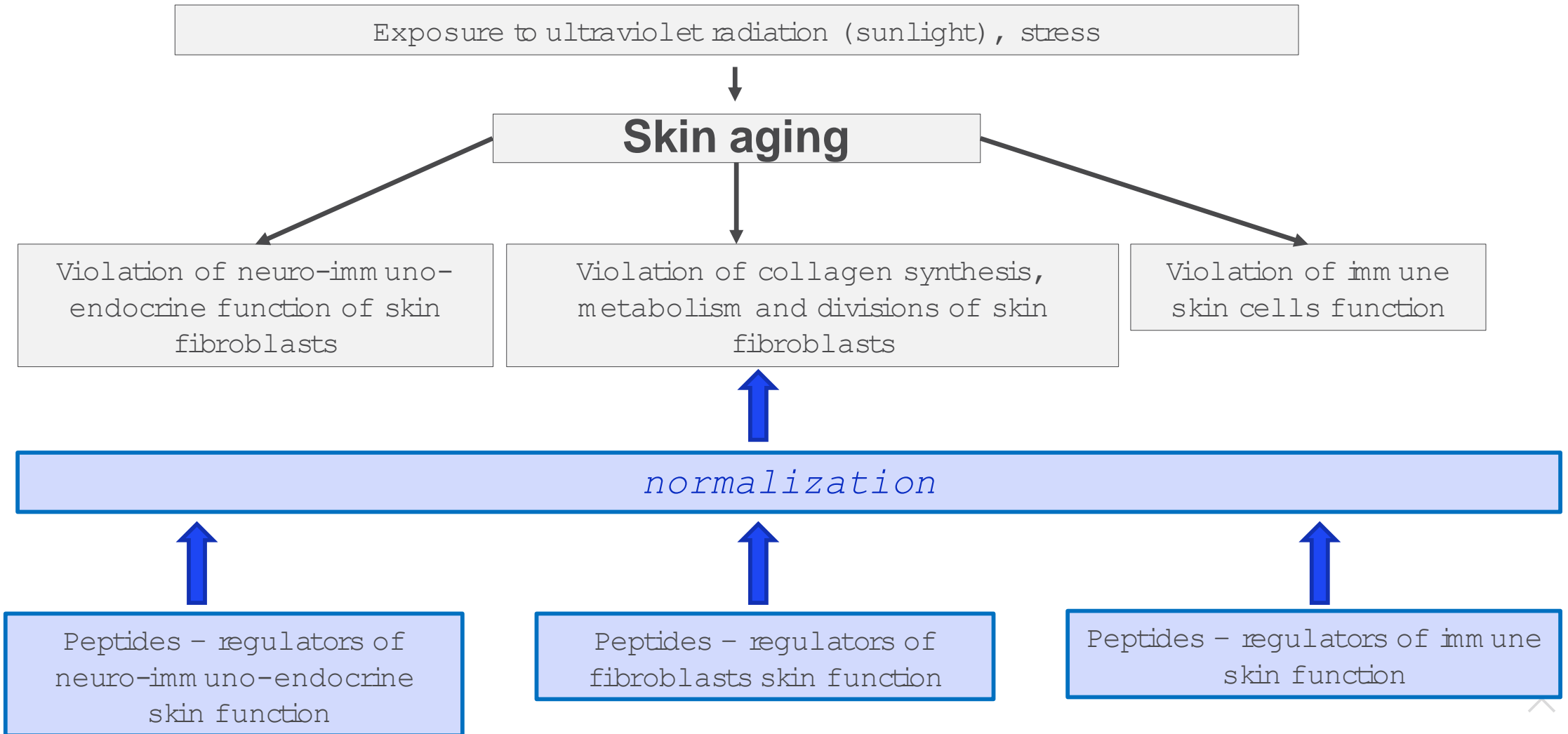
# PEPTIDE BIOREGULATORS APPLICATION IN COSMETOLOGY: CLINICAL AND MOLECULAR ASPECTS

PROFESSOR NATALIA LINKOVA





# The role of peptide regulation in slowing down skin aging |





# Endoluten® is a polypeptide complex from pineal gland, the regulator of the neuro-immuno-endocrine system and a geroprotector.



It was detected in the polypeptide complex from pineal gland by mass spectrometry and high-performance liquid chromatography:

- free amino acids (3.3%),
- dipeptides (23.2%),
- tripeptides (50.7%),
- tetrapeptides (22.1%),
- pentapeptides (0.7%).

The AEDG peptide was identified among tetrapeptides in pineal gland polypeptide complex by the method of selective monitoring of reactions (SRM).

**The biological activity inherent in pineal gland polypeptide complex is due to the effect of the AEDG peptide included in its composition.**

Khavinson V.K., Kopylov A.T., Vaskovsky B.V., Ryzhak G.A., Lin'kova N.S. Identification of Peptide AEDG in the Polypeptide Complex of the Pineal Gland. Bull Exp Biol Med. 2017. Vol. 164. N 1. P. 41-43.







# Sigumir® is a polypeptide complex from cartilage and bone tissues, a regulator of connective tissue cells (cartilage and skin fibroblasts) functions



Sigumir® was analyzed at the Institute of Toxicology of the FMBA of Russia for the content of the AED peptide (**Cartalax®**) by matrix-activated laser desorption/ionization (MALDI) and ultra-efficient liquid chromatomass spectrometry (UEFC-MS).

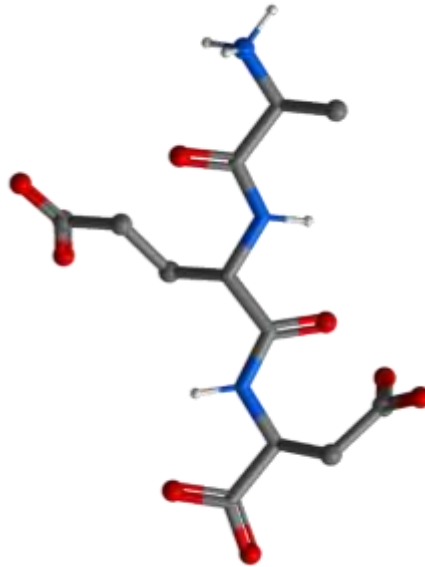
**The AED peptide was found in the composition of Sigumir® in an amount of 0.2 mg/g and is its active component.**

Zhurkovich I.K., Kovrov N.G. Ryzhak G.A., Mironova E.S., Khavinson V.Kh. Identification of Short Peptides as Part of Polypeptide Complexes Isolated from Animal Organs. Biology Bulletin Reviews. 2020. Vol. 140, N 2. P. 140-148 (In Russian).





# The AED peptide (Cartalax®) – an active component of Sigumir®



Chondroprotective effects of Cartalax® (efficacy in osteoarthritis, osteoporosis, etc.) are due to the activation of proliferation (cell division) of cartilage fibroblasts.

Fibroblasts have similar properties in all organs and tissues. Thus, Cartalax® has a stimulating effect on the metabolism of skin fibroblasts.





# Vladonix® is a polypeptide complex from thymus, a regulator of the functions of immune system



Vladonix® was analyzed at the Institute of Toxicology of the FMBA of Russia for the content of the EDP (**Crystagen®**) peptide in it by matrix-activated laser desorption/ionization (MALDI) and ultra-efficient liquid chromatomass spectrometry (UEFC-MS).

**The EDP peptide was found in Vladonix® in an amount of 0.04 mg/g and is its active component.**

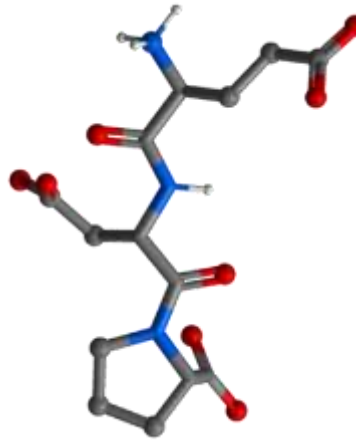
Khavinson V.Kh., Zhurkovich I.K., Ryzhak G.A., Mironova E.S., Kovrov N.G. Identification of short peptides: optimization of targeted therapeutic properties of a thymus drug. *Molecular Medicine*. 2021. Vol. 19, N 3. P. 32-37 (In Russian).







