Experts' opinion on current approaches in anti-ageing medicine and gerontology
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INTRODUCTION

The world is rapidly ageing, entailing significant consequences for the global society and economy, while the fast developing biomedical science and technology stand in the forefront of defense against the potential risks. These two processes bring gerontology, describing the challenges of ageing and at the same time seeking means to address those challenges, to the focus of the global scientific, technological and economical discourse.

Ageing can be understood at various levels, from evolutionary and biological levels to psychological and sociological ones. At the molecular biological level ageing is characterized by the stochastic occurrence and progressive accumulation of molecular damage. Altered cellular functioning and reduced stress tolerance are the determinants of health status, probability of diseases and the duration of survival. The inefficiency and imperfection of the maintenance and repair systems underlie the biological basis of ageing. Gene therapy, stem cells, and modulation through functional foods, calorie restriction, nutraceuticals, cosmeceuticals and other life style alterations are examples of ageing interventions.

In view of the immense significance of degenerative ageing processes for the emergence of virtually all diseases, both communicable and non-communicable, and in view of the accelerating development of potential means to intervene into and ameliorate these processes for the sake of achieving healthy longevity, contributions to this field have ever greater global significance.

Inhibition of ageing and prolongation of life in laboratory animals prompts similar possibility for humans due to unique mechanisms of ageing. Expanded knowledge on ageing is in the first line of prevention of such exhaustive pathologies as cardio-vascular diseases, cancer, diabetes mellitus type II, Alzheimer disease. The therapy based on fundamental mechanisms of ageing will contribute to health maintenance.

Achieving the goal of extended health-span will depend on elucidating and exploiting successful and dynamic interactions among
biological, psycho-social and environmental factors. This understanding of ageing should transform our approach towards interventions from therapeutic “anti-ageing” to maintaining health which is according to WHO definition is a state of complete physical and mental independence in activities of daily living. Age-related health problems for which there are no clear-cut causative agents, except the complex process of ageing, may be better tackled by focusing on health mechanisms and their maintenance, rather than disease management and treatment. Supporting health-oriented research is the urgency of our time.

This Symposium is aimed to overview evidence-based references and scientific argumentation in favor of means and methods preventing age-related pathology and prolonging sustainable health, as well as to estimate prospects and efficacy of explored geroprotectors with their further recommendation for medical practice.

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ABSTRACTS
CURRENT APPROACHES TO TESTING ANTI-AGING DRUGS

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Relevance. Extending the human healthy lifespan is one of the main purposes of gerontology and modern preventive medicine. Researchers managed to slow down aging and to prolong healthy lifespan using genetic, dietary and pharmacologic approaches in many model organisms: yeasts, worms, fruit flies, insects, short-living fishes, birds, rodents (mice, rats and hamsters), minipigs, dogs, and monkeys. Recent experimental studies demonstrate that medications targeting aging (antioxidants, calorie restriction mimetics, autophagy inductors, etc.) can substantially promote health and extend lifespan [1-3]. Pharmacologically targeting aging appears to be more effective in preventing age-related pathology compared with treatments targeted to particular pathologies. The development of new anti-aging drugs represents a great opportunity for the pharmaceutical and healthcare industries. However, if people will better survive into later life and live longer, the increase in incidence of age-associated diseases including cardiovascular diseases, diabetes type 2 and cancer will be a great challenge for the mankind. The search for adequate selection models of effective and safe methods of life extension became the most urgent matter in biology of aging.

There are at least two accepted definitions for compounds applicable to pharmacological intervention into the aging: a) anti-aging drugs, which presumably are able to reverse the aging process (rejuvenation) and b) geroprotectors, which being administered lead to prevention of premature aging and/or slow down or postpone aging. Spindler [2] introduced term “longevity therapeutics” for drugs that intervene in the process of aging to extend mean and/or maximum life span, maintain physiological function, and mitigate the onset and severity of a broad spectrum of age-associated diseases in mammals. Vaiserman et al. [3] subdivided potentially geroprotective agents into several groups: those demonstrating anti-aging effect, but without any evidence of life span increase; drugs which increase life span reducing incidence of age-associated pathology, and agent which
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extend lifespan because they suggested to reverse the aging process itself. While laboratory animals are similar to humans in some respects (such as patterns of aging at the molecular, cellular/tissue, and physiological levels, responses to hazardous exposures), there is a growing pool of experimental evidence indicating important differences (genetic, metabolic, ontogenetic etc.) among mammalian species that make valid interpretation and extrapolation of the animal experiments to humans difficult. Issues of concordance of responses between rodent species and between rodents and humans – as well as repeatability and site-specificity – are important considerations in evaluating laboratory animal results [4].

In 2003 the U.S. National Institute of Aging (NIH) started the Aging Interventions Testing Program (ITP), which proposed to test compounds with the potential to extend lifespan and to delay (postpone) age-associated diseases and dysfunctions. Among such means are pharmacological drugs, nutriceutics, food products, diets, food additives, plant extracts, hormones, peptides, amino acids, chelating agents, antioxidants, etc. In the framework of the ITP aspirin, nordihydroguaretinic acid, nitrofluorodiprophen, rapamycin, resveratrol and some other drugs were studied. Priority was paid to preparations which are easily available, have a reasonable price and can be administered with food (preferentially) or with drinking water. An ITP protocol includes two phases. During the first phase the capability of the drug to increase lifespan is studied. In addition, other parameters, such as the animal’s activity in young and old age, metabolic hormone levels, and T-lymphocyte levels are also studied. During the second phase, drugs that show promising results are studied more intensively to reveal candidates for further clinical studies. Behavioral and cognitive experiments, measurement of the oxidation level and pathomorphological studies of the dead animals take place during the second phase.

In 2000, an international program (project) on the assessment of efficacy and safety of geroprotectors has been suggested. Its activity could be carried out under the control of the United Nations Program on Aging, World Health Organization and the International Association of Gerontology and Geriatrics. The aim of this program is the preparation of international critical reviews by an expert working group providing systems and guidance of evidence relating to the activity and efficacy of geroprotective drugs. Experts could give recommendations for additional studies, if required. The
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categorization of agent is a matter of scientific judgment that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

Group 1: The drug is geroprotector for humans. This category includes drugs with sufficient evidence of lifespan increase in humans. Evidence is confirmed by epidemiological multicenter randomized studies;

Group 2: This category includes drugs for which, on the one part, the degree of evidence of geroprotective activity in humans is almost sufficient, as well as those for which, on the other part, there are no human data, but for which there is evidence of lifespan extension in model animals.

Group 3: The drug is not classifiable as to its geroprotective effect in humans. This category is used most commonly for agents for which the evidence of geroprotective effect is inadequate in humans and inadequate or limited in experimental animals.

Group 4: The drug is probably not a geroprotector in humans. This category is used for drugs for which there is evidence suggesting lack of lifespan extension in humans and in experimental animals.

Publication of the results received by the expert group would assist national and international health institutions to plan and perform programs of rehabilitation and prevention of premature aging, as well as to make a decision about risk-benefit ratios of such programs. Experts in the working groups need to develop a scientific report about the evidence of geroprotector efficacy and safety of the drugs. They should not give any recommendations directly to national or international health institutions about regulation or legislation of drug usage, this remains the exclusive priority of this organizations. Currently, there is no substance which could be evaluated as a group 1 agent (i.e. geroprotector activity of the drug had been proved in humans). Drugs that could be in group 2 are probably metformin, rapamycin, melatonin, pineal peptide preparations Epithalamin and Ala-Glu-Asp-Gly (Epitalon). There are numerous data confirming the geroprotective effect of these drugs in animal experiments and, in some cases, in clinical studies (Table 1). These drugs are probably the most reliable candidates for testing in multicenter randomized clinical studies. The evaluation of safety of a drug in rodents is a crucial aspect of its preclinical trials. Long-term assays for carcinogenicity in rodents are an integral method which evaluates toxicity and some adverse effects of the drug being tested.
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Table 1. Summary on some most significant effects of promising geroprotectors observed in rodents

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Metformin</th>
<th>Rapamycin</th>
<th>Melatonin</th>
<th>Epitalon*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifespan</td>
<td>↑</td>
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<td>↑</td>
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<tr>
<td>Antioxidant potential</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Susceptibility to insulin</td>
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<tr>
<td>Low-density lipids</td>
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<tr>
<td>Resistance to stress</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Reproductive function</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Cognitive and learning capacity</td>
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<tr>
<td>Physical endurance</td>
<td>↑</td>
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<tr>
<td>Age-related pathology</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Cancer risk</td>
<td>↓</td>
<td>↓</td>
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</tbody>
</table>

↑ - increase; ↓ - decrease; *Ala-Glu-Asp-Gly.

Combination in one study of both safety and geroprotective potential of drugs significantly decreases the cost of the study. GeroScope is an *in silico* project that can aid prediction of novel anti-aging drug from existing human gene expression data. The design of the majority of studies in the field was found to suffer from confounds and defects.

**Conclusion.** Accordingly, there is the need to create standard guidelines for testing such drugs and for evaluation of life extension potential as well as other late effects including tumor development. Guideline for the testing should include such significant points as animal models, regime of testing, and biomarkers/endpoints. The system of experimental preclinical study of such drugs could include a study on their effects on biomarkers of aging, lifespan and the development of various age-associated pathologies, especially tumors. The study should be conducted in rats and mice (inbred, outbreed or genetically-modified animals) treated by drugs in different doses for their whole life [5]. The ultimate goal in this field is the choice of geroprotectors for studies in humans. To achieve these goals, the international standards for preclinical and clinical studies of agents for pharmacological interventions into the aging, as well as for evaluation of results of such studies, should be
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developed. In the coming years, the perspective direction could be the development of new biomarkers, based mostly on biochemical and genetic methods, for short-term screening of such drugs. At present, cooperative studies on anti-aging drugs and geroprotectors conducted in various laboratories could be promising.

References:

MEDITERRANEAN DIET AND LIFESTYLE: A PROVED APPROACH TO HEALTHY LONGEVITY

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Relevance. Aging is a multifactorial and progressive process, universal and irreversible, that takes place at different levels, affecting practically all living organisms. Human life expectancy increased extraordinarily during the last century worldwide. Currently, there is growing evidence that there are modifiable factors that contribute to ageing per se, and particularly to longevity (i.e. diet, physical and mental activity). These factors may interact with the ageing process and may alter the susceptibility of an individual to develop age-associated diseases. There is currently much promise in research that provides information about the underlying biology of ageing and longevity, which has unveiled possible interventions to slow the ageing process, including a healthy lifestyle in terms of nutrition, exercise, and smoking cessation, as well as new
discoveries that result from basic research. Diet is a major determinant of the ageing process itself and of the development of age-associated diseases. There is strong evidence of the influence of diet on the health status. Epidemiological and experimental evidence have emphasized the traditional diet from the Mediterranean basin as a possible contributor to longevity and good quality of life. After the first positive data from the Seven Countries Study, numerous investigations in different populations have confirmed a beneficial role for the Mediterranean dietary pattern on the reduction of overall mortality and mortality from cardiovascular diseases and cancer, hence, increasing longevity (1, 2). A meta-analysis including a total of over 1.5 million subjects confirmed a significant reduction in all-cause mortality associated to increased adherence to the Mediterranean diet (2). These investigations have over and over again confirmed that the Mediterranean diet is a model of healthy eating which contributes to a favourable health status and a good quality of life (3). The PREDIMED (Prevención con Dieta Mediterránea) study, a prospective randomized controlled trial, which included 7,447 volunteers aged 55 to 80 years at high cardiovascular risk, demonstrated that a Mediterranean diet supplemented with extra virgin olive oil or nuts significantly reduced major cardiovascular events after a median follow-up of 4.8 years (4). Using data from the SUN (Seguimiento Universidad de Navarra) project, a large, prospective cohort, we found that the risk of the combined outcome of incident cardiovascular events, incident diabetes incidence or all-cause mortality, was significantly lower with a higher adherence to Mediterranean diet, even after adjustments for total energy or weighting each item's evidence-based contribution to coronary heart disease protection (5).

What is Mediterranean diet? The Mediterranean diet is a balanced dietary pattern, high in foods of plant origin, which are rich in antioxidant compounds, vitamins and fiber, similar to that followed by traditional populations living in the Mediterranean basin during the post-World War II period. It is mostly based on whole bread and grains, extra virgin olive oil, legumes, fresh vegetables and fruits, nuts, fish, herbs and spices, and wine in moderate amounts. There is mounting evidence that this dietary pattern have remarkable effects reducing total mortality, cardiovascular mortality, and cancer-related mortality. The name “Mediterranean diet” was coined in the 60’s by Ancel Keys, a researcher of
the University of Minnesota, who arrived in Italy after the World War II. He carried out a 20-year investigation exploring the effects of diet on the incidence of various diseases in some Southern Italian regions and Greece compared to the incidence of those diseases in the USA, Finland, Japan, the Netherlands, and former Yugoslav. Less similar the dietary pattern used compared to the Mediterranean diet, the higher was the incidence of the diseases of “abundance”, i.e., cardiovascular disease and some types of cancer (1). The definition of a single Mediterranean diet as a unique entity is not simple because there are countries with cultural, ethnical, religious and economic diversity in the region. However, the general pattern is similar and the use of extra virgin olive oil as the main source of dietary fat is universal in the basin, although it varies according to socioeconomic and demographic characteristics in some countries. The use of whole complex carbohydrates derived from wheat (bread, pasta, couscous) along with a minimal percentage of simple sugars, constitute 50-60 percent of the total caloric intake in the Mediterranean diet. The fat consumption (based almost exclusively on extra virgin olive oil) represents 25-30 percent, and protein intake (fish, legumes and meat) the remaining 10 percent of the total caloric intake.