# The EDP peptide (Crystagen®) – an active component of Vladonix®



The EDP peptide

- stimulates differentiation and activation of B-cells, macrophages,
- proliferation of immune cells during aging,
- reduces apoptosis of stem and immune cells during aging.

At the systemic level, these effects of the EDP peptide are expressed in regulation of immune system functions, antioxidant action and stress-protective effect.

The skin performs a protective (barrier) function and contains a large number of immune cells. Vladonix® and its constituent the EDP peptide (Crystagen) can contribute to the normalization of the functions immune cells in the skin.

Khavinson V.K., Popovich I.G., Linkova N.S., Mironova E.S., Ilina A.R. Peptide Regulation of Gene Expression: A Systematic Review. *Molecules*. 2021.Vol. 26. Issue 22. 7053. 22 p.





# Peptide bioregulators application in cosmetology: study design

The study was conducted on the basis of the anti-aging medicine clinic ArtMedia (St. Petersburg). There were 15 middle-aged and elderly women in each group. To assess the effectiveness of peptide therapy we use the questionnaire "Dermatological Index of Quality of Life". Also it was used an assessment of the thickness (echogenicity) of the dermis and epidermis of the skin by ultrasound scans method.

- ❖ **The 1<sup>st</sup> group (control)** – a saline solution application by electrophoresis on the facial skin area, once a week during 8 weeks.
- ❖ **The 2<sup>nd</sup> group** – peptides **Cartalax®** and **Crystagen®** (sublingual biologically active food supplements) application by electrophoresis on the face area, once a week during 8 weeks.
- ❖ **The 3<sup>rd</sup> group** – peptides **Cartalax®** and **Crystagen®** (sublingual biologically active food supplements) application by electrophoresis on the face area, once a week during 8 weeks. Women from this group received oral polypeptide complexes (biologically active food supplements) **Sigumir®** and **Endoluten®** simultaneously with electrophoresis.

Fridman N., Bojko L., Trofimova S. Peptide bioregulators applying perspectives for skin structure restoration in middle aged women. Vrach. 2020. Vol. 31, N 9. P. 63-67 (In Russian).

Fridman N.V., Linkova N.S., Bojko L.V., Kacheli M.A. The influence of peptide bioregulators on the structural and functional specific of face skin in elderly women. Molekulyarnaya meditsina. 2021. Vol. 19, N 4. P. 42-46 (in Russian).



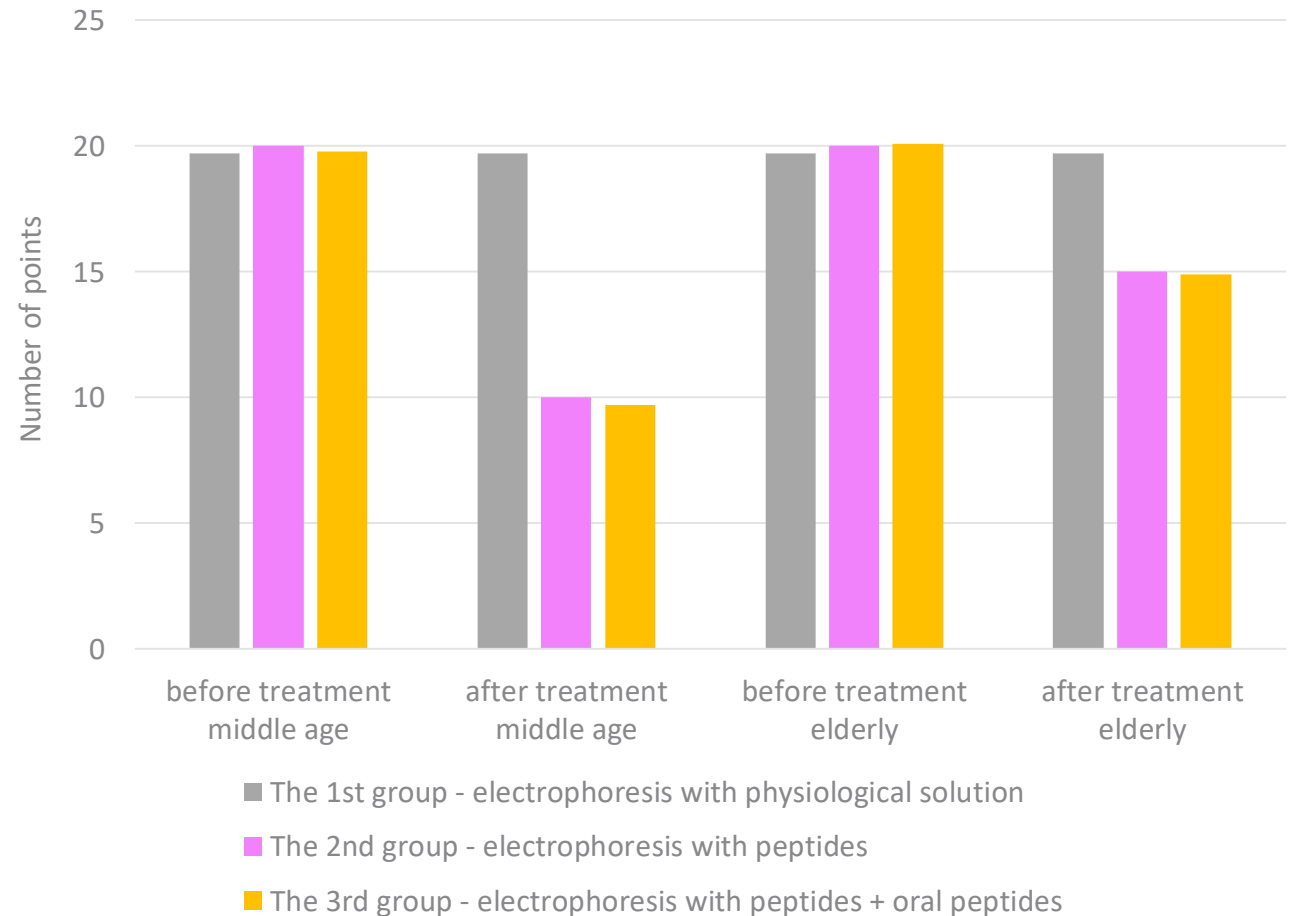


# The influence of peptides on the skin condition of different ages women according to the questionnaire

The evaluation criteria were the sensations of dryness or tightness of the skin, the color from normal to dull, the surface relief of the skin, smoothness, the presence of enlarged capillaries, the severity of wrinkles, age spots and the general feeling of the appearance - freshness, radiance or tired appearance.

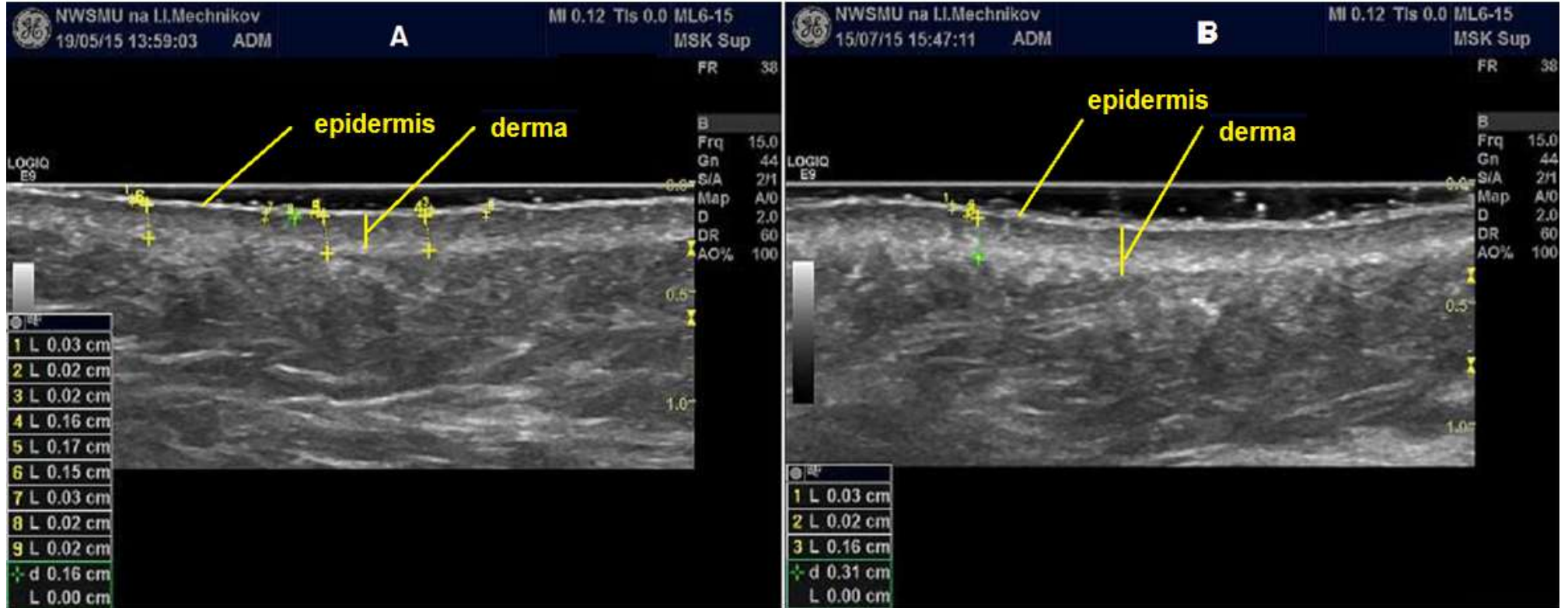
Interpretation of results: 0 points - absence of age-related changes in all signs, 40 points - the most pronounced age-related changes in all signs. The questionnaire was filled out by women and cosmetologist.

**The maximum positive effect was observed in middle-aged women in both investigated groups.**





# The influence of peptides on the thickness (echogenicity) of the dermis in a middle-aged woman



Before treatment

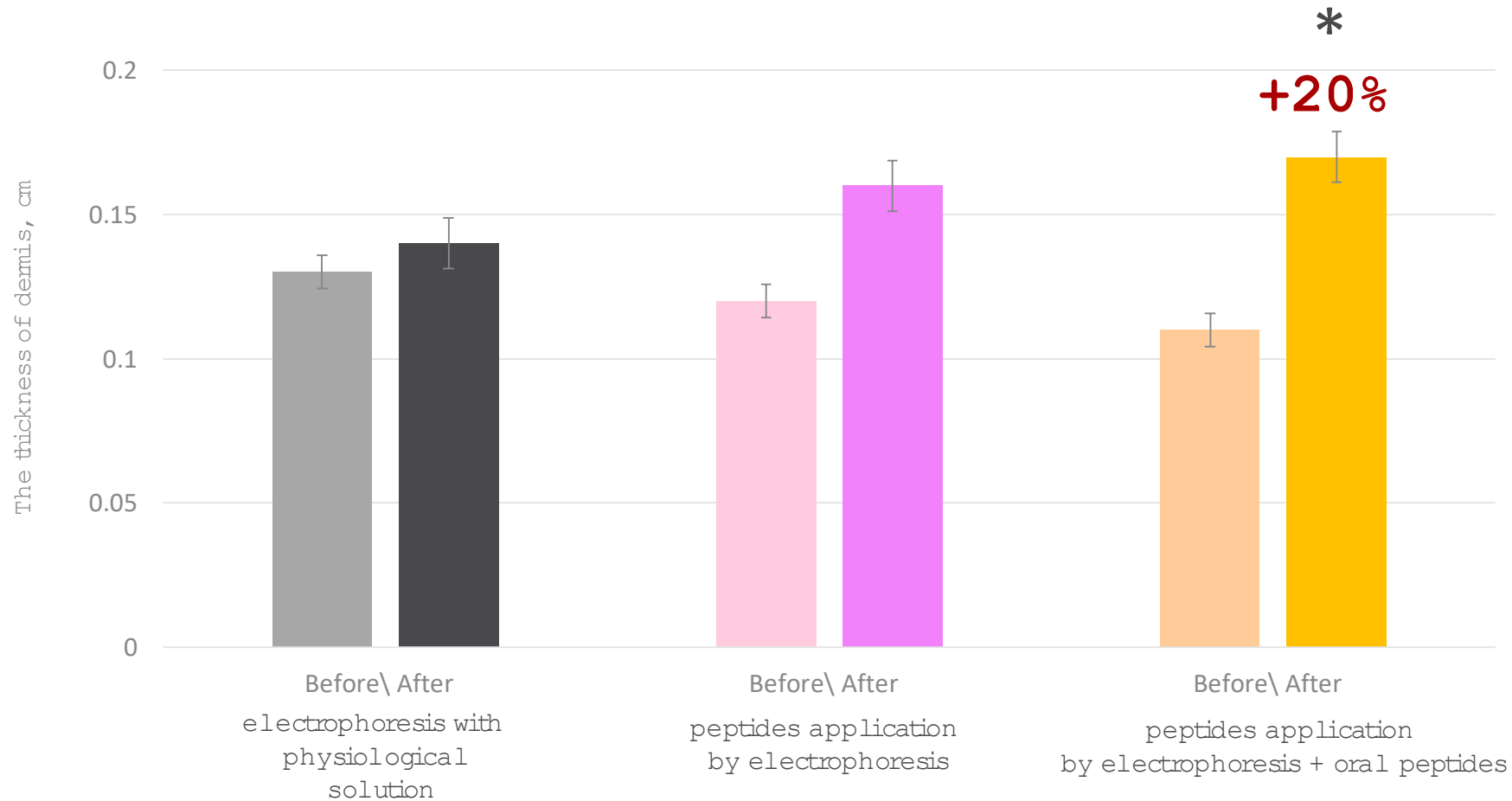
After treatment, the 3<sup>rd</sup> group

L1-L3 - epidermis, L4-L6 - derma. Figure B clearly shows a brighter and thicker white stripe in the dermis layer. We can see its thickening and increased collagen synthesis after the use of peptides.





# The influence of peptides on the thickness (echogenicity) of dermis in a middle-aged woman



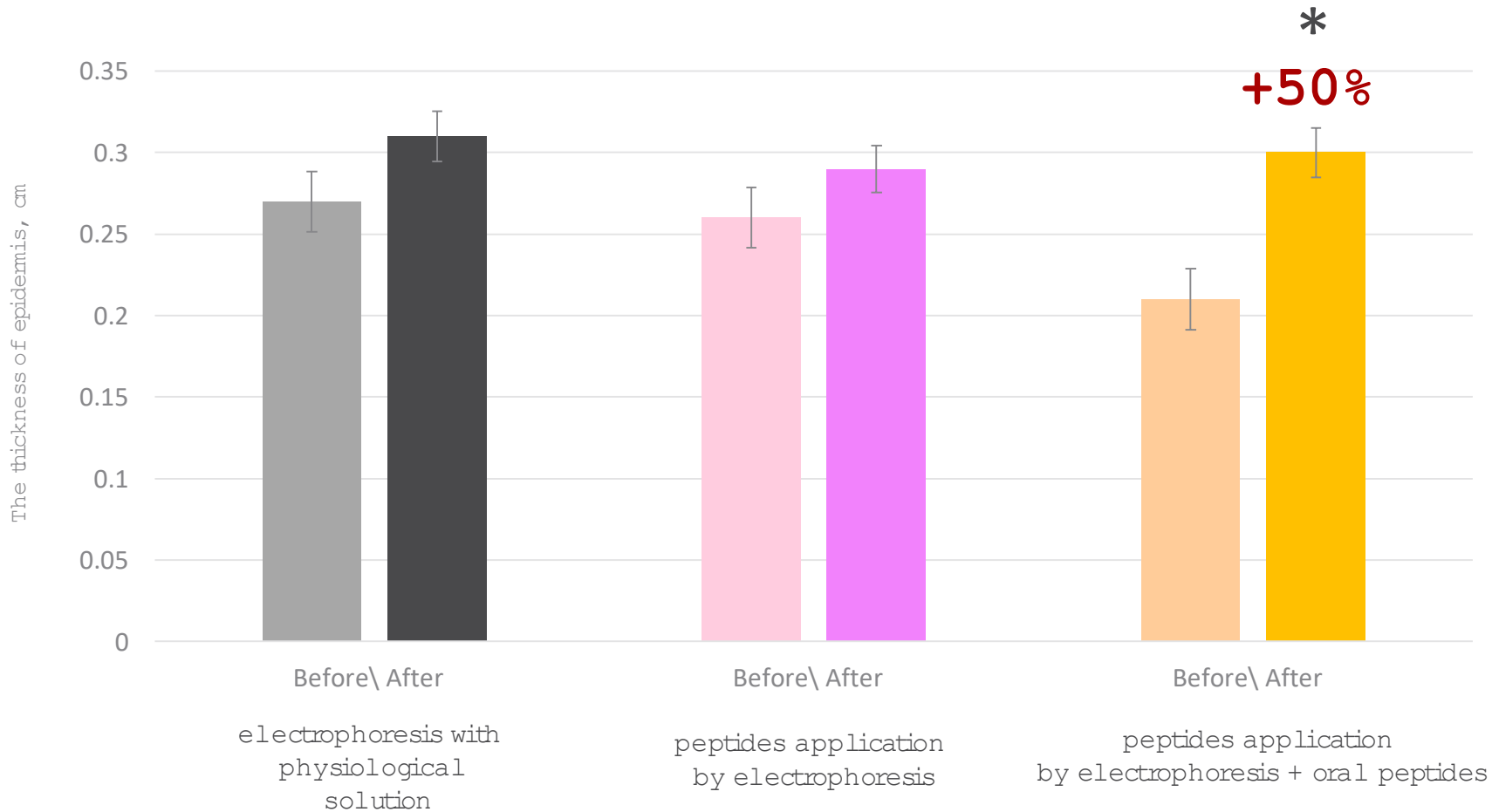
Application electrophoresis with Cartalax® and Crystagen® in combination with Endoluten® and Sigumir® in capsules contributed to a significant increase on 20% the thickness of dermis in middle aged women.