**Mechanisms of the Beneficial Effect of the Mediterranean Diet.** The antioxidant and anti-inflammatory action of several components of this dietary model have been linked to its favourable effects regarding the prevention of degenerative diseases in old age. One of the most accepted mechanism to help explain the ageing process is the excessive mitochondrial production of free oxygen radicals (ROS: reactive oxygen species) with damage to cellular structure and subsequent chronic inflammation. Oxidative stress accumulates when prooxidants overwhelm the antioxidant defence mechanisms. Oxidative stress and chronic low-grade inflammation are involved in several age-associated conditions including hypertension/cardiovascular disease, type 2 diabetes mellitus, neurodegenerative diseases, including Alzheimer's and Parkinson's disease, dyslipidaemia, and cancer. ROS serve as precursors to the formation of oxidized LDL, essential for the formation of atherosclerotic plaques. Elevated ROS have been also associated with an increased expression of pro-inflammatory cytokines such as TNF-alpha, plasma activator inhibitor (PAI)-1 and interleukin-6.
These concepts have lead to seek for factors that may reduce ROS or contrast the cellular damage or enhance the repair mechanisms. Mediterranean dietary pattern includes a significantly large amount of plant foods rich in antioxidant compounds, which may help to explain its multiple benefits. Many of the components of the Mediterranean diet, including extra virgin olive oil, fresh vegetables and fruits, nuts, wine, and fish, contain molecules with anti-oxidant and anti-inflammatory properties such as monounsaturated fatty acids, omega 3 fatty acids, polyphenols, flavonoids, phytosterols, antioxidant vitamins, and antioxidant minerals and micronutrients. Among the components of this dietary model, the use of extra virgin olive oil as the main source of lipids has been linked to diverse favourable effects regarding the prevention of degenerative diseases in old age because of its antioxidants and antinflammatory properties. Resveratrol, a potent free radical scavenger, contained in particular (but not exclusively) in red wine has been shown to modulate insulin secretion and action, and that it may extend lifespan in animal models. However, all the studies on the Mediterranean diet emphasize the effects of the whole dietary pattern with the combination of different nutrient-rich and antioxidant food rather than individual elements. In fact, the effects of the combination of different components of a balanced diet may potentiate the effect of single elements and may better help to explain the unquestionable evidence of the multiple benefits of this dietary pattern on longevity and against the development of age-associated diseases.

**Conclusion.** The Mediterranean diet is now considered worldwide as an ideal nutritional model, based on numerous scientific studies showing that people who follow this traditional lifestyle live longer and are healthier, with lower incidence of cardiovascular disease, obesity, atherosclerosis, diabetes, hypertension and cancer, compared to those adherents to a “western” type diet. The Mediterranean diet is balanced, high in foods of plant origin, which are rich in antioxidants, vitamins and fibers, and so rich in fresh fruits and vegetables, nuts, olive oil, fish, herbs and spices, and wine in moderate amounts. Adherence to a Mediterranean dietary pattern represents a promising option for the prevention of diverse diseases associated to aging contributing to maintain a favourable health status and a good quality of life.
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References:

METABOLISM AMELIORATING REMODELING INDUCED BY ATMOSPHERE (MARIA)

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Relevance. Lower metabolic expenditure is more than often associated with declined rate of damage and longer lifespan. Such inverse relationship between metabolic and aging rates is well documented on numerous phylogenetic and ontogenetic models and could be a basic concept for many hypotheses of aging (wear and tear, waste accumulation, free radicals etc). Unfortunately, long-term decrease in metabolism in warm-blooded species remains an unresolved problem. At least all our efforts to decrease the metabolic rate and extend life span of laboratory animals by application of the known inhibitors of mitochondrial or nuclear replication, transcription, translation and uncoupling resulted in only marginal effects, presumably because of the adverse side-effects of the chronic usage of antibiotics/xenobiotics [Frolkis, Muradian, 1991]. Our recent findings, however, show that metabolic expenditures could be decreased/optimized by maintaining animals in hypercapnic/hypoxic atmospheres (HHA) [Timchenko et al., 2008; Muradian, 2013; 2015]. There is another important issue: basic life-supporting systems evolved and most part of their
evolution occurred in severe hypercapnia and hypoxia. Therefore, maintenance of modern animals in environments resembling primordial earth hypercapnic/hypoxic atmospheres could result in optimization of metabolic expenditures.

**Methods.** Experiments were performed on young (3-5 months) and old (22-25 months) male C57Bl/6 or female CBA mice. Effects of artificial atmospheres on viability in stress conditions, survival dynamics and lifespan were studied on drosophila (Oregon). Acute (3-4 hours) and chronic (several weeks) exposures of mice to HHA were studied. Acute HHA were modeled by keeping individual mice in hermetically closed glass jars. Chronic exposures were modeled by maintaining mice groups in boxes with regulated ventilation, so that animals themselves created necessary atmosphere (auto HHA). Specifically, mice were kept in auto HHA typical for the naked-mole rat (around 10% CO$_2$ and 10% O$_2$). In experiments with drosophila, flies were kept lifetime in hermetically closed 100 ml syringes with addition of 5-15% CO$_2$ to the air. Feeding medium and atmosphere were refreshed on every other day basis. In experiments with mice, food and water consumption, motor activity, real time PCR of hypoxia inducible factor (Hif1) and two hypothalamic genes associated with appetite stimulation (neuropeptide Y and agouti like protein), as well as oxygen consumption and CO$_2$ production rates ($V_{O_2}$ and $V_{CO_2}$), body temperature, concentrations of the blood T3 and T4, glucose, total cholesterol, fatty acids, lactate and pyruvate were determined. Type 1 diabetes was modeled by injection of streptozotocin (40 mg/kg) during 5 days.

**Results.** It was found that dependence of $V_{O_2}$ and $V_{CO_2}$ on partial pressure of CO$_2$ or O$_2$ ($P_{CO_2}$ and $P_{O_2}$) is characterized by three phases. At lower atmospheric concentrations (<2%), CO$_2$ induces paradoxical stimulation of $V_{O_2}$ and $V_{CO_2}$. Middle range concentrations of CO$_2$ in the air (>2% and <5%) did not significantly change metabolic rate whereas $P_{CO_2}$ higher than in the blood of mice (CO$_2$ > 5%) resulted in significant decrease of $V_{O_2}$ and $V_{CO_2}$. Surprisingly, exposure to HHA typical for naked-mole rats was sufficient to induce analogous metabolic modifications in mice, i.e., decreased $V_{O_2}$ and $V_{CO_2}$, food and water consumption without significant changes of the motor activity. Body surface temperature decreased by 2-4 °C, as it is typical for the naked-mole rats. To our best knowledge, MARIA is the only reliable mammalian model of chronic body temperature decrease. It is remarkable, that highly significant positive correlations were found
between Vco\textsubscript{2} and body temperature. Analogous correlations with Vo\textsubscript{2} were often insignificant indicating that Pco\textsubscript{2} could have more direct relations with mice thermoregulation. Alterations of the blood glucose, fatty acid, pyruvate, and lactate concentrations indicate possible activation of glycolysis and lipolysis. The suggestion is additionally supported by the prompt decline of body mass. The decreased Vo\textsubscript{2} and Vco\textsubscript{2}, as well as food consumption and body mass are apparently physiologically controlled, as it follows from the decreased blood T3 (but not T4) and lower expression of the hypothalamic peptides stimulating appetite. HHA could be efficient in treatment of metabolic syndrome- and age-associated diseases, as it follows from our experiments with the streptozotocin model of type 1 diabetes. During the whole observation period (12 weeks), hyperglycemia in the streptozotocin-treated mice kept in the air undulated within 230-300\% compared with the blood glucose level of the same animals before the streptozotocin injection, whereas in analogous experiments on mice kept in HHA hyperglycemia sustained within 100-160\%. Maintenance of drosophila in hypercapnic or hypoxic atmospheres also resulted in decreased Vo\textsubscript{2} and Vco\textsubscript{2}, enhanced viability and extended life span [Timchenko et al., 2008].

**Conclusion.** All, or nearly all, biological processes are energy-dependent. Therefore, purposeful modulation of energy generation could be a universal ‘master key’ for targeted changes of virtually all biomedical processes, aging included. Maintenance of mice in HHA typical for the earth primordial atmospheres or modern naked-mole rats decreased energy expenditures without apparent changes of motor activity presumably due to optimization of metabolic expenditures. In terms of gross-metabolic indices (food and oxygen consumption, body temperature, etc.), HHA actually ‘transformed’ mice into naked-mole rats. MARIA could be promising models of voluntary food restriction and chronic body temperature decline relevant for treatment of the metabolic syndrome and age-associated pathology. Further research is clearly warranted to study HHA effects on aging pattern and longevity in mammals.

**References:**

RESTORATION OF THYROID GLAND MORPHOLOGY IN SENILE PATIENTS ADMINISTERED WITH THYREOGEN (PEPTIDE COMPLEX)

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Relevance. Thyroid gland is the only source of thyroid hormones which produce various effects on all organs and systems. With age thyroid gland undergoes certain structural and functional changes.

The size of the gland reaches its maximum at the puberty and diminishes with aging. Anatomic involution of the thyroid gland is caused by atrophic and dystrophic processes in the organism, primarily by reduction in follicles diameter and decrease in their number. On the contrary, the amount of connective tissue increases, thus replacing normal tissues.

Functional changes in the thyroid gland are caused by the age-related decrease in the number of hormone synthesizing cells. As a rule, those changes are manifested in moderate decrease in triiodothyronine level, less frequently – decrease in thyroxine level. However, reverse functional deviance affected by compensatory reactions may also occur.

Thyroid hormones level is significantly affected by comorbidity (poorly controlled diabetes, cardiovascular insufficiency, neoplasia, etc.) and medications taken by senile patients (amiodarone, lithium, metamizol).
Statistically 20% of females and 15% of males aged over 70 suffer clinically significant hypothyreosis. Frequency of subclinical cases is much higher.

Thyroid hypofunction in the elderly is mainly caused by chronic Hashimoto thyroiditis, thyroid gland surgeries, radioactive iodine treatment, head and neck irradiation. Lack of iodine in food and use of anti-thyroid medications also play a significant role in the process.

The purpose of investigation consisted in studying the effect of Thyreogen on restoration of thyroid gland morphology in senior patients. Peptide bioregulators are widely used in contemporary clinical gerontology for restoration of pathologically changed tissue of the thyroid gland.

**Methods.** 90 patients aged 48-70 years with various thyroid pathologies were assessed. The results of ultrasound investigation revealed age-related increase in the frequency of nodal abnormalities in thyroid gland, particularly in women. Nodular abnormalities appeared in patients aged 60 and older in 80% of cases. In this respect peculiarities of clinical progression have been determined. Elderly patients reveal mostly atypical forms of thyroid gland pathologies. Quite often senile hypothyroidism can be disguised as depression.

Ultrasound investigation revealed nodular abnormalities and cyst formations of 0,6-1,0 cm in 80 patients. Thyreotrophin and thyroxine indices were within the age norm. Increased thyroperoxidase antibodies level was registered in 10 patients.

The patients were divided in 2 groups. A control group comprised 20 patients, who were treated with Iodomarin by 100-200 mg once a day for a period of 3 months. 60 patients in the main group were treated with Thyreogen by 1 capsule twice a day for one month. The patients were followed up for 3 months.

**Results.** In 3 months all patients underwent ultrasound investigation of thyroid gland. 20 patients of the control group revealed no changes and the size of nodes remained the same.

50 patients in the main group revealed a decreased number of palpable abnormalities alongside restoration of the thyroid gland tissue. In 10 patients the size of palpable abnormalities remained the same, but some improvements in the structure of thyroid gland tissue were registered.
Conclusion. Administration of Thyreogen in case of thyroid nodes resulted in their regression and morphological improvement of the thyroid tissue. Thyreogen is well-tolerated by elderly patients and can be recommended for administration to middle-aged, elderly and senile patients in case of structural pathologies of thyroid gland. It also contributes to the environmental adaptation of the elderly.

References:

PROSPECTS FOR USING PEPTIDES IN VIVAX FOR ENHANCING HUMAN BODY RESOURCES

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Relevance. Physical stress is part of human life. It helps to restore good health and well-being, keep intact mental and physical activity and reduce the risk of infection and getting ill. There is, in fact, solid evidence that physical stress plays a great role in lowering the risks of cardiac failure, cerebral hemorrhage, diabetes, hypertension, colon and breast cancer, getting overweight, traumatic falls, cognitive deterioration and depression. Physical stress while constituting the basic framework of physical training plays an important prognostic role in the factor of total mortality.

Methods. Fundamental research conducted at the St. Petersburg Institute of Bioregulation and Gerontology in the area of increasing the spare capacities of the human body resulted in developing of new VIVAX medications that are based on short peptides allowing an increase in physical performance as well as an acceleration of restoring a sportsman’s physical resources after extreme physical and psychoemotional stress.
Currently great priority is provided to peptide bioregulators which protect cells from the impact of toxins, boost metastasis in cells and remove decay products from them, contribute to saturating them with nutrients. They feature profound tissue specificity, and they regulate functions of only those organs and systems from which they were extracted. Peptides that can condition the functions of practically all human organs and systems are already available today. Medications with peptide basis act on the cellular level, e.g. active synthesized peptide complexes “program” the genesis of a damaged cell with ultimate atomic-scale precision thus providing on it bioinformative effect. With exogenous administration of regulative peptides substances are released for which the initial peptide serves as an inducing agent helping to start the peptide cascade that lies at the core of biological regulation. Thus the treatment effect can be felt some time after peptide application, yet it lasts long enough.

**Results.**Synthesized peptides are not toxic, boast high chemical purity and thus act in a systematic and unhampered way due to their structure. Most importantly, active synthesized peptide complexes do not supersede the functioning of infected tissues: they only set going own cell resources for their regeneration. Notably, peptide-activated changes in biochemical processes within the human body are natural and thus safe for humans.

**Conclusion.**Reconstructive and rehabilitative VIVAX Sport medication that was developed in collaboration between the St. Petersburg Institute of Bioregulation and Gerontology and the Academy of Scientific Beauty is a safe and effective means of preparing for physical stress helping a quick body rehabilitation after intensive physical stress and is used for urgent rehabilitation during traumas and general fatigue. It can be used by professional sportsmen as well as by anyone who is practicing an active life style and looks after own health.