\* -  $p < 0.05$  - compared with the indicator before the study in the corresponding group





# The influence of peptides on the thickness (echogenicity) of epidermis in a middle-aged woman



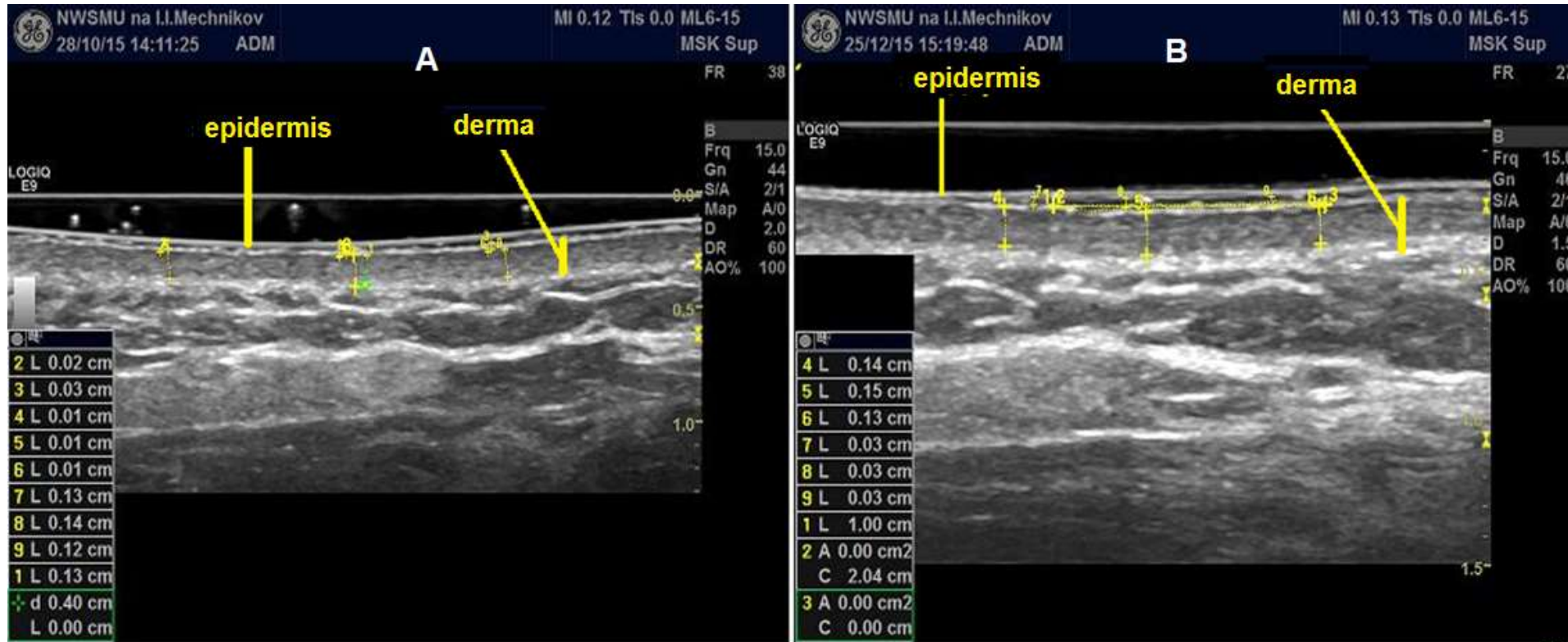
Application electrophoresis with Cartalax® and Crystagen® in combination with Endoluten® and Sigumir® in capsules contributed to a increase on 50% the thickness of epidermis in middle aged women.

\* -  $p < 0.05$  - compared with the indicator before the study in the corresponding group





# The influence of peptides on the thickness (echogenicity) of the dermis in an elderly woman



Before treatment

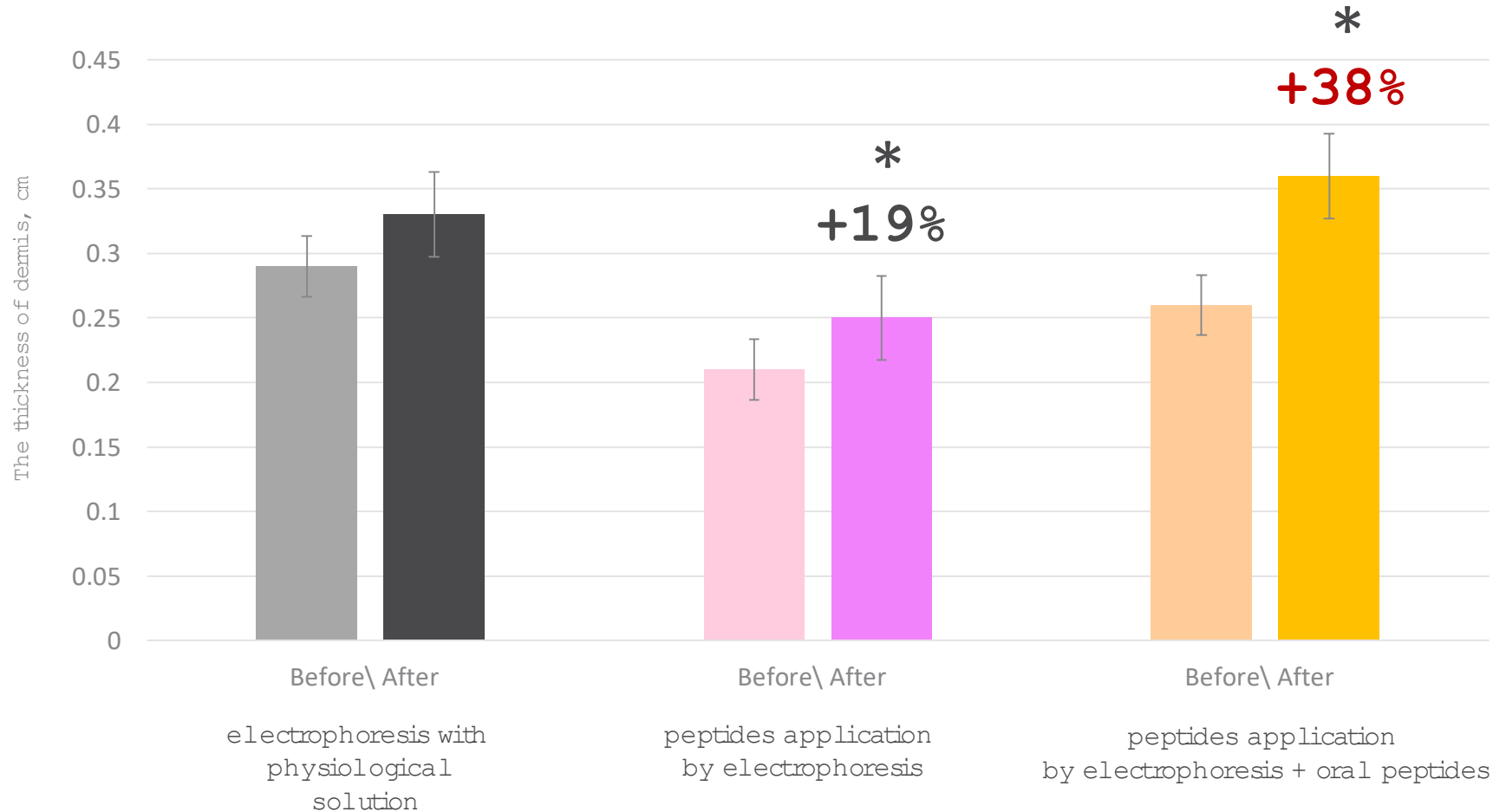
After treatment, the 3<sup>rd</sup> group

L1-L3 - epidermis, L4-L6 - derma. Figure B clearly shows a brighter and thicker white stripe in the dermis layer. We can see its thickening and increased collagen synthesis after the use of peptides.





# The influence of peptides on the thickness (echogenicity) of dermis in a elderly woman



Single application electrophoresis with Cartalax® and Crystagen® and its use in combination with Endoluten® and Sigumir® in capsules increase on 19% and 38% the thickness of dermis in elderly women.

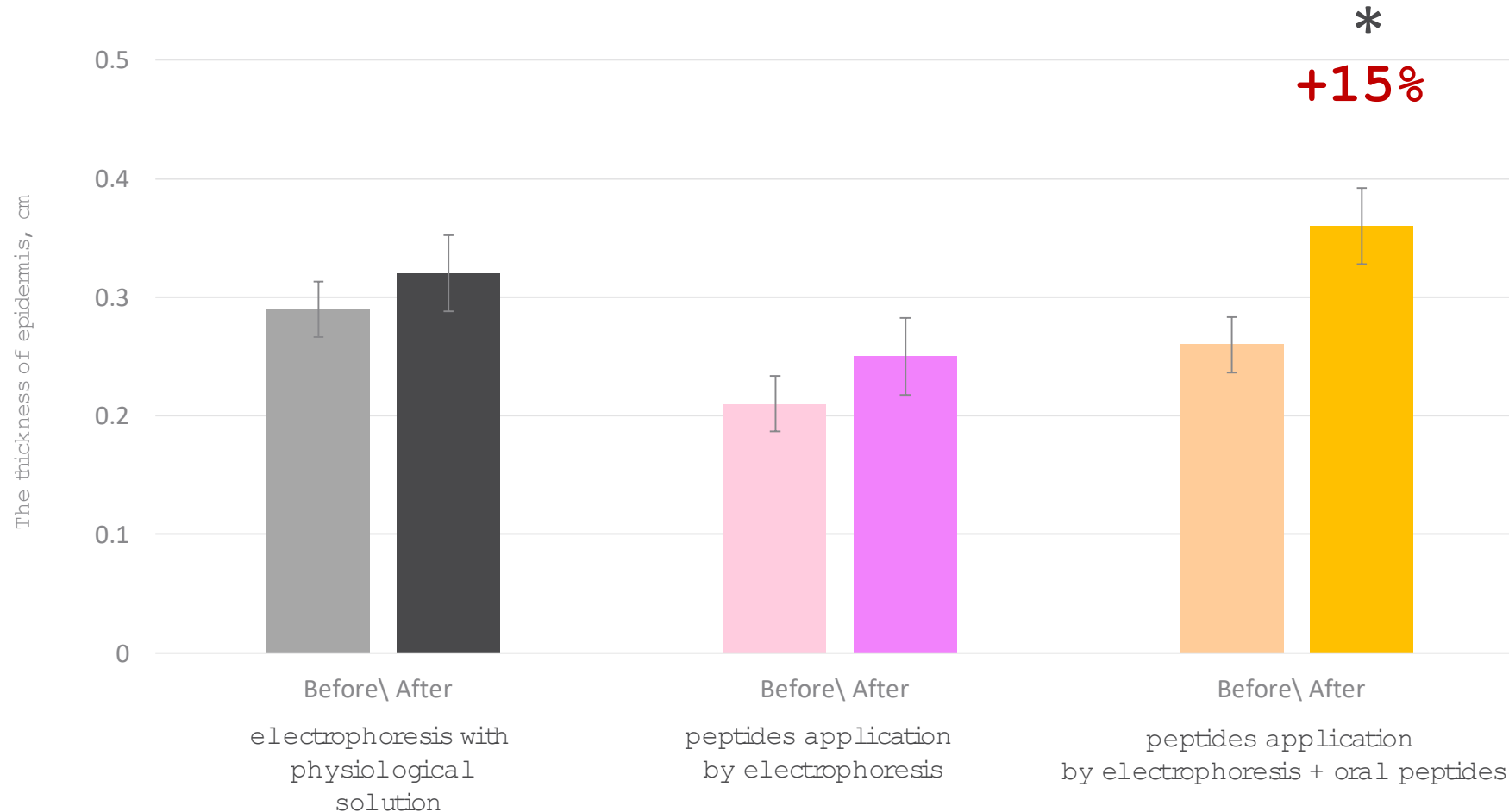
\* -  $p < 0.05$  - compared with the indicator before the study in the corresponding group







# The influence of peptides on the thickness (echogenicity) of epidermis in a elderly woman



Application electrophoresis with Cartalax® and Crystagen® in combination with Endoluten® and Sigumir® in capsules increase on 15% the thickness of epidermis in elderly women.

\* -  $p < 0.05$  - compared with the indicator before the study in the corresponding group





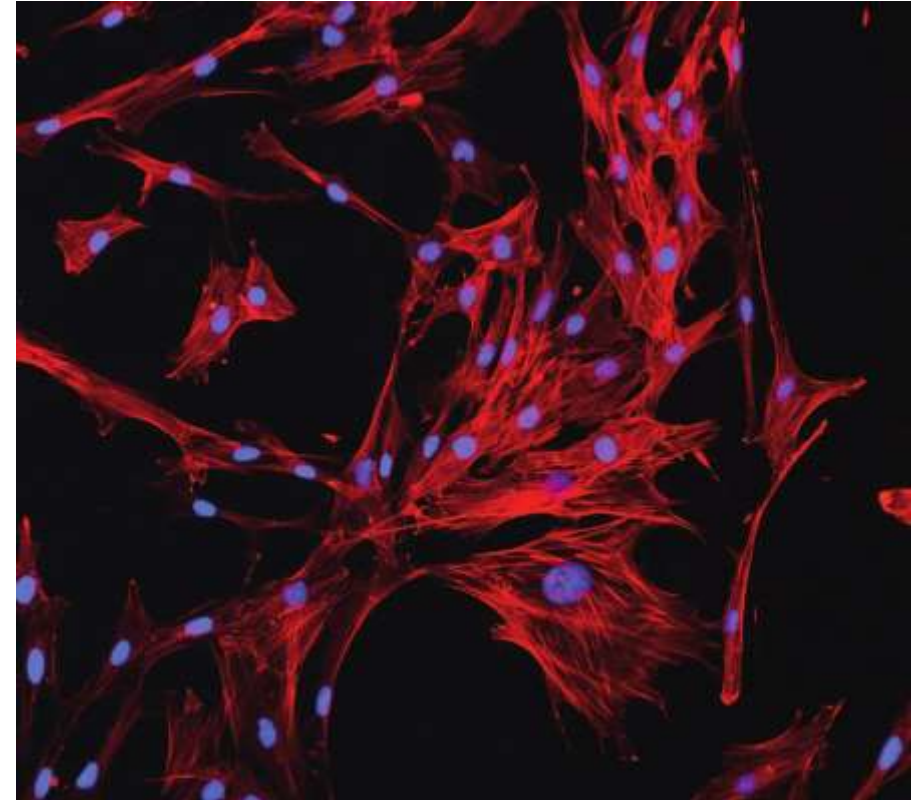
# AEDG peptide (an active component of Endoluten®) regulates the expression of genes encoding enzymes of the antioxidant system<sup>1</sup> in human skin fibroblasts in the model of accelerated aging induced by UR radiation

The study was used **skin fibroblasts of 45 years old woman** (DF2 cell line) obtained from the Collection of cell cultures of the Institute of Cytology of the Russian Academy of Sciences (St. Petersburg).