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ACTIVE & HEALTHY AGING: THERAPY versus PREVENTION

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Relevance. Innovation in strategies in the senior comprehensive care is especially important due to the enormous growth in the size, and changing needs, of the older population. Adopting a culture of innovation will enable stakeholders and care providers to take full advantage of the latest ideas and proposals to both enhance existing methods of prevention and care and develop new ones to improve the quality of life of older adults. On average across OECD countries, the share of the population aged over 65 years has increased from less than 9% in 1960 to 15% in 2010 and is expected to nearly double in the next four decades to reach 27% in 2050. Life expectancy at the age of 65 has increased significantly for both men and women over the past few decades, rising by 5.5 years on average since 1970. Some of the factors explaining these gains in life expectancy at age 65 include advances in medical care combined with greater access to health care, healthier lifestyles and improved living conditions before and after people reach age 65 [1]. Increased life
expectancy at age 65 does not mean that the extra years lived are in good health; today older people may experience a longer period of unhealthy life and they are more likely to develop disabilities and need support from family, friends, social and medical institutions and long-term care services. The majority of aged people are suffering chronic diseases because they have one or more of the following characteristics: permanent disorders; were caused by non-physiological changes leading to irreversible damage to a tissue, organ or system, required rehabilitation, or required long term care. The demographic gero-boom is associated with rising of the global burden of disease and disability, considering the world population aged 80 years and above will more than triple by 2050. However, recent studies have confirmed that leisure time activities, especially physical activity, are positive factors for a longer healthy survival. Physical activity could be considered as one of preventive and therapeutic measures against pathological aging at any age. Healthy aging interventions are needed to reduce the burden of disease and protect entire population. Aging per se has to be considered as a disease, and requires an appropriate therapy.

A biological dream. Would you like to stay within your biological age of 25 forever?

To some extent, this dream may become a reality in the near future by reversing in part aging-related diseases. There are many theories explaining the origin of human aging and premature aging, including alteration in the immune system, inflammation, fibrosis, mineralization of connective tissue and many others. But is this biology or pathology?

Many genetic and epigenetic changes involved in aging and longevity are associated with human diseases. Numerous studies have already shown the aging of our bodies is inherently modifiable, and a therapeutic intervention that slows down aging in people is a plausible target for science and public health [2]. The aging process is an assilant and functional consequences of this process have to be timely quantified in people still young [3] and in older adults for prevention of aged related disease. This provides a scenario for healthy aging therapies: caloric restriction, physical activity, brain training, food supplements (peptides, minerals, vitamins etc), or drugs (metformine, rapamicyn etc).

Cell-penetrating peptides are able to transport different types of cargo molecules across plasma membrane acting as molecular delivery vehicles. They have found numerous applications in medicine, and the
peptidergic system is a functionally relevant complex consisting of a cell that synthesizes and releases the peptide, a cell that responds to that peptide by some change in function, and a means whereby the peptide is transferred from its site of synthesis to its site of action [4].

Aging as a disease. According to numerous studies, mechanisms involved in cancer development are also associated with aging. This fact supports numerous proposals to prevent cancer and other age-related diseases using drugs or supplements that increase lifespan in preclinical studies. Many scientists throughout the world have argued aging should be classified as a disease [5]. However, today there are no visible global or national efforts to classify aging as a disease. This is primarily due to the structure and clear separation of the supplement and wellness markets and disease treatment markets. Improving healthcare safety, quality, and coordination are important goals for enhancing the health of the seniors with multiple chronic conditions and/or functional limitations. Person-centered care is an approach to meeting these aims, irrespective of different definitions of this type of global and personalized approach.

Conclusion. Decreased physical functioning, cognitive and emotional decline, and other comorbidities in older adults significantly diminish their quality of life. Educational and economic status, health behaviors, and social participation at the individual level are robust factors for predicting healthy aging. In considering what factors impact healthy aging, a person-centered approach would be useful and critical for policy makers to understand the compositions of health profiles and the influencing factors in view of a life-course perspective. Based on the factors identified as influencing healthy aging at the individual level, it is imperative from a policy-making perspective to maximize opportunities for healthy aging. The ultimate goal of biomedical research consists in the development of therapeutic drugs, and food supplements, particularly based on peptidergic regulation of aging. There will be definite benefits for many stakeholders in having aging classified as a disease with multiple actionable “non-garbage” disease codes. These can then be used to target therapeutic interventions. Dietary supplements encompass a wide variety of products ranging from vitamins, minerals, probiotics to peptides. These are important components of modern health care as demonstrated by robust consumption in all developed countries. Everybody would benefit by improving the hallmarks of aging: genomic instability, telomere attrition,
epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication.

References:

CLINICAL APPLICATION OF PEPTIDE BIOREGULATORS IN PATHOLOGICAL TREATMENT OF RETINITIS PIGMENTOSA

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Relevance. Retinitis pigmentosa is a rare degenerative disease of the retina characterized by a progressive degeneration of photoreceptor cells- initially the rods, followed by abnormalities of the adjacent retinal pigment epithelium and deterioration of cone photoreceptor cells. As a rule it occurs in young age and leads to increasingly compromised peripheral vision. Patients experience progressive "tunnel vision" and eventual blindness. Affected individuals may additionally experience defective light-dark adaptations, nyctalopia (night blindness), and the accumulation of bone spicules in the fundus. Until recently there was no effective treatment of retinitis pigmentosa in European medicine. It is known that in the USA there are actively conducted studies in the field of genetic engineering, but the achieved results haven’t been applied in clinical practice yet. Patients do not receive a proper medical care.
Methods. In Russia there is a continuous evolution in a new field of clinical medicine- bioregulating therapy- which has started in the mid 1980’s. V. Khavinson and V. Morozov have created and actively studied during all these years peptide bioregulators- preparations with a high biological activity due to epigenetic gene regulation. The authors have developed a unique method of peptide isolation from the organs and tissues of animals. These peptides have tissue-specific effects. The produced drugs are peptide complexes with low molecular weight (under 10 KDa). The utilization of modern technologies during the drug production precludes the possibility of presence of viruses and prions. Further scientific studies that were continued at Saint Petersburg Institute of Bioregulation and Gerontology under the guidance of Prof. Vladimir Khavinson allowed the creation of a new group of peptide regulators and peptide complex analogues preparations with a 100 fold stronger activity than their predecessors. There were obtained more than 200 Russian and international patents on peptide bioregulators. Many of these preparations are included in the pharmacopeia of the Russian Federation. [Khavinson V., 2002; Anisimov V.N., Khavinson V.Kh, 2010].

The first real advances in treatment of retinal diseases were achieved in the early 90s’ in the research laboratory of bioregulators (Head- Professor colonel of the medical service V.Kh.Khavinson) of the Military Medical Academy, which is named after S. M. Kirov. The experimental and clinical studies that were conducted in the laboratory, revealed a high retinoprotective activity of peptide bioregulators for severe pathologies such as retinitis pigmentosa.

Results. Many years of experimental clinical studies allowed us to apply peptide bioregulators in clinical practice for patients with retinitis pigmentosa. Results of the studies conducted on Campbell rats (from the 20th day of their life a genetically predetermined pigment degeneration develops) pointed that peptide retinoprotectors have a high ability to slow down the genetically programmed death of pigment epithelium cells, to stimulate regenerative processes of neuroreceptor apparatus of retina and to restore its functional activity. Administration of peptide bioregulators in Campbell rats allowed to increase the bioelectric and the functional activity of retina by stabilizing its morphological structure. This fact maintained their visual functions twofold compared to control. The main mechanism of action of retinoprotectors may be revealed by the results of the
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experimental studies cited below. It is known that retina contains 30% of differentiated cells (stems cells) [Khavinson V.Kh. et al., 2002]. Many years of studies about mechanisms of action of peptide bioregulators showed that peptides have a strictly specific inducional activity. The study of retinal peptides' inducional activity on the cells of ectoderm polypotent tissue of early gastrula Xenopus Laevis proved that these peptides have a neural inducional activity, unlike other bioregulators which have for instance mesodermal activity. The effect of retinal peptides on the differentiated tissue triggers a neural differentiation, which leads to an emergence of neural cells, retinal cells and pigment epithelium. [Khavinson V.Kh. et al., 2002, 2003; Takano M., 2002].

The conducted immunocytochemical studies with the use of monoclonal antibodies for markers of retinal cells' differentiation showed that short peptides can epigenetically regulate synthesis of pigment epithelium and retinal neurons differentiation markers. [Khavinson V.Kh. et al., 2012]. Therefore mechanism of action of peptide preparations is related to the regulation of expression of signal molecules that are the markers of retinal cells differentiation.

Understanding of mechanisms of action of retinal peptides explains how it is possible to increase visual functions in patients with eye diseases in clinical practice. [Khavinson V.Kh., Malinin V.V.,2005; Vanyushin B.F., Khavinson V.Kh., 2016].

30 years of clinical studies have also showed high retinoprotective activity of peptide bioregulators in patients with retinitis pigmentosa. Maintenance of visual functions was observed in more than 80% of patients with retinitis pigmentosa. At the same time during a 30-years observation period only 28% of patients in control group maintained their visual functions.

Many years of clinical administration of peptide bioregulators in patients with retinitis pigmentosa in Russia allowed us to begin a joint project on the treatment of patients with retinitis pigmentosa with our Greek colleagues. A 4 year clinical research was conducted in a group of patients with retinitis pigmentosa, in “OMMA Ophthalmological Institute of Athens”, using technologies of Saint Petersburg Institute of Bioregulation and Gerontology [Datseris Y. et al., 2016].

**Conclusion.** This 4 years' experience of application of peptide bioregulating technologies in treatment of retinitis pigmentosa revealed
not only a stabilization of pathological process, but also an improvement of visual functions, manifested by an extension of the visual field and a gain in visual acuity. There was a tendency of visual acuity increase at each course of treatment. One additional and significant contributing factor to the visual acuity increase is the stage of the disease. Patients with the first stage of retinitis pigmentosa had an increase in visual acuity after the very first course of peptide preparations. In some of the eyes visual acuity increased up to 3 lines at the ETDRS chart. At the terminal stages of retinitis pigmentosa the increase in visual acuity was observed after a few courses of treatment with peptide preparations, i.e. the positive result potentiated. Under the effect of peptide therapy an expansion of the visual fields was observed. It should be noted that alongside with the expansion of the constricted visual fields, the existing scotomas decreased in size, after the next courses they became relative and then disappeared. Thereby a positive effect of the treatment was correlated with the number of conducted courses.

Positive results of the treatment were confirmed by the data of electroretinography. Analysis of the results of the retinal electrophysiological study showed a significant improvement of the indices due to an increased activity of neurons and a decrease in their latent period.

No adverse events were reported during the course of treatment with peptides.

At present bioregulating therapy is a unique method of treatment for retinitis pigmentosa.

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EUROPEAN TRENDS IN AGEING POLICY

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Relevance. Population ageing has come to dominate the demographic scenarios of all continents. Demographic surveys demonstrate how during the second half of the 20th Century most countries’ population structure evolved out of a traditional pyramidal shape to an even-shaped block distribution of equal numbers at each age cohort except at the top [15]. Whilst the global ageing index (the number of persons aged 60-plus per hundred persons under age 15) is projected to rise from 24 to 101 in the 1950-2050 period, the global median age is projected to rise from 24 to 36 years (ibid.).

Europe is certainly at the forefront of population ageing trends and the population pyramid for European Union (EU) Member States is currently narrow at the bottom and more rhomboid at the top, as the baby boomer cohorts are moving speedily to the top of the age pyramid. Indeed, the demographic old-age dependency ratio (people aged 65 or above relative to those aged 15-64) is projected to increase from 27.8 to 50.1 per
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cent in the EU as a whole over the 2013 - 20160 period [8]. This implies that the EU would move from having four working-age people for every person aged over 65 years to about two working-age persons. It is thus not surprising that demographic change is high on the European policy agenda. This will be especially true in the coming decade as the baby boom cohorts will start retiring from the labour market - hence, increasing the pressure on income security, and social and health care systems.

Results. United Nation’s Economic Commission for Europe

The European follow-up to the Madrid International Plan of Action on Ageing was the first amongst all world regions. In September 2002, government delegations from 52 countries of the United Nations Economic Commission for Europe [UNECE] gathered at the Ministerial Conference in Berlin, Germany, and produced a Ministerial Political Declaration that recognised both the multiple challenges as well as numerous opportunities resulting from population ageing [10]. While welcoming the continual growth in longevity as an outstanding achievement of society, the Declaration emphasised the importance of enabling older persons to fully participate in all aspects of life. The Declaration confirmed that signatory governments commit themselves to the ultimate goal of a society for all ages that relies on respect for human rights, on protection against age discrimination, on social cohesion, and on equal opportunities for men and women of all ages. It also advocated that active citizenship is vital for achieving a society for all ages as this has the potential to promote social cohesion, help overcome age discrimination, and empower older and younger persons to act for themselves and to work together. A favourable environment is one that promotes active ageing, lifelong learning, access to modern information and communication technologies, volunteerism and civic engagement, as well as the adjustment of social protection systems that prevent and reduce poverty and material exclusion. In signing the declaration, governments reaffirmed the United Nations’ (1991) Principles for Older Persons. Indeed, the Declaration committed governments to protecting the rights of persons of all ages and the prevention of age discrimination and social exclusion, whilst promoting a positive image of older persons through the education system and media campaigns so as to enhance an appreciation of the social contributions of older persons. The UNECE conference formulated and adopted a Regional Implementation Strategy (RIS) [13] for Europe which advocated a number of key policy
principles, but foremost of all, the need to mainstream ageing in all policy fields with the aim of bringing societies and economies into harmony with demographic change to achieve a society for all ages.