Investigated skin fibroblasts cultures:

- 1 – control (intact cells)
- 2 – control + GGGG control peptide, 400 ng/ml
- 3 – control + AEDG peptide, 400 ng/ml
- 4 – exposure to ultraviolet radiation (UR)
- 5 – exposure to UR + GGGG control peptide, 400 ng/ml
- 6 – exposure to UR + AEDG peptide, 400 ng/ml

UV is the part of the spectrum of sunlight that is the cause of accelerated skin aging.

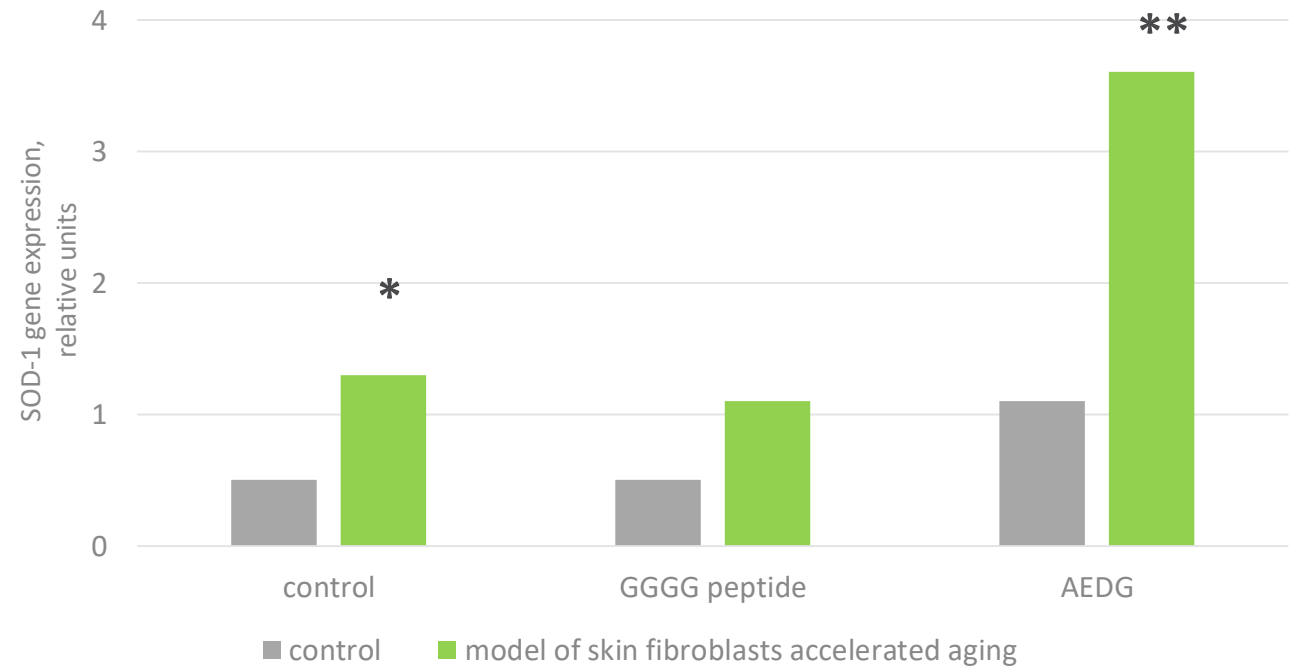




# The AEDG peptide activates *SOD1* gene expression in the model of skin fibroblasts accelerated aging

**Superoxide dismutase 1 (SOD1)** is an antioxidant enzyme that protects the intracellular space from superoxide anions by catalyzing their conversion into molecular oxygen and hydrogen peroxide. *SOD1* gene expression decreases in human skin fibroblasts during aging.

- An increase of *SOD1* gene expression in human skin fibroblasts is one of the mechanisms of response to the effects of oxidative stress (UR). In mice with a deficiency in the synthesis of SOD1 enzyme, there is a decrease in the thickness of the dermis, a violation of the migration and proliferation of fibroblasts and accelerated skin aging.
- **The AEDG peptide increased *SOD1* gene expression by 3.5 times.** It is shown the protecting peptide activity on skin fibroblasts from the action of free radicals arising under the action of UR.



\* -  $p < 0.05$  in comparison with the control

\*\* -  $p < 0.05$  in comparison with the control in the model of skin fibroblasts accelerated aging

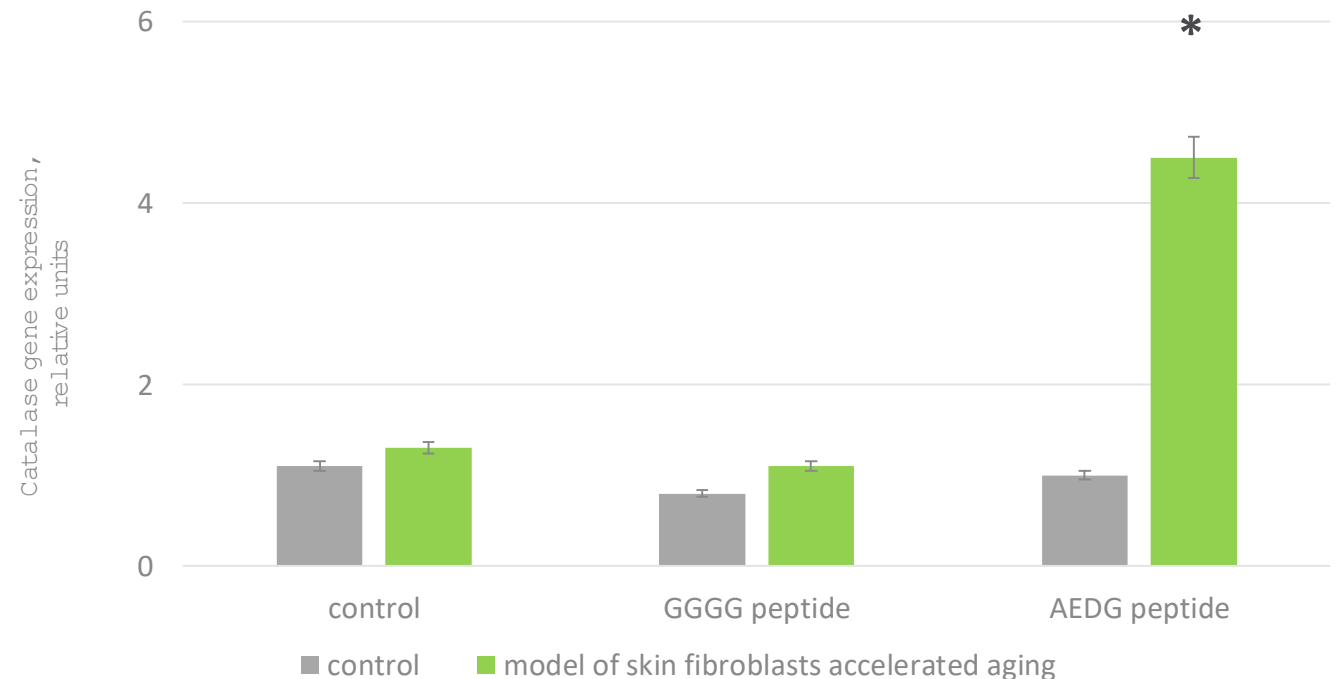




# The AEDG peptide activates *Catalase* gene expression In the model of skin fibroblasts accelerated aging

**Catalase** is an enzyme of the oxidoreductase class, which is part of the antioxidant system of the cell. It performs the function of antiperoxide protection. This enzyme catalyzes the decomposition of hydrogen peroxide formed during biological oxidation into water and molecular oxygen.

- The increased of catalase and SOD1 synthesis associated with aging is one of the protective mechanisms that prevent DNA damage in skin fibroblasts under the action of oxidative stress.
- Compensatory increase of catalase synthesis in fibroblasts was revealed with accelerated skin aging under the influence of UR. **The AEDG peptide stimulates this effect by activating the antioxidant system.**



\* -  $p < 0.05$  in comparison with the control in the model of skin fibroblasts accelerated aging



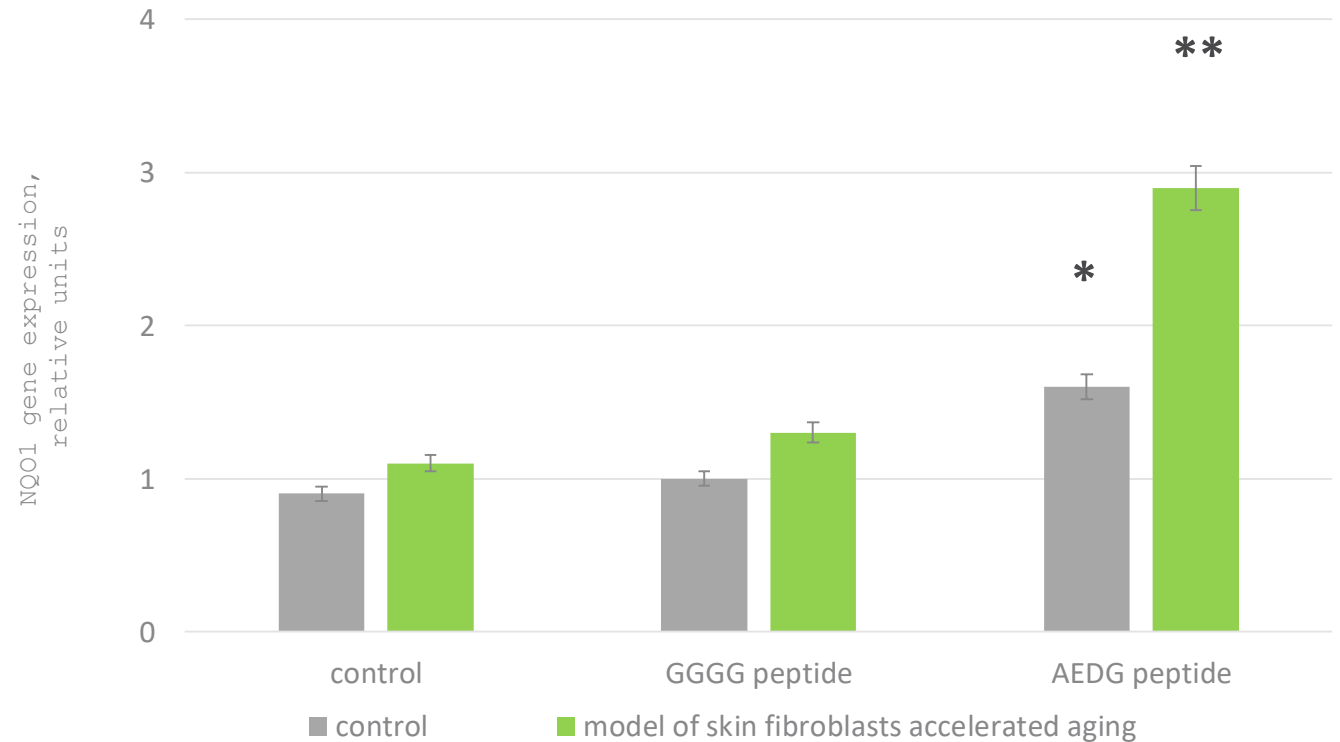


# The AEDG peptide activates *NQO1* gene expression In the model of skin fibroblasts accelerated aging

**NQO1** (NADP (H) – quinone oxidoreductase 1) enzyme is localized in the cytosol (inside the cell), catalyzes the two-electron reduction of quinone compounds and prevents the formation of semiquinone free radicals and active oxygen molecules, protecting cell from oxidative stress.

*NQO1* gene overexpression is observed in various cell types when exposed to oxidants and heavy metals.

**The AEDG peptide stimulates *NQO1* gene expression of the antioxidant system.**



\* -  $p < 0.05$  in comparison with the control

\*\* -  $p < 0.05$  in comparison with the control in the model of skin fibroblasts accelerated aging





# AED (Cartalax®) and AEDG peptides regulate protein synthesis in skin fibroblasts during aging in cell culture

| Peptide | The influence on protein synthesis | Effect   |
|---------|------------------------------------|--|
| AED     | SIRT6 ↑ in 11,5 times              | Geroprotective<br>Antioxidant<br>Collagen synthesis stimulation                                  |
|         | Collagen 1 ↑ in 2,7 times          |  |
|         | SIRT1 ↑ in 2 times                 |  |
| AEDG    | Ki67 ↑ in 1,4 times                | Antiapoptotic<br>Proliferotropic<br>Geroprotective<br>Extracellular matrix remodeling regulation |
|         | MMP9 ↓ in 1,3 times                |  |
|         | CD98hc ↑ in 1,5 times              |  |
|         | p53 ↓ in 3 times                   |  |
|         | p16 ↓ in 1,7 times                 |  |
|         | Caspase3 ↓ in 1,4 times            |  |

Fridman N.V., Linkova N.S., Kozhevnikova E.O., Gutop E.O., Khavinson V.K. Comparison of the Effects of KE and AED Peptides on Functional Activity of Human Skin Fibroblasts during Their Replicative Aging. Bull. Exp. Biol. Med. 2020. Vol. 170, N 1. P. 154-157.

Lin'kova N.S., Drobintseva A.O., Orlova O.A., Kuznetsova E.P., Polyakova V.O., Kvetnoy I.M., Khavinson V.Kh. Peptide Regulation of Skin Fibroblast Functions during Their Aging In Vitro. Bull. Exp. Biol. Med. 2016. Vol. 161, N 1. P. 175-178.



# Molecules, which regulates skin homeostasis – targets for AED (Cartalax®) and AEDG peptides

**Collagen type 1** is one of the main structural proteins synthesized by skin fibroblasts, which indicates the high functional activity of these cells. Its synthesis in fibroblasts decreases during skin aging. Sirtuin-1 (Sirt1) is a multifunctional protein that participates in stress response, cellular metabolism and aging through deacetylation of various substrates, including histones and transcription factors.

**Sirtuin-1** regulates energy homeostasis, cell cycle, apoptosis, inflammatory reactions and level of reactive oxygen species in cell. **Sirtuin-6** (Sirt6), by weakening the action of the **cytokine NFkB**, inhibits cellular aging. Sirtuin-6 contributes to an increase in life expectancy. It participates in DNA repair, activates cell metabolism.

**Ki67 (proliferative protein)** synthesis decreases in fibroblasts during skin aging.

**CD98hc** protein is synthesized on the membrane of skin fibroblasts and participates in their proliferation (division). CD98hc synthesis in skin fibroblasts decreases during aging, which leads to a slowdown in its regeneration.

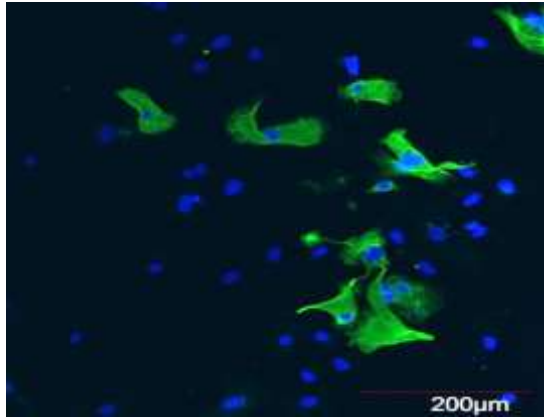
**Matrix metalloproteinase-9 (MMP-9)** is involved in the remodeling of the intercellular environment by skin fibroblasts. The UV-induced synthesis of MMP-9 contributes to the destruction of type 1 and 3 collagens in the dermis. The synthesis of MMP-9 leads to the degradation of non collagenic components of the dermis, including glycoproteins and proteoglycans of the basement membrane.