More recently, the UNECE (2012) issued the 2012 Vienna Ministerial Declaration titled *Ensuring a society for all ages: Promoting quality of life and active ageing*. In fostering the implementation of a third review of the United Nations [12]) *Madrid International Plan of Action on Ageing* (2013-2017), UNECE is determined to reach the following policy goals and objectives by the year 2017: (i) encouraging longer working lives and the ability to work by supporting healthy lifestyles and wellbeing in work, preventing and controlling non-communicable diseases, and ensuring safe and healthy working conditions, and including measures for appropriate work-life balance with flexible working time schemes, through the entire working career; (ii) promoting the participation, non-discrimination and social inclusion of older persons by reducing material deprivation, poverty and social exclusion among older persons, especially older women, and facilitating the access of older persons to resources to meet their needs; (iii) increasing the levels of dignity, health and independence in older age by safeguarding the dignity of older persons, particularly those with disabilities, and fostering their sense of belonging and self-esteem through measures aimed at combating prejudice, neglect, abuse and discrimination; and (iv), enhancing intergenerational solidarity by strengthening multigenerational dialogue and intergenerational learning by all stakeholders, including governments, non-governmental organisations, the private sector, the media and the general public. Achieving these goals is also tantamount to meeting individual and societal challenges triggered by population ageing through adequate and sustainable policies on financial security, life-long continuum of health and social care, long-term care, inclusive and supportive systems for active ageing, lifelong learning, and participation in various spheres of society without discrimination, particularly with regards to older women. Indeed, ageing can no longer be seen solely through the angle of retirement and pension issues, and designing ageing policies means that we have to make active provisions for the future, in the interest of all generations.

*The European Union.* Ageing became a major European policy issue in the early 1990s when the EU’s Commission established an observatory to study the impact of national policies on ageing and older people. The
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dedication of the year 1993 as the European Year of Older People represented the first proclamation at this level of the key elements of the new active and participative discourse on ageing, albeit the Observatory on Ageing and Older People promoted the discourse of deservingness. A subsequent major milestone in the development of EU policy on ageing occurred in 1999, during the United Nations Year of Older People, which served as a catalyst to sensitise European governments to the continent’s forthcoming ageing imbalance in a wide array of areas, but especially, employment. The now famous Lisbon Council in March 2000 - which took the decisive step of establishing a 10-year strategy to make Europe the most competitive and dynamic knowledge-based economy in the world - committed the Union to full employment by 2010 [2]. As Walker [15] notes, “it was obvious to everyone that this ambitious goal could not be achieved unless the employment rate of older workers was raised substantially in most Member States” so that “the next logical step was taken at the Stockholm Summit in March 2001, when a specific target employment rate for older workers was set at 50%”. Although most EU policies on ageing until the early 2000s emphasised employment rather than a holistic positive discourse on ageing, the document Confronting demographic change (European Commission, 2005) and The demographic future of Europe - From challenge to opportunity [4] both introduced a more positive element into EU policy discourses on ageing, as they declared the need to recognise that policies on ageing should have a wider remit than employment, striving to redefine ageing from a ‘social problem’ to a ‘window of opportunity’. At the same time, the EU’s renewed emphasis on intergenerational solidarity could not be more commendable since the coming of ageing populations necessitate novel ways of liberating the potential of young people and older citizens, as well as locating innovative strategies on which to found social, economic, and health care services.

The European Commission [5] has committed itself to work towards an Age-Friendly EU by 2020 which would incorporate the following ideals: (i) positive attitudes to ageing that recognises the value of all age groups’ identities and contribution to society, (ii) an inclusive labour market that ensures the participation in paid work of older people, including those with disabilities or chronic conditions, (iii) accessible outdoor spaces, buildings and transport as well as facilities promoting independent living and participation in society for longer, (iv) goods and services that are
adapted to the needs of all, (v) digital inclusion to enable older persons to participate in the increasingly ICT-based society, (vi) the possibility for older adults to have a voice in the decision-making and research processes that affect them, (vii) the opportunity to actively participate in volunteering, cultural, sport and recreational activities, (viii) access to lifelong and intergenerational learning to acquire new skills and knowledge at any age, (ix) social protection systems based on intra- and inter-generational solidarity, and (x) conditions and opportunities to grow and age in good mental and physical health through disease prevention and the promotion of physical activity. In fact, the European Commission's [6] Council declaration on the European Year for active ageing and solidarity between generations (2012): The way forward recommended that the achievement of active ageing and solidarity between generations requires:

(i) strengthening social cohesion, inclusion and participation across a person's lifetime by ensuring access to services and to political, social, recreational and cultural activities, (ii) promoting participation in the labour market, (iii) recognising the values of all age groups and their contribution, (iv) promoting research and innovation to improve the lives of older people, including promotion of e-inclusion and e-health, (iv) health promotion, disease prevention and early diagnosis throughout the lifecycle, as well as rehabilitation, and (v) adjusting social security systems...to provide sustainable and adequate pensions [6].

The same year also saw the European Commission [7] launching a Strategic plan of the European innovation partnership on healthy and active ageing, based on three pillars: prevention, screening and early diagnosis; care and cure; and active ageing and independent living. The Plan is a prospect to accelerate innovation and exploit synergies within/across the different priorities and policies at EU, national and regional levels. It has a valuable role in delivering critical mass and attracting political recognition to active/healthy ageing. Nowadays, it has already delivered added value by establishing a shared positive vision on ageing and providing a comprehensive framework for action as agreed by stakeholders.

**Conclusion.** This concise review attempted to provide an overview of the key issues in European policy-making with regard to population ageing. Although countries are at different starting points, and at different rates, as far as their population pyramid is concerned, all are experiencing similar demographic trends. These trends are combining to present a
number of issues and challenges for national policy-makers: (i) as the ratio of retired to working people shifts towards the former, who is available to provide the services demanded and needed by retired people? (ii) how can the rising number of older persons be helped and encouraged to take upon a larger role in society, using their skills and experience to remain as active citizens? (iii) can the family remain the focus of care of frail older relatives? and (iv), as the ratio of retired to working people shift increasingly toward the retired, how will pensions to be funded? [1]. Meeting these challenges requires a multi-dimensional, as well as inter-linked response, one that spans various sectors such as employment, income security, social and health care, poverty, social exclusion, ageism, and age discrimination, to mention some. The key pre-requisite here is not to embed rationales in a ‘biomedicalised’ framework but, rather, ensure that the challenges of an ageing society are met in an equitable and sustainable manner. At the same time, it is important that ageing policies are not conceptualised and implemented in a ‘top-bottom’ approach but, rather, that they take shape in collaboration with older persons. As Maltby and Deuchars (2005) [9] underline, “it is time ...to focus on the positive contributions that older people can and do make to society and to a reconstruction of the meaning of old age”. One potential vision for the future of ageing policies in both high- and low-income countries lies with the concept of ‘empowerment’ which rejects the notion of the ‘old’ as an undue social burden and the capitalist ideals of ‘productivity’ that exclude them. The definition of ‘empowerment’ varies with professional context but maintains a focus on agency. People must be involved in those decisions that affect their lives, thus gaining confidence, self-esteem and knowledge, and developing new skills. The process is cumulative: the more skills, the more the person is able to participate and the more she can gain...Participation must be the sort that facilitates learning, action, and the achievement of goals [11]. It is hoped that future policy workings are earnest in involving professionals in the construction of ageing policies, whilst also remaining in negotiation with older persons, their carers, and other multiple stakeholders.

References:


A CRITICAL APPRAISAL OF THE USE OF MELATONIN IN THE ELDERLY HUMAN

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Relevance. Melatonin is a highly pleiotropic regulator of numerous physiological functions (1). It is synthesized in various organs. As far as it is secreted by the pineal gland, it exhibits a high-amplitude circadian rhythm, mediates the information ‘darkness’ and feeds back to the circadian master clock, the suprachiasmatic nucleus. Pineal melatonin declines by age, a change that is frequently associated with deteriorations of the circadian multi-oscillator system. The relationship to ageing has led to the suggestion of applying a hormone replacement therapy. Countless pre-clinical studies have revealed improvements of health, anti-oxidative protection, immune-stimulatory effects, suppression of symptoms of metabolic syndrome, anti-diabetic effects, oncosstatic actions, as well as life extension in senescence-accelerated mice.

Fundamental problems of translation to humans. Despite the encouraging preclinical findings, corresponding data for humans are relatively scarce and not generally in favor of a health-promoting role. One of the major problems consists in the chronobiological difference between nocturnally active laboratory rodents and the diurnally active human. In either case, melatonin levels peak at night. Consequently, melatonin is associated in laboratory rodents with enhanced metabolic, muscular and neuronal activities, with alertness and food intake, whereas it is related in humans to reductions in these functions and to sleep. With regard to these inverse relationships between rodents and humans, it may be naïve to
assume that effects on metabolic and neuronal parameters found in the laboratory animals should be equally present in man. The circadian differences between nocturnal and diurnal species represent a truly fundamental problem of translation. Nevertheless, there are two areas in which melatonin seems to act in a similar way, (a) in the support of the circadian system, by enhancing circadian amplitudes in both central and peripheral oscillators (2), and (b) in anti-oxidative protection (1).

**Aspects of dose and application method.** Melatonin can be easily applied to nocturnal animals via the drinking water, a procedure that allows a continuous elevation of melatonin throughout the night. This cannot be done in a similar way in the human, since the available melatonin preparations including controlled/extended release formulations do not generate the physiological pattern of melatonin. One of the consequences is apparent in the poor outcome concerning sleep maintenance, contrary to the relatively reliable effect of reducing sleep onset latency. Synthetic melatonergic agonists have not overcome this problem. Pre-clinical studies have often used melatonin in very high pharmacological doses, sometimes, in cells or tissues, in the upper μM or mM range. With regard to physiological melatonin levels in the circulation, mostly somewhat below 1 nM, the high pharmacological concentrations do not tell much about protective effects at reasonable doses applicable to humans.

**Melatonin and metabolic disorders: focus on type 2 diabetes.** Although melatonin and some synthetic melatonergic agonists have been shown to antagonize various aspects of metabolic syndrome in rodents, including obesity, hypertension, cardiovascular problems and insulin resistance, these findings cannot be simply translated to the human. This has to be seen on the background of the inverse relationship of metabolism and melatonin between rodents and humans. Some clinical studies have shown reductions of nocturnal blood pressure, platelet aggregation, LDL cholesterol, weight gain, serum levels of malondialdehyde and inflammatory parameters such as TNF-α and IL-6. However, several of these effects may not be related to metabolism control, but rather to anti-oxidant actions that are present in both humans and rodents. Although a few reports have claimed positive influences on blood glucose, several more recent publications indicate just the opposite. In non-diabetic young individuals, melatonin has now been shown to reduce glucose tolerance. A variant (“G allele” containing SNP rs10830963) of the melatonin receptor
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gene MTNR1B, which encodes the receptor MT₂, represents a risk allele for type 2 diabetes. Its presence was also shown to worsen the reduction of glucose tolerance by melatonin, in still normoglycemic young women. Its diabetogenic action consists in an MT₂ overexpression in β cells. This causes enhanced MT₂-mediated Gα_i signalling, supra-normal adenyl cyclase inhibition and, thus, strong reductions of cAMP, which is required for insulin secretion. Interestingly, the overexpression of the G allele is strongly age-dependent. In young adults, the MT₂ expression level is more variable but not significantly elevated in homozygous G allele carriers, compared to non-carriers. However, at an age above 45 years, presence and, even more, homozygosity of the G allele causes manifold higher expression levels. The change in MT₂ expression seems to be associated with concomitant reductions in melatonin levels and a lingering deterioration of the circadian system (2). Notably, the findings on the G allele contrast with other results on melatonin in type 2 diabetes. First, melatonin is known to be more strongly reduced in diabetics than in non-diabetic individuals. A pronounced reduction has been discussed as a risk factor for type 2 diabetes. Second, insulin resistance, a hallmark of this disease, is not explained by MT₂ overexpression. Third, some dysfunctional MTNR1B variants, which are incapable of binding melatonin or of interacting with G_i proteins, have been shown to be also associated with type 2 diabetes. Therefore, melatonin signalling seems to be required for avoiding the development of this disease. As discussed elsewhere (2), the age-dependent impairments of the circadian system and melatonin secretion may represent a cause of MT₂ overexpression. Future work should focus more strongly on insulin resistance, also with regard to its inflammatory aspect, which even extends to brain insulin resistance ("type 3 diabetes") as an early pathophysiological change in Alzheimer's disease.

**Melatonin’s dual role in the immune system.** Melatonin is an immune modulator, which influences numerous subtypes of leukocytes and controls expression and secretion of various cytokines. Depending on conditions, it can stimulate various pro-inflammatory and suppress anti-inflammatory cytokines. In this context, melatonin also behaves as a pro-oxidant agent, contrary to the otherwise frequently discussed action as an anti-oxidant. However, in endotoxemia, sepsis, excitotoxicity, ischemia/reperfusion or brain trauma, melatonin is a clearly anti-inflammatory compound. The precise conditions under which melatonin
acts in the one or the other way, remain to be further elucidated, especially with regard to immunosenescence and the development of a pro-inflammatory phenotype. However, relatively common pathologies are known to be worsened by melatonin. This concerns particularly an aggravation of rheumatoid arthritis. All autoimmune diseases represent a caveat for the use of melatonin.

**Conclusion.** At near-physiological and low pharmacological levels, benefits of melatonin treatment can be mainly expected from its role in the circadian system, by enhancing rhythm amplitudes and readjusting circadian phases. Although melatonin is mostly very well tolerated (300 mg/day for 2 years did not cause problems in ALS patients), melatonin should not be used in autoimmune diseases including rheumatoid arthritis, in other forms of chronic inflammation, and in type 2 diabetes. These considerations set limits to gerontological applications. With regard to metabolic syndrome and pre-diabetic states, it may be discussed whether an early onset of treatment, at a time when endogenous melatonin begins to decline, may be beneficial and prevent, directly or via the circadian system, the development towards MT2 overexpression and, perhaps, insulin resistance.

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INNOVATIVE TECHNOLOGIES IN THE REGULATION OF PINEAL GLAND FUNCTION

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Relevance. Pineal gland (also known as epiphysis) is the central endocrine organ that synthesizes a number of hormones and regulatory peptides involved in the regulation of the functioning of the neuroimmune system of the body. Among all the substances synthesized by pineal gland, one of the leading roles in this regulation belongs to melatonin (MT). MT is the essential regulator of all types of biological rhythms underlying the processes of vital activity, taking place at any level of organization: cellular, tissue, organ and system. As a result of aging, an involution of pineal gland is taking place and, as a consequence, the synthesis of MT decreases, which leads to age-related changes in a number of metabolic processes and to the emergence of age-related diseases. The results of modern studies have shown that MT is synthesized not only in pineal gland. Extrapineal melatonin is actively synthesized in many organs and tissues of the human body, including skin integuments. While aging the synthesis of pineal and extrapineal melatonin in the skin decreases. An age-related decrease in the synthesis of extrapineal melatonin has a negative effect on the aging rate of human skin.

Methods. It is well-known that lifestyle is an important factor that affects melatonin level in human body. The main regulator of physiological rhythms in humans and animals is the change in circadian cycle of day and night. Disruption of biorhythms leads to a suppression of melatonin synthesis. Therefore, careful attitude to biorhythms is the most important epigenetic factor of the neuroimmunoendocrine system's physiological work maintaining. A special diet also promotes activation of endogenous melatonin synthesis. First and foremost, these are the products rich in essential amino acid tryptophan, which is a predecessor of melatonin. Such products are nuts, beans, oats, bananas, beef, etc.

Pharmaceutical industry currently has remedies that increase the melatonin level. Some of them are registered as medicines, the others belong to dietary supplements. The most advanced medicines that regulate
melatonin level in blood are melatonin preparations and pineal gland peptides.

**Results.** Researchers of St. Petersburg Institute of Bioregulation and Gerontology (Russia) have developed a new group of medical preparations that regulate the synthesis of endogenous hormones, including melatonin. The results of long-term experimental and clinical studies have shown that pineal gland peptides can stimulate the synthesis of endogenous melatonin, which leads to restoration of neuroimmunoendocrine system and to slowing down of aging processes. It is established that the molecular action of peptide preparations is based on epigenetic regulation of gene expression and protein synthesis, which are markers of the functional activity of cells. The results were confirmed by numerous biomolecular (cell culture, immunocytochemistry, microscopy), physicochemical (spectrophotometry, molecular modeling) methods, animal experiments and clinical trials. Furthermore, the results of experimental researches have shown that short peptides are able to penetrate into skin, exerting regulating effect on the synthesis of not only extrapineal, but also pineal melatonin. Results of these studies allowed to create a new product – the facemask with pineal gland peptides.

**Conclusion.** The results of clinical studies of the facemask with pineal gland peptides have shown that a treatment course with this product helps to improve skin structure (according to ultrasonic scanning of the skin). This happens because an amount of collagen and elastin in the skin increases, and skin hydration improves. In addition, the course of facemasks activates the synthesis of both extrapineal and pineal melatonin in the human body. As the result, the facemask with pineal gland peptides helps to slow down the aging processes in the skin.

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CENTRAL, INFLAMMATORY AND OXIDATIVE STRESS RESPONSE IN AGEING

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Relevance. Central and systemic stress response is altered during ageing. Regulation is in part controlled by the hypothalamo-pituitary-adrenal (HPA) axis. Adrenal steroids have rapid effects on inflammation by controlling release of cytokines. The underlying mechanisms are still a matter of controversy since nuclear glucocorticoid receptors (GCR) are unlikely to be involved in membrane actions. Recent studies showed the intrinsic expression of corticosteroid binding globulin (CBG) in various glucocorticoid (GC) target tissues. Biosynthesis of steroids is closely linked to mitochondria. GCs affect mitochondrial metabolism while mitochondrial impairment results in decreased systemic cortisol levels and subsequent increased inflammation. Ageing of cells and tissues and related health issues seem to be closely linked to an altered stress response axis.
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Methods. Light- and electronmicroscopical immunocytochemistry, immunoassays and reverse transcriptase PCR were used to assess expression and localization of GCR, corticotropin releasing hormone (CRH), CBG and neutrophil elastase (N-el) in post mortem samples of 36 subjects who had died either from chronic heart failure (CHF), from Alzheimer dementia (AD) or from Parkinson’s disease (PD). We studied samples from hypothalamus, pituitary and adrenal glands. We further examined myocardial biopsies (n=430) from patients afflicted with various cardiac ailments. Serum samples of 81 patients diagnosed with atrial fibrillation (a-fib), of patients who received chronic treatment with high doses of GC agonists (n=18) and of matched healthy controls (n=65) were assessed for levels of GC, CBG and N-el with special emphasis on circadian rhythm. Levels of circulating IL6 and TNF alpha were determined by immunoassays. Measurements of blood levels of reactive oxygen species (ROS), antioxidative enzymes (endogenous peroxidase like activity EPLO) and of mitochondrial membrane markers were used to determine levels of oxidative stress response in context with age, neurodegenerative diseases and cardiac health.

Results. Hypothalamic nuclei of healthy controls contained numerous neurons immunoreactive for CRH, frequently colocalized with CBG. GCR was mostly absent in these cells. Hypothalami of AD and PD patients were mostly devoid of CBG but showed high expression of GCR. Similar observations were made in CHF patients. Only in CHF patients histology of anterior pituitary lobe and of adrenal cortex was impressively altered. Serial semithin sections of myocardial biopsies revealed expression of CBG and N-el in Purkinje fibres. Again these cells were GCR negative. Serum GC levels were significantly elevated in a-fib patients. Circadian changes of systemic GC levels appeared to be suppressed while levels of CBG and of N-el were decreased in these subjects. Similar observations were made in patients subjected to longterm treatment with high GC doses. These patients are known to have increased risk for cardiac ailments and for neurodegenerative diseases. Amounts of ROS were elevated in most of the patient samples while EPLO was diminished. Levels of TNF and of IL6 were elevated in samples that showed high GC but low CBG and low N-el. These effects were more pronounced in elderly patients.

Conclusion. GCs link central and systemic stress response. They also seem to play multiple roles in control of inflammatory- and oxidative
stress. Myocardial aging and certain neurodegenerative ailments are in part linked to GC toxicity, which seems to initiate impairment of mitochondrial performance. Purkinje fibres of the cardiac excitation system are particularly vulnerable to such changes, triggering arrhythmia. GCs readily cross the blood brain barrier to act on GC target regions and to affect the brain functionally and structurally. CBG is expressed in most of the GC target organs to buffer steroids and to enhance GC bioavailability thus increasing anti-inflammatory capacity and glucose utilization. A membrane receptor for CBG/GC has been postulated which in part could account for known non-genomic GC effects including the blockade of cytokine secretion. Nuclear GCR and direct genomic effects may be less important in this context in healthy subjects while down regulated CBG and up regulated GCR seems to correspond with degeneration of cells and tissues. Intracellular CBG could be important for cytoplasmic steroid transport to and from the mitochondrial compartment. Free radicals generated in course of mitochondrial metabolic activity are handled by the cells antioxidative capacity, which also depends on GC and CBG. Cellular ageing and cell death may therefore largely be influenced by chronic actions of various factors of the stress response cascade. This should be of great significance for early diagnosis, prevention and therapy of certain cardiac ailments and neurodegenerative diseases.

References:
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GENERAL MECHANISM FOR PEPTIDE REGULATION OF GENE EXPRESSION, PROTEIN SYNTHESIS AND HUMAN VITAL RESOURCE

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Relevance. Possible increase in life expectancy up to its specific limit (110-120 years) seems to be one of the most urgent tasks contemporary physicians, gerontologists and molecular biologists focus on. Biological reserve of human lifespan does not materialize completely due to the influence of various adverse factors. This is related to disturbed biorhythms, alterations in gene expression, protein synthesis and decreased functions of body main systems. The use of peptide bioregulators (KHAVINSON PEPTIDES®) is one of scientifically based methods of increasing life span. Six peptide preparations (Thymalin - from thymus, Epithalamin - from pineal gland, Cortexin - from brain cortex, Samprost – from prostate gland, Retinalamin - from retina and Thymogen – a synthesized dipeptide) have been registered as medications, while the other 50 – as different kinds of bioregulators. Ascertainment of cellular and molecular mode of action is an important issue in biology and medicine.

Results. Investigation of peptides on 17 different types of organisms resulted in a hypothesis of a unified peptide regulation mechanism of gene expression, protein synthesis in a wildlife. It was revealed that short peptides (2-4 amino-acid residue) can penetrate into cell and then into karyon. Complementary interaction of short peptides with promotor zone of genes is a signal for transcription, translation and protein synthesis. The
sequence of these processes leads to body functions improvement and human life recourse increase.

**Effect of peptides on plants.** Short peptides modulate action of endonucleases (WEN1, WEN2) isolated from fraction of cytocindery vesicles of coleoptily wheat. Short peptides regulate gene expression of growth, development and differentiation of *Nicotiana tabacum* (tobacco).

**Effect of peptides on bacteria.** EDG peptide suppressed growth of the *Helicobacter pylori* isolates received from patients. This effect was observed with peptide dropping application on a surface of Petri cup with agar on which the strain of *Helicobacter pylori* grew. Adding peptide in agar was similar to action on the strain of *Helicobacter pylori* of antibiotic of Tetracyclinum.

**Effect of peptides on insects.** EDR peptide provided a stimulating effect on short-term and long-term memory maturing for honey bee *Apismelliferacarnica Pollm* with basically low conditional reflexes background. EDR peptide normalized the memory and locomotory behavior, regulation of gene (limk, rok, park) expression at the mutant line drosophila agn$^{ts3}$ (model of a Parkinson disease with dementia and Levi's bodies). AEDG and KE peptides increased antioxidant activity and contributed to increase in life expectancy of drosophila.

**Effect of peptides on Amphibia.** Adding retina peptides to pluripotent cells of ectoderm (of an early gastrula of *Xenopus laevis*) led to emergence of retina cells and pigmentary epithelium. Adding other short peptides to pluripotent cells of ectoderm led to emergence of various tissues for the same experimental group. That defines ability of peptides to stimulate processes of cell differentiation.

**Effect of peptides on birds.** Adding AEDG and KE peptides in embryonic cultures of chicken's retina cells contributed to induction of differentiation of various types of retinal neurons (activation of protein Brn3, Pax6, Prox1, Vsx1 expression) and a pigment epithelium (activation of transthyretin synthesis). Thymalin showed a normalizing effect on indices of nucleic and protein metabolism. This medicine showed the same effect on T-lymphocytes activity in a chicken immunity suppression model (exposure to cyclophosphahan).

**Effect of peptides on rodents.** Administration of Thymalin, Epithalamin and also KE, EW, AEDG peptides to mice and rats led to an increase in average and maximum life span. The maximum increase in life
span by 42.3% was registered in case of AEDG peptide treatment of CBA mice. The peptides isolated from the pineal gland and thymus decreased incidence of malignant tumors (spontaneous or induced by radiation or carcinogens) in mice and rats 1,4 - 7-fold. Administration of AEDG and KE peptides to transgenic mice inhibited her-2/new (breast cancer in human) gene expression 2-3,6 times. Inhibition of gene expression was followed by reduction of tumor’s diameter. Effect of KE, EW, AEDG, AEDP peptides on expression of 15247 heart and brain genes of mice was researched. It was established that each peptide specifically regulated expression of a certain group of genes. EDG peptide regulated MRNK expression of various genes coding proteins of cellular metabolism (SOD, TNFα and COX-2) in a model of induced stomach ulcer in rats. Administration of Epithalamin to old female rats led to restoration of their reproductive function followed by pregnancy in a number of rats. This fact is unexampled.

**Effect of peptides on primacies.** Administration of AEDG peptide (Epitalon) to old monkeys (Macaca mulatta, 20-26 years) contributed to full restoration of melatonin secretion to the norm in young animals (6-8 years) (initially the level of melatonin was reduced twice). A rhythm of adrenal gland main hormone – cortisol secretion was restored to normal range in the same group of old monkeys after peptide treatment. Administration of Epitalon or Epithalamin to an old animal led to restoration of glucose tolerance (which falls down during aging).

**Effect of peptides on human.** Application of Epithalamin and Thymalin led to decreased aging rate in elderly people suffering cardiovascular pathology (in a randomized comparative study). It is established that long-term use of Epithalamin (6 courses within 3 years) reduces aging of cardiovascular system, restores physical working capacity, exerts normalizing effect on a daily production of melatonin, carbohydrate and lipid metabolism. Epithalamin geroprotective effect was confirmed by reduction of mortality according to Kaplan — Meier survival curves.

General molecular mechanism underlies peptide effect on vitality and lifespan. Regulatory peptides increase the content of euchromatin in karyon providing for transcription of a bigger number of genes. Transcription occurs more intensively and protein synthesis increases. Adding AEDG peptide in culture of human pulmonary fibroblasts led to the induction of telomerase gene expression and a 2.4-fold increase in telomere length. It has been found out that short peptides stimulate tissue-
specific differentiation and proliferation of cells and decrease apoptosis in cell cultures in aging. Peptides’ activity is related to the structure of its molecule. AEDG peptide in pluripotent cells stimulated the emergence of the nerve tissue, KEDP peptide (Prostamax) affected epidermis, KE peptide influenced retina. Peptides induced differentiation of human embryo bone marrow stem cells (CD34+) in CD14+ cells (myelocytes), cell CD3+ (precursors of T-lymphocytes), cell CD4+ (T-helpers), cell CD8+ (cytotoxic T-lymphocytes). KEDW peptide stimulated expression factor of differentiation of acinal (Pdx1, Ptfla) and insular (Pdx1, Pax6, Pax4, Foxa2, NKx2.2) cells of the pancreas in "young" and "old" cultures. The peptide reduced the expression of proapoptotic protein p53 and strengthened the expression of proliferative markers Ki67 and PCNA. KE and AEDG peptides induced differentiation of embryonic retina cells into neurons (Brn3, Prox1, Vsx1, Pax 6) and pigmented epithelium (TTR). Molecular modeling elucidated interaction of peptides with certain DNA regions. Complementary fixation models of 20 peptides with the DNA sequences (for example, AEDG peptide with ATTTC, GTTTC, CTTTC and KE peptide with CGAG) were calculated by the method of molecular docking.

**Conclusion.** The research of biological activity of peptides in different organisms’ types pointed out changes in gene expression and normalization of protein synthesis. That was followed by the improvement of different functions of organs and systems, including a decrease in tumor incidence, a life span increase. The peptide mechanism of vitality regulation in different species must be underlying the bases of evolution.

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EFFICACY OF LONG-TERM APPLICATION OF PINEAL GLAND PEPTIDES IN ELDERLY PATIENTS: 15-YEAR FOLLOW-UP STUDY

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Relevance. During recent years extensive discussions have been made about the significance of pineal gland (PG) functioning changes in aging and age related pathologies [1-3]. The decrease of melatonin production in aging is primarily linked with functional rather than structural changes of the PG [1,4]. Hence, recovery of the pineal function in old age merits attention. In animals an improvement of melatonin (MEL) production was demonstrated under influence of PG peptide preparation “Epithalamin” [5], which was developed in the St. Petersburg Institute of Bioregulation and Gerontology. Many works stated that Epithalamin slowed age-related changes in neuroendocrine control and reproductive function, favorably influenced on the metabolic processes and immune system, improved organism’s antioxidant status, reduced incidence of malignant tumors and increased average and maximal animal lifespan [6,7]. However, the data about survivorship of elderly patients receiving PG peptides are lacking. Purpose was to evaluate the effects of PG peptides on the circadian rhythm of plasma MEL level in elderly subjects, and to analyze the results of long-term use of PG peptides in elderly patients with coronary artery disease.

Methods. The protocol and research program were approved by local ethic committee. The patients gave their signed informed consent to participate in the study. Altogether 79 elderly patients took part in clinical studies.

The following inclusion criteria were considered:
1) Coronary artery disease (CAD), 2nd functional class stable angina; 2) Low nocturnal blood plasma MEL level (below 40 ng/l at 3 am).

The patients, who were selected in 1992 year, were randomized into two similar groups according to their age, gender and initial functional status. During the next 15 years, both groups of patients were administered same treatment for coronary artery disease: acetylsalicylic acid (100-125 mg/day), angiotensin-converting enzyme inhibitors, beta-blockers, statins, and, if necessary, nitrates. In addition to this basic therapy, the patients of 1st group (n=39) received 6 courses of PG peptides (Epithalamin) during three years: from 1992 to 1996. PG peptides were administered in the morning along the following scheme: 10 mg with 2 ml saline intramuscularly, every three days, five injections per one course, with six-month intervals between courses. Overall, the PG peptides dose per course was 50 mg and total dose was 300 mg. The patients of 2nd group (n=40) received only basic therapy preparations between 1992 and 2007 years. The PG peptides were not administered.

Examination program included the following assessments:
- Plasma MEL concentration assessed by radioimmunoassay (DPC, USA) at different times of the day (3 and 9 a.m., 3 and 9 p.m.);
- Exercise working ability (bicycle ergometry);
- Functional age by working ability [8];
- Blood plasma glucose concentrations at fasting and 2 hours after standard oral glucose tolerance test; and
- Serum concentrations of total cholesterol (TC) and low density lipoprotein cholesterol (LDL);

Besides, over 15 years we have tracked mortality in these groups and verified causes of death. Data were analyzed by using the method for life table construction, survival curves and Kaplan-Meier estimates. Statistical significance of the differences was assessed by means of the Breslow (generalized Wilcoxon) and Log Rank (Mantel-Cox) statistical tests.

**Results.** At baseline, all elderly patients in both groups had low nocturnal plasma MEL levels (< 40 ng/l). Administration of PG peptides led to a significant two-fold increase of nocturnal plasma MEL level as well as to restoration of MEL circadian rhythm. These changes were observed after the first course and persisted during long-term use of PG peptides. At the same time, no changes in plasma MEL concentrations were observed in the 2nd group patients. The functional age of elderly patients at baseline
exceeded their chronologic age by 10 years or more. So, we registered the accelerated type of aging in them. After three years of treatment with PG peptides, the functional age of elderly patients did not differ from that at baseline. Moreover, the degree of aging, that is the difference between functional and chronologic age, was reduced by 3.6 years in this group (p<0.05). The functional age of control group patients, who did not receive PG peptides, increased within 3 year and the degree of aging during this period increased by 4.5 years (p <0.05). The functional age of patients in both groups was again assessed in the year 2006. It appeared that functional age of patients receiving previously PG peptides was significantly lower in comparison with non-receivers of PG peptides. These results may indicate about anti-aging effect of PG peptides in elderly patients. There were also beneficial changes in the physical performance during long-term use of PG peptides. After first course of treatment with PG peptides the threshold work load power increased significantly in 60% of patients with an average of 21%. In patients, who did not receive PG peptides, the physical performance remained unchanged. During 3-year use of PG peptides the physical performance remained at a high level but in the control group it decreased significantly. Prior to treatment with PG peptides 56% of patients had impaired glucose tolerance. After 3-year treatment such disturbances were detected only in 24% patients. In control group, the frequency of impaired glucose tolerance did not change (48 and 42% of cases, respectively). Favorable changes in lipid spectrum of blood serum were registered due to long-term use of PG peptides: the levels of total cholesterol and low density lipoprotein cholesterol were significantly reduced. In patients, who were not treated with PG peptides, the TC and LDL-C concentrations over a 3-year observation period increased significantly. After the expiry of a 3-year application of PG peptides, there was a long-term follow-up of both groups of patients. During twelve years period they were constantly receiving basic therapy. By 2007 year there were only 16 of 40 survivors (40%) in the control group and 26 of 39 survivors (66.7%) among those who had previously received PG peptides. Construction of life tables and survival curves showed a statistically significant decrease in the deaths from all causes in the patients who previously received the PG peptides. In the group treated with PG peptides, myocardial infarction and stroke were the causes of death for 6 of 13 patients (46.2%). In the control group 20 of 24 patients (83.3%) died from
these causes. Consequently, the long-term use of PG peptides significantly reduced mortality related to cardiovascular disease.

**Conclusion.** The results of our study have shown that long-term use of PG peptides (Epithalamin) slows the rate of aging in elderly CAD patients. The pineal peptides promote restoration of daily rhythms of MEL production and reduce the loss of physical performance with age. They also produce normalizing effects on carbohydrate and lipid metabolism. A significant decrease in the mortality rate was detected in the group of patients receiving PG peptides. Our experience shows that long-term use of PG peptides can be recommended as part of complex therapy of elderly patients with cardiovascular diseases.

**References:**

EXPERIENCE OF THE CLINICAL APPLICATION OF PEPTIDE BIOREGULATORS

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Relevance. Peptide medicines derived from various organs have been called Cytomedines (Thymalin, Epithalamin, Cortexin, Retinalaminum, Prostatilen, etc.). These medicines render therapeutic effect at pathological conditions on that organ from which they are received. Short peptides - Cytogenes possess same effect (Thymogen, Epitalon, Vilon, etc.). Cytomedines and Cytogenes possess immunomodulatory effect, increase antioxidant activity, strengthen reparative processes, normalize blood coagulation.

Methods. The effect of peptide bioregulators on a condition of various systems and functions of an organism, including cellular and humoral immunity, inflammatory acute phase protein, vascular thrombocytic hemostasis, blood coagulation and fibrinolysis was researched during 30 years at more than 10000 patients with different diseases. Thymalin and Thymogen was applied for acute pneumonia, chronic obstructive bronchitis, acute respiratory diseases, chronic liver diseases, purulent peritonitis, burning and cold injury, bone fractures, meningococcal infection, sacred fire, typhoid, dysentery, hepatitises A and B, diabetes mellitus of 1 type, psoriasis, eczema, anemia and gestosis of pregnant women, periodontal disease, stomatitis. Cortexin applied in case of ischemic hemorrhagic stroke, epileptiform neuralgia, traumatic brain injury, diabetes of 1 type, atrophy of optic nerve, macular dystrophy, clottage of the central retinal artery. Retinalamin applied in ophtalmology for diabetic retinopathy, retina vein thrombosis, combined damage of retina and optic nerve, exudative-hemorrhagic damages of retina, for late stages of age macular dystrophy. Prostatilen applied at acute and chronic prostatitis. The clinical condition of all the patients was analyzed before and after the treatment. And also in remote terms. Except the standard laboratory and tool methods of testing we analyzed the condition of cellular and humoral immunity, lipid peroxidation, indicators of a blood coagulation, defined concentration anti-inflammatory cytokines (IL-1α, ILβ,
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IL-8, TNFα, IL-4, IL-10, TGFβ), the content of inflammatory acute phase protein.

**Results.** Improvement of clinical condition was noticed in majority of patients after peptide medicines application. Acute period of disease reduced, intensity of pain syndrome decreased, amount of complications abated either after the usage of peptides. Remission terms were enlarged at chronic diseases. Patients at the post-operation period had no complications and the terms of stay in a hospital were reduced. Usage of peptides for patients with secondary immunodeficiencies led to normalization of quantity of CD4⁺, CD8⁺, CD16⁺, CD19⁺ lymphocytes and the maintenance of immunoglobulins. Pro-inflammatory cytokines increased in blood by influence of peptides in case of acute inflammatory and infectious diseases. Application of Thymalin, Thymogen, Epithalamin and Cortexin also contributed to decrease in pro-inflammatory cytokines and increase in anti-inflammatory cytokines. There was found normalization of inflammatory acute phase proteins level in blood by the effect of bioregulators and the indicators of antioxidative system were improved. Majority of patients were noticed excessive hypercoagulation with increase in fibrogenic and D-dimer. Activity of antithrombin III and protein C decreased in case of burning or cold injury, bone fracture, peritonitis, lung abscess, inflammatory diseases proceeding with a background of atherosclerosis or diabetes. Usage of peptide bioregulators for these patients led to normalization of the main indicators of blood coagulation, rejuvenation of anticoagulants level and intensification of fibrinolysis. Usage of Thymalin or Thymogen in combination with Epithalamin for pregnant women abated expression of intoxication, and increased probability of giving a birth to a healthy child in case of premature birth or prolonged pregnancy. Usage of peptide bioregulators for diseases of eye retina contributed to quick elimination of acute pathological process, rejuvenation function of retina and led to increase in visual acuity. Precautionary usage of Thymalin or Thymogen in extreme Siberian climate was followed by decrease in acute respiratory diseases of adults and children by 2 – 4 times. Cytomedines and Cytogenes are epigenetic regulators of congenital and adaptive immunity, system of lipids peroxidation, heat shock protein, major factors of hemostasis system, anticoagulants and fibrinolytic agenes. All mentioned facts determine positive effect on the diseases of different etiology. It should be noted that no one among all the patients had any allergic...
reaction or side effect before and after peptide bioregulators administration.

**Conclusion.** Use of Cytomedines and Cytogenes at various diseases leads to normalization of cellular and humoral immunity, blood coagulation and fibrinolysis, antioxidant system that is followed by improvement of patient’s condition, reduces amount of complications and disease terms.

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**Relevance.** Expression of genes participating in cell differentiation is regulated by transcription factors. They stimulate or suppress the synthesis of regulatory proteins through interaction with specific nucleotide sequences of the gene. Synthesis of proteins (including differentiation factors) in cells decreases with age and under pathological conditions, which leads to dysregulation of gene expression and a decrease in functional activity of organs and tissues [1-5]. Peptides Pancragen (Lys-Glu-Asp-Trp), Bronchogen (Ala-Glu-Asp-Leu), and Vesugen (Lys-Glu-Asp) created at St. Petersburg Institute of Bioregulation and Gerontology promote functional recovery of the pancreas and organs of the respiratory and cardiovascular systems, respectively. However, their effect on expression of differentiation factors has never been studied. Here we studied the effects of Pancragen, Bronchogen, and Vesugen on the expression of transcription factors WEDC1, Hoxa3, and CXCL12 in cultures of human lung and pancreatic cells and fibroblasts during aging.

**Methods.** Experiments were performed on embryonic cultures of acinar cells of the pancreas MIA PaCa-2 (Institute of Cytology, Russian Academy of Sciences) and bronchial epithelium FLECH (Research Institute of Influenza, Ministry of Health and Social Development) of passages 1, 7, and 14. Cultures of passages 1, 7, and 14 were considered as young, mature, and aged cultures, respectively (according to recommendations of International Association of Cell Culture Studies, San Francisco, USA, 2007). We also used culture of human prostate fibroblasts (clone CX3CR1, Cambrex Bioscience) passing 1, 4, and 7 passages, which corresponded to 1, 7, and 14 passages for embryonic cultures. Physiological saline or one of the test peptides was added to control and experimental cultures respectively: Bronchogen to culture of bronchial epithelial cells, Pancragen
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to culture of acinar cells of the pancreas, and Vesugen to fibroblast cultures. Preliminary experiments showed that peptides not specific for the tissue (Bronchogen for acinar cells, Pancragen for bronchial epithelial cells, and Cardagen for fibroblasts) do not affect the expression of transcription factors. Acinar cells of the pancreas were cultured in 25-cm² flasks (JetBiofil) in 5 ml DMEM supplemented with L-glutamine (Biolot), 15% fetal calf serum SC-BIOL (Biolot), and 1% penicillin-streptomycin at 37°C. Bronchial epithelial cells were cultured in MEM supplemented with L-glutamine (Biolot), 10% fetal calf serum SC-BIOL (Biolot), and 1% penicillin- streptomycin. Fibroblasts were cultured in 24-well plates (Costar) at 37°C and 5% CO₂ in RPMI-1640 (Flow) supplemented with 10% ECS (Serva), 300 pg/ml L-glutamine (Flow), 0.02 M HEPES buffer (Sigma), and 100 pg/ml gentamicin (Pharmakhim). Initial concentration was 10⁶ cell/ml. For immunocytochemical study, primary monoclonal antibodies WEDC1 (1:150, Vectorlab), CXCL12 (1:200, Vectorlab), and Hoxa3 (1:150, “Vectorlab”) and secondary antibodies, biotinylated anti-mouse immunoglobulins (Novocastra) were used. Permeabilization was performed with 0.1% Triton-X100. The reaction was visualized with horseradish peroxidase and diaminobenzidine (EnVision Detection System, Peroxidase/DAB, Rabbit, Mouse). The results of immunocytochemical analysis were evaluated morphometrically using a computer- assisted microscopic image analysis system consisting of Nikon Eclipse E400 microscope, Nikon DXM1200 digital camera, and Videotest-Morphology 5.0 software. In each case, at least 5 fields of view were analyzed at >=200. The area of expression was calculated as the ratio of the area occupied by immunopositive cells to the total area of cells in the field of view and expressed in percents. This parameter characterizes the intensity of the synthesis of the studied transcription factors in cells. Immunofluorescent confocal microscopy was performed on non-fixed cell suspensions. Cell smears were treated with primary antibodies (Torrey Pines Biolabs) to CXCL 12 and WEDC1 proteins. Expression of signal molecules was visualized using Vector Red kits (Vector Lab) for immunofluorescent visualization of alkaline phosphatase. Levamisole (1.25 mM) was added during incubation with alkaline phosphatase of the kit for blockade of endogenous enzyme. The preparations were examined under Leica TCS SP5 confocal microscope at *400 and *1000 using an MRC-1024 system equipped with LaserSharp 5.0 software (Bio-Rad) for confocal image analysis. In each case, the area of
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Expression was analyzed in 10 fields of view at *400. Statistica 7.0 software was used for comparison and evaluation of between-group differences.

**Results.** Expression of CXCL12 and Hoxa3 markers was verified in control cultures of pancreatic acinar cells and bronchial epithelial cells and CXCL 12 and WEDC1 markers in fibroblast culture. In culture of pancreatic acinar cells, the area of Hoxa3 expression significantly decreased with age. In young cultures, the area of Hoxa3 expression was 1.2- and 2-fold larger than in mature and aged cultures. The area of CXCL 12 expression decreased by 22 and 12% in mature and aged cultures, respectively. Area of WEDC1 expression in fibroblast cultures of passages 4 and 7 was reduced by 60 and 81% in comparison with passage 1. Addition of Pancragen to the culture of pancreatic acinar cells reduced the area of expression of differentiation factor CXCL 12 by 21% in young cultures and by 86 and 72% in mature and aged cultures, respectively. Expression of Hoxa3 significantly increased after addition of Pancragen: by 60% in young cultures and by 90% in mature cultures. The most pronounced effect was observed in aged cultures: Hoxa3 expression area increased by 2.8 times in comparison with the control. Enhanced Hoxa3 expression was also observed after addition of Bronchogen to the culture of bronchial epithelial cells: by 1.7, 1.4, and 1.7 times in young, mature, and aged cultures. Bronchogen had no effect of the expression of differentiation factor CXCL12 in all studied passages of bronchial epithelial cells. Addition of Vesugen to fibroblast culture increased expression of differentiation factors CXCL12 and WEDC1: in young cultures by 1.2 and 1.5 times, respectively, in mature cultures by 8 and 8.2 times, and in aged cultures by 7.6 and 16 times.

**Conclusion.** Senescence of cell culture of bronchial epithelium, pancreatic acinar cells, and prostate fibroblasts is associated with a decrease in the expression of differentiation factors CXCL12, Hoxa3, and WEDC1, which attests to reduced differentiation capacity of these cells and can be a cause of reduced functional activity of the pancreas, bronchi, and prostate during aging. Stimulating effects of peptides Pancragen, Bronchogen, and Vesugen on the glandular and endothelial tissues was reported previously. In our experiments, these peptides induced the expression of differentiation factors CXCL12, Hoxa3, and WEDC1 in cells of the pancreas, bronchi, and prostate fibroblasts. Since these differentiation factors participate in transcription, we can hypothesize that stimulation of
cell differentiation by short peptides is mediated by an epigenetic mechanism underlying peptide regulation of aging processes.

Thus, the short peptides Pancragen, Bronchogen, and Vesugen have geroprotective features and may be considered as potential substances for profilaxis and treatment of the diseases, associated with aging.

References:
MOLECULAR MECHANISMS OF PEPTIDES ACTION ON CELL SENESCENCE

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Relevance. Expression of genes participating in cell differentiation is regulated by many signaling molecules (hormones, transcription factors, cytokines, etc.). Synthesis of proteins (including differentiation factors) in cells decreases with age and under pathological conditions, which leads to dysregulation of gene expression and a decrease in functional activity of organs and tissues. Melatonin (MT) is the main hormone of the pineal gland, regulating the biological rhythms of the organism and involved in mechanisms of aging by coordinating the immune and endocrine system activities (1,2). During aging, the MT level in blood in humans reduces significantly in comparison with young age (3,4). MT could be considered as the target for short peptides action. Because the molecular and cellular mechanisms of MT synthesis recovery under the effects of short peptides are unknown, we have studied the effects of Epitalon and Vilon peptides on the synthesis of melatonin and factors involved in this process, arylalkylamine-N-acetyltransferase (AANAT) enzyme and pCREB transcription protein in rat pinealocyte culture.

Methods. Pinealocytes were isolated from the pineal glands of adult male Wistar rats decapitated with guillotine at 10:00. Dissociated pinealocyte culture was grown in Petri dishes on a sublayer of slides in a C0₂ incubator at 36.7°C and 5% C0₂ for 3 days. Culture material was then divided into 3 groups: 1) control specimens without treatment of any kind; 2) specimens treated with NE in a concentration of 1 pg/ml (positive control); and 3) specimens treated with one of the studied peptides in a concentration of 100 ng/ml. The chosen concentration corresponded to the physiological concentration exhibiting biological activity. The cultures were treated with the studied bioactive substances for 3 h. Peptides were synthesized at Saint Petersburg Institute of Bioregulation and Gerontology: Vilon (Lys-Glu) and Epitalon (Ala-Glu-Asp-Gly). Pinealocyte cultures for
immunocytochemical study were fixed in 95% ethanol. Immunocytochemical study was carried out with antibodies to pCREB (Upstate Biotechnology Inc., 1:500) and AANAT (Sigma, 1:1000). The reaction was visualized with peroxidase (Peroxidase VECTASTAIN Elite ABC Kit Standard, Alexis Biochemicals). Densitometric analysis of nuclear expression of pCREB and cytoplasmic expression of AANAT in pinealocyte culture was carried out using VIDAS image analyzer (Kontron) by optical density. Melatonin secretion from pinealocytes into cell medium supernatant was measured by ELISA with IHF GmbH kit (Hamburg). The data were statistically processed by Statistica 7.0 software. All experiments were carried out in accordance with regulation for studies on experimental animals, approved by Instruction of the EEC Scientific Council 86/609/EEC.

Results. Studies by the immunocytochemical method showed a positive reaction to pCREB and AANAT for the majority of syncytium-forming cells in the control pinealocyte culture. After peptide treatment only some cells remained immunopositive to these markers; these cells were located in clusters forming round cell chains. Studies of optical density of pCREB transcription protein expression in pinealocyte culture showed no changes in this parameter in the control over 3 h. Norepinephrine treatment (positive control) promoted a somewhat 16-fold increase of pCREB expression during the first 2 h and 4-fold after 3-h incubation. Epitalon peptide promoted stable stimulation of pCREB expression (4-fold throughout the entire period of incubation. Vilon increased this parameter depending on incubation duration. After 1 h pCREB expression increased 7-fold under the effect of vilone, while after 2- and 3-h incubation the expression of this transcription factor reduced to the control level. These data indicated that addition of NE and Vilon into pinealocyte culture induced a short-term stimulation of pCREB expression, while Epitalon treatment caused a more lasting effect of this kind. The data on potentiation of the effects of peptide and NE added into pinealocyte culture simultaneously are interesting; in our study the effects of Epitalon and Vilon on pCREB expression were much similar. Optical density of AANAT expression in the control somewhat increased over 2-3 h of observation. Vilon stimulated AANAT expression during 1-h incubation. However, prolongation of incubation showed that Vilon effect on AANAT was short-term. Epitalon stimulated the expression of AANAT throughout the entire period of incubation and its effect was higher than that of NE.
Hence, the findings of immunohistochemical studies indicate a significant effect of Epitalon (pinealocyte tropic peptide) on the expression of pCREB transcription factor and AANAT enzyme in pinealocyte culture. Presumably, Epitalon effect on AANAT and pCREB underlies the peptide regulation of pineal cell activity.

**Conclusion.** These results suggested study of the direct effect of Epitalon on the level of MT released by pinealocytes into culture medium. Norepinephrine stimulated an increase of MT concentration in culture medium from 1897±273 to 7000±1350 pg/ml (3.5 times; p<0.05). Epitalon increased significantly (p<0.05) MT level, which reached 2451±121 pg/ml. Epitalon obviously stimulated the expression of the key molecules involved in MT synthesis by pinealocytes. Epitalon stimulated the synthesis of AANAT present in the cell cytoplasm and the expression of pCREB located in the nucleus. Hence, intensification of MT synthesis under the effect of Epitalon was explained by its modulation of the cellular cytoplasmic and nuclear structures. Potentiation of NE effect on the expression of AANAT and transcription factor pCRHB trigger-related to cyclic AMP, and the formation of cell clusters under the effect of Epitalon indicated that Epitalon stimulated the MT synthesis by pinealocytes.

Thus our investigation allows to consider Epitalon as promising geroprotective substance for profilaxis of aging-associated diseases.

**References:**
EFFICACY OF PINEAL GLAND PEPTIDES (ENDOLUTEN) IN LOW DOSE LONG TERM PROTOCOL AND HIGH DOSE SHORT TERM PROTOCOL IN TWO SUBJECTS

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Relevance. Studies have shown pineal gland peptides, Epitalon, Epithalamin and Endoluten, developed at the St. Petersburg Institute of Bioregulation and Gerontology, induces telomerase activity and telomere elongation in human somatic cells. These peptides have been proven to regulate pineal gland function and increase lifespan in animals and provide geroprotective effects in humans. The purpose was to evaluate the effect of Endoluten on a low dose long term (2 year) protocol and on a high dose short term (6 month) in inducing telomerase activity and telomere elongation in two older age humans.

Methods. Two healthy subjects, a male and female were selected. The male, age 68 at inception, was randomly selected for the low dose long term program. The female, age 60 at inception, was selected for the high dose short term program. Neither subject was taking any pharmaceutical medications, but only poly vitamins. Both subjects appeared healthy and reported no medical issues. Telomere testing was performed at SpectraCell Laboratories (Houston, Texas, USA) using Quantitative Real Time PCR (polymerase chain reaction) technology. From samples the white blood cells were broken apart, the DNA was extracted and the telomeric DNA was amplified into a measurable signal. This signal was compared to a control gene of known length and a Telomere Score was generated.

Male protocol:
Twelve boxes, each containing twenty capsules 10 mg Endoluten peptide were administered during a twenty-four month period. Dosage was two capsules for first ten days of each alternating month. Time frame was June 2014 to June 2016.

Female protocol:
Eighteen boxes, each containing twenty capsules 10 mg Endoluten peptide were administered during a six months period. Dosage was two capsules each day for the entire six months.
There was a follow up addendum of two months with only the female subject in which an additional six boxes were administered at dosage of two capsules per day.

**Results.** LOW DOSE LONGER TERM (2 YEAR) ENDOLUTEN PROTOCOL (Male subject)

Baseline (June 2014), male subject telomere base pairs measured 6.38 (6,380bp) positioning subject at 44th percentile relative to male population of same age (68).

Results of the low dosage protocol after twenty-four months of Endoluten application (June 2016) were subject’s telomere base pairs measured 6.54 (6,540bp), an increase of 160 base pairs, positioning subject at 55th percentile relative to male population of same age (70).

**HIGH DOSE SHORT TERM (6 MONTH) ENDOLUTEN PROTOCOL (Female subject)**

Baseline (June 2016), female subject telomere base pairs measured 6.84 (6,840bp) positioning subject at 49th percentile relative to female population of same age (60). Results of the high dosage protocol after six months of Endoluten application (January 2017) were subject’s telomere base pairs measured 7.38 (7,380bp), an increase of 540 base pairs, positioning subject at 69th percentile relative to female population of same age (60). Two month follow up to original six month protocol resulted in further telomere lengthening. Extended protocol telomere testing was performed by Telomere Diagnostics (TeloYears-Elizabeth Blackburn) also using Quantitative Real Time PCR (polymerase chain reaction) technology. Results reported placed subject at 89.3 percentile relative to female population of same age (60). TeloYears estimated subject’s telomere biological age as twenty-eight years (28).

**Conclusion.** The results obtained presented evidence that pineal peptides, as constituents of Endoluten, acted as telomerase activators in human subjects. Application of the pineal peptides on subjects with below average telomere indices resulted in significant lengthening of initially reduced telomeres in somatic cells of elderly humans, thus bringing them to normal, or above normal, indices. Endoluten application has significant geroprotective effect as it lengthens human telomeres. Endoluten, in both long-term regimen in lower doses and by short courses with increased doses, is recommended for regular use by older people to lengthen telomeres.
References:


Relevance. Compacted (heterochromatinized) chromatin regions occur in aging and are generally hypoacetylated and methylated indicators of an epigenetic change. Hypermethylation causes heterochromatinization and thus results in gene silencing. The fact that such histone modifications are reversible – offers potential usage in therapy (1,2). In the present investigation are considered eligible the modification of heterochromatin (total heterochromatin, constitutive – pericentromeric and telomeric heterochromatin, nucleolus organizer regions - NORs and facultative heterochromatin) under the influence of peptide bioregulators (tetrapeptides Ala-Glu-Asp-Gly-Epitalon; Lys-Glu-Asp-Ala – Livagen; Ala-Glu-Asp-Pro – Cortagen and dipeptide Lys-Glu - Vilon) in lymphocytes culture old individuals.

Methods. We used molecular-cytogenetic methods. In lymphocyte cultures were studied: of total heterochromatin with differential scanning calorimeter - (DSC); facultative heterochromatin (sister chromatid exscanges – (SCE) with 5-bromodeoxyuridin (BrdU), activity of ribosomal genes of acrocentric chromosome – NORs (Ag-band), polymorphism of structural pericentromeric C-heterochromatin (C-band) and mutation (chromosome aberrations) (2,3) from individuals at the age of 80 and older.

Description of the preparation. Epitalon (Ala-Glu-Asp-Gly) reinforces the organism’s resistance to stresses, regulating neuro-endocrine system and prolongs the average life expectancy;

Livagen (Lys-Glu-Asp-Ala) increases the average level of protein synthesis in aging, renovates liver proteins and induces the activation of protein synthesis in hepatocytes;

Cortagen (Ala-Glu-Asp-Pro) in humans demonstrated a pronounced therapeutic effect on the structural and functional recovery of the damaged peripheral nerve tissue.
Vilon (Lys-Glu) stimulates lowering for he risk of premature aging, has an antitumor activity and stimulates functioning of the immune system and reparative processes, strengthens the resistance of organismsto stress activities, favours prolongation of the average life span. The bioregulators kindly was provided by professor Vladimir Khavinson (Institute Bioregulation and Gerontology, St. Petersburg, Russia).

**Results.** Differential scanning calorimeter The heat absorption curves corresponding to denaturation processes in intact lymphocytes and in lymphocyte cultures treated by synthetic peptides (Ala-Glu-Asp-Gly; Lys-Glu-Asp-Ala, Ala-Glu-Asp-Pro, and Lys-Glu) indicate that the treatment of cells with synthetic peptides induced heat redistribution and should be attributed to the local decondensation (deheterochromatinization) of loops of up to the 30 nm fibers and partial decondensation of transcribed chromatin transformation of 10 nm filaments into 5 nm filaments in comparison with a healthy 80 year and over. Thus, we can conclude that the synthetic peptide bioregulators (Ala-Glu-Asp-Gly; Ala-Glu-Asp-Pro, Lys-Glu-Asp-Ala and Lys-Glu) unfolds the highest levels of chromatin organization, that induces deheterochronatinization of total (structural and facultative) chromatin in intact cells of old individuals.

**Table.** Influence of peptide bioregulators – Epitalon (Ala-Glu-Asp-Gly), Livagen (Lys-Glu-Asp-Ala), Cortagen (Ala-Glu-Asp-Pro), Vilon (Lys-Glu)
Variability of facultative heterochromatin based on the SCE test

The results of studies on the induction of SCEs by peptide bioregulators (tetrapeptides Ala-Glu-Asp-Gly, Lys-Glu-Asp-Ala, Ala-Glu-Asp-Pro, and dipeptide Lys-Glu) in lymphocyte cultures of aged individuals are shown in Table.

The analysis showed that Epitalon induced a significant increase in SCE counts in A, C, D and G group chromosomes (Epitalon-treated cells from old individuals corresponding to an average of 8.4±0.5 - per cell (for intact cultures of the same individuals, this value was 6.2±0.2 SCE/cell); Livagen (Lys-Glu-Asp-Ala) induced a significant increase in SCE counts in A, B, C, D, E and G groups with statistic relevance (an average of 9.2±0.4 - per cell ); Cortagen (Ala-Glu-Asp-Pro) significantly increased SCE counts in A, C and D chromosomes in comparison with intact cells (an average of 10.1±0.3 - per cell ) and the bioregulator Vilon (Lys-Glu) significantly increased SCE counts in A, C, D, E and G chromosomes (an average of 9.9±0.6 - per cell ).

This data indicates that each of the studied peptide bioregulators has a selective effect on definite chromosomes. The SCE processes do not occur or are less in heterochromatin or heterochromatinized chromosome regions. Therefore, the increased frequency of SCEs under the influence of bioregulators demonstrates the decondensation (deheterochromatinization) of the condensed during the aging chromosome regions, followed by the release of the repressed genes located there (2,3-5).

Transcriptional activity of ribosomal genes. The associative activity of the strands positively correlates with the intensity of Ag-staining that depends on the activity of the ribosomal genes located in NORs. The absence of silver staining (caused by condensation of the stalks) also testifies to the inactivation of ribosomal genes.

The data obtained from the analysis of Ag-positive NORs in cultured lymphocytes, intact and treated with bioregulators, obtained in the case of old donors, are shown in the Table. It was shown that peptide bioregulators (Ala-Glu-Asp-Gly, Lys-Glu-Asp-Ala, Ala-Glu-Asp-Pro, and Lys-Glu) strongly increased the amount of Ag-positive NORs in all acrocentric chromosomes involved or not involved in associations, in comparison with intact cells (p<0.001). In particular, the number of Ag-positive NORs of acrocentric chromosomes involved in association corresponded to Epitalon - 2.32±0.12; to Livagen - 2.49±0.14; to Cortagen - 2.20±0.11 and Vilon-
2.39±0.11 per bioregulators - treated cells, which is significantly higher than the corresponding index for intact culture cells (see Table). Our results are in accordance with the previous data. In particular, hormones, various growth factors and chemicals induced chromosome decondensation (in old age as well) resulting in increased transcriptional activity of nucleolar organizer regions (2,5). An increase in the amount and size of Ag-positive NORs, and an increase in the number of acrocentric chromosomes involved in associations, in the cultures obtained from old individuals and treated with peptide bioregulators, indicated deheterochromatinization of satellite stalks, when compared with control values. This can lead to the intensification of protein synthesis because of the activation of ribosomal genes in aged individuals (4,5).

Heteromorphism of structural pericentromeric C-heterochromatin

The data on heteromorphism of structural pericentromeric heterochromatin (C-segments) in intact lymphocytes and in lymphocytes treated by peptide bioregulators (Ala-Glu-Asp-Gly; Lys-Glu-Asp-Ala, Ala-Glu-Asp-Pro, and Lys-Glu) (of old individuals) for chromosomes 1, 9 and 16 are presented in the Table. The data reflecting variability of large (d and e) and small (a and b) C-segment variant frequencies in separate chromosomes appeared to be equal in the case of the tested bioregulators. It should be noted that in the cells, treated with Cortagen (Ala-Glu-Asp-Pro) and Vilon (Lys-Glu), the distribution of C-segment variants for chromosomes 1, 9 and 16 remained stable and did not differ in old people from the corresponding intact cells for chromosome 1, 9 and 16, respectively (p>0.05). In particular, chromosome 1 and 9 appeared to be deheterochromatinized (the decrease of large bands in size) in Epitalon and Livagen- treated cells. The rate of heteromorphism for appointed chromosomes was significant (p<0.001). A difference from the control indices was noticed for chromosome 16 (p>0.05). It should be noted that in the cells, treated with Cortagen (Ala-Glu-Asp-Pro) and Vilon (Lys-Glu) (Table), large and small C-segment variants in chromosomes 1, 9 and 16 were registered with approximately the same frequency in intact cells, and differences between the indices compared were not significant (p<0.05) (Table).

Conclusion. Epigenetics process – heterochromatinization progress with aging and can deactivate many previously functioning active genes. It blocks certain stages of normal metabolic processes in the cell, which inhibits many specific enzymes and leads to aging pathologies. The
action of genetic systems reveals general rules in the behavior of such systems, such as the connection between the structural and functional interrelationships between the “directing” and “directed” structures. In this respect, it should be noted that heterochromatinised regions in chromosomes can reversed by many physical and chemical agents, hormones and peptide bioregulators. Peptide bioregulators (tetrapeptides Ala-Glu-Asp-Gly; Lys-Glu-Asp-Ala, Ala-Glu-Asp-Pro, and dipeptide Lys-Glu) generally affects the remodeling of facultative heterochromatin and therefore such actions may be helpful in the treatment of aging diseases.

The proposed genetic mechanism responsible for constitutive and facultative heterochromatin remodeling (deheterochromatinization pericentromeric and telomeric region) of old age may lead for the prolongation of the life span and to the development of therapeutic treat of the aging pathologies.

References:
APPLICATION PROSPECTS OF SHORT PEPTIDES FOR NEUROPROTECTION IN ELDERLY PEOPLE

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Relevance. Neurodegenerative diseases define the group of neuropathologies occurring predominantly among the elderly people. The overall incidence of neurodegenerative diseases has a clear tendency to increase since aging of population is observed worldwide. Therefore, the social significance of this problem is obvious. Despite an intensive search for the causes of these disorders, they are still incurable. The main reason of difficulties in the development of medicines for neurodegenerative disorders is the lack of understanding of the precise molecular mechanisms underlying disease progression except rare cases of inherited forms. Therefore, approved drugs for the treatment of these diseases result in modest improvements, primarily by alleviating disease-related symptoms for a relatively short time, however many of these remedies have serious side effects which significantly limit their usage in elder people. Nowadays treatment or even a temporary improvement of symptoms of neurodegenerative disease is very expensive. New group of compounds which are now studied as potential treatment for neurodegenerative diseases called short peptides.

Results. There are some promising neuroprotectors among short peptides - tripeptide EDR (Glu-Asp-Arg, Pinealon), tetrapeptide AEDP (Ala-Glu-Asp-Pro, Cortagen) and a drug heptapeptide MEHFPGP (Met-Glu-His-Phe-Pro-Gly-Pro, Semax). The peptide EDR reduced the level of reactive oxygen species in neuronal cultures under oxidative stress, stimulated the synthesis of serotonin and increased the spines number in the cortico-striatal neurons in a culture model of Huntington’s disease. During the “Morris water maze” test in the experimental model of prenatal hyperhomocysteinemia in rats intramuscular injection of the peptide EDR improved the spatial orientation and learning of their progeny. Adding Pinealon (oral administration) to standard therapy in 72 patients of different ages with the effects of traumatic brain injury and
encephalasthenia resulted in regression of focal symptoms and improvement of speech function. Patients with encephalasthenia after receiving the peptide Pinealon showed less errors while proofreading work and increased the integral index of efficiency. Oral application of Pinealon in athletes promoted the normalization of the antioxidant system's functions, improving level of adaptation to physical stress, the body's fitness and energy metabolism. Oral application of Pinealon in 75 elderly people contributed to improving short-term and long-term memory and decreasing the severity index of mental and emotional and functional condition of the central nervous system. The tetrapeptide AEDP by intraperitoneal injection to rats reduced the intensity of lipid peroxidation in the brain cortex at ischemia model (occlusion of the common carotid arteries). In the rat's electrical painful stress model the peptide AEDP reduced the level of serotonin's metabolite - 5-oxyindolacetic acid and increased the level of serotonin. In old rats (20-24 months) at conditions of amnesia (electroconvulsive shock and ethanol intoxication) the peptide AEDP after intraperitoneal administration improved memory performance and learning. In acute traumatic brain injury in rats the peptide AEDP promoted the normalization of conditioned reflexes, coordination and muscle tone. The heptapeptide MEHFPGP stimulated the growth of neurons of the cholinergic group in experiments on neurons in organotypic cultures. Effect of the Semax on cholinergic neurons was accompanied by increased activity of acetylcholine in the specific structures of the brain that correlates with the improvement of learning and memory formation. In clinical studies the Semax applied in 30 patients with mild to moderate symptoms of disorders of attention, memory, motor skills caused by ischemic cerebrovascular disorders, head injuries without complications from Parkinson's disease and in phase with no signs of dementia. After application of the Semax was an improvement of cognitive, motor and visual-motor skills, attention and the electrical activity of the brain. In another study of the neuroprotective properties of the Semax participated 303 patients suffering from vascular disorders of the brain, Huntington's chorea, and those who underwent surgery after traumatic brain injury. After applications of the Semax in 80% of patients with marked cerebrovascular disorders were indicated by increase of efficiency, improvements of cognitive functions and audio-verbal memory, sleep and mood. In 87.5% of patients with Huntington's chorea in the form of
hyperactivity were observed decreases in headaches, sleep recovery, reducing the number of involuntary movements, improvements in the auditory-verbal memory, mood, health and electrocardiography. In those patients who underwent neurosurgical interventions after a traumatic brain injury were improved short-term and long-term memory.

**Conclusion.** Scientific research is aimed at the maintenance of human's activity during elongated life and prevention measure gain the highest importance worldwide. Due to the observed persistent growth of the population's age in the developed countries the importance of biomedical scientific projects increases, especially those that study the molecular mechanisms of emergence and development of pathologies, typical mostly for elderly people, and also projects devoted to development of effective therapies for such pathologies. Based on the literature and our own data we conclude that short peptides like Pinealon, Cortagen and Semax can be successfully applied for the treatment of a variety of CNS disorders with ischemic stroke, Alzheimer's disease, traumatic brain injury, Huntington's disease and other neuropathologies. Short peptides have proven neuroprotective effect and no side or toxic effects, what is very important for the development of pharmaceuticals for the elderly people. Thus, neuroprotective short peptides are highly effective in the correction of violated brain function and treatment of age-related pathology.

**References:**


KHAVINSON PEPTIDES IN PROTEOMES OF LONG- versus SHORT-LIVED RODENTS

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Relevance. The lifespan of the naked mole-rat (Heterocephalus glaber) is known to reach 30 years [4], being 10 times longer than in the brown rat (Rattus norvegicus) and house mouse (Mus musculus). H. glaber is resistant to cancer [1], presumably due to a special stability of its proteins [5]. The Khavinson short-chain peptides epigenetically inhibit some processes related to ageing [2, 3]. We compared the proteomes of the above long- and short-lived rodents in search for the Khavinson peptides.

Methods. Using