**Caspase-3 and p16, p53 transcription factors** are the most important components of the cascade of apoptosis (programmed cell death) in skin fibroblasts.

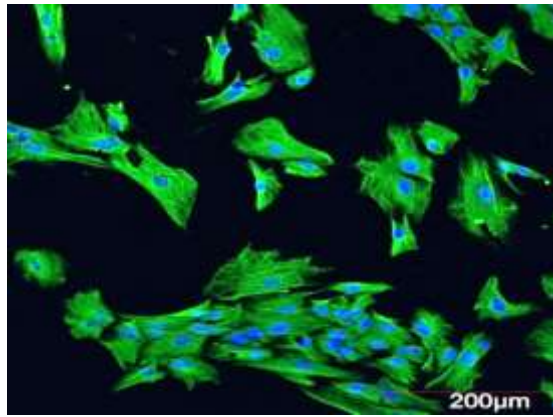




# The AED peptide (Cartalax®) stimulates collagen 1 in skin fibroblasts in aging of cell culture (replicative aging)



**Control**



**The AED peptide**

Immunofluorescence confocal microscopy of skin fibroblast culture, passage 14 ("old" cells), x400. Cell nuclei are colored Hoechst 33258 (dark blue fluorescence), green fluorescence is the expression of collagen 1 (Alexa Fluor 488).

**Type 1 collagen** is one of the main structural proteins synthesized by skin fibroblasts, which indicates the high functional activity of these cells. Its synthesis in fibroblasts decreases during cell aging.

**The AED peptide (Cartalax®) restores the synthesis of type 1 collagen in skin fibroblasts during aging.**

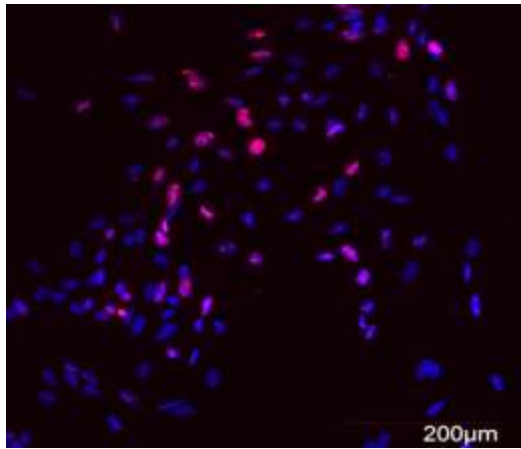
Fridman N.V., Linkova N.S., Kozhevnikova E.O., Gutop E.O., Khavinson V.K. Comparison of the Effects of KE and AED Peptides on Functional Activity of Human Skin Fibroblasts during Their Replicative Aging. Bull. Exp. Biol. Med. 2020. Vol. 170, N 1. P. 154-157.



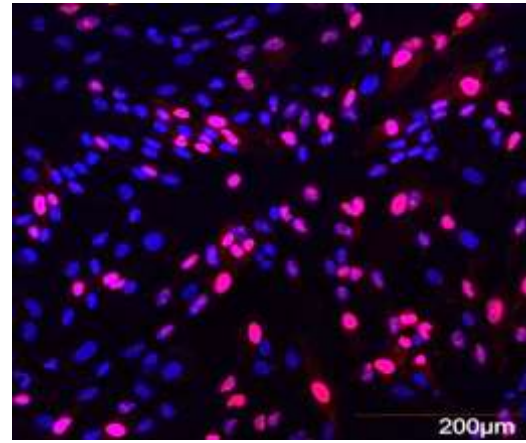




# The peptide AEDG stimulates the protein Ki67 synthesis in fibroblasts of the skin during their senescence in culture (replicative senescence)



Control



The AEDG peptide

**Ki67** is one of the main proteins necessary for the proliferation (division) of skin fibroblasts. Its synthesis in fibroblasts decreases during aging.

**The AEDG peptide restores Ki67 protein synthesis in skin fibroblasts during aging.**

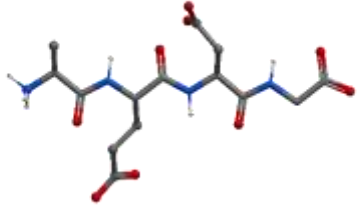
Immunofluorescence confocal microscopy of skin fibroblast culture, passage 14 ("old" cells), x400. Cell nuclei are colored Hoechst 33258 (dark blue fluorescence), pink fluorescence is the expression of Ki67 (Alexa Fluor 647).

Lin'kova N.S., Drobintseva A.O., Orlova O.A., Kuznetsova E.P., Polyakova V.O., Kvetnoy I.M., Khavinson V.Kh.  
Peptide Regulation of Skin Fibroblast Functions during Their Aging In Vitro. Bull. Exp. Biol. Med. 2016.  
Vol. 161, N 1. P. 175-178.

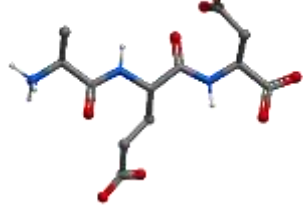




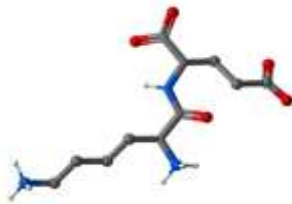
# The scheme of peptide regulation of skin functions



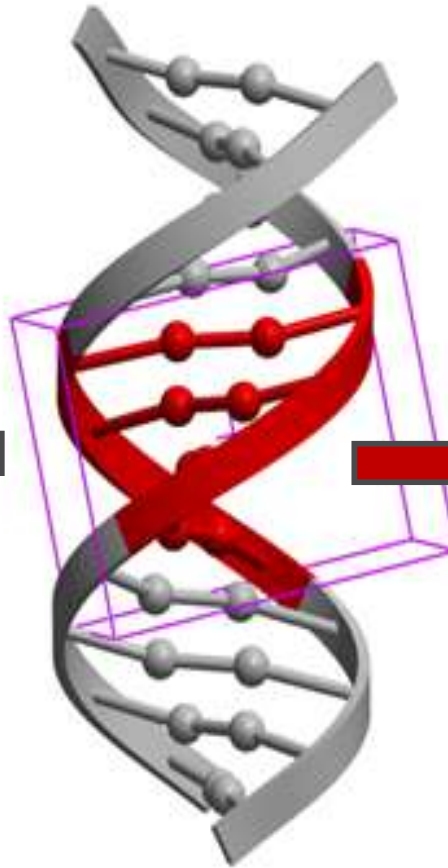
The AEDG peptide -  
an active  
component of  
Endoluten®



The AED peptide (Cartalax®) -  
an active component  
of Sigumir®



The EDP peptide (Crystagen®) -  
an active component  
of Vladonix®



DNA



Gene  
expression  
and protein  
synthesis  
regulation in  
skin  
fibroblasts



Normalization  
of the  
thickness of  
dermis and  
epidermis in  
skin  
during aging

COX-2, SIRT1, SIRT6, p16, p53,  
Caspase-3, CD98hc, MMP-9, 1<sup>st</sup>  
type collagen, Catalase, SOD1,  
NQO1





# Conclusion

- Polypeptide complexes of cartilage (**Sigumir®**), pineal gland (**Endoluten®**), thymus and AED (**Cartalax®**), EDP (**Crystagen®**) peptides increase the thickness of the epidermis and dermis in middle-aged and elderly women according to the questionnaire and evaluation of the thickness of the epidermis and dermis by ultrasound diagnostics. This helps to slow down the aging of skin cells and improve its appearance.
- AED (**Cartalax®**) and AEDG short peptides interact with promoters of genes encoding proteins SIRT1, SIRT6, p16, p53, Caspase-3, CD98hc, MMP-9, Ki67, type 1 collagen, catalase, SOD1, NQO1 - markers of skin fibroblasts functional activity. This contributes to the normalization of skin fibroblasts functions with their natural and accelerated (caused by the action of sunlight) aging.
- **The obtained data indicate the effectiveness of peptide bioregulators Sigumir®, Endoluten®, Crystagen® and Cartalax® to slow down age-related skin changes.** These peptide bioregulators can be used to improve the quality of life and satisfaction with their appearance in middle-aged and elderly women.





Thank you for your attention!

Saint Petersburg, 2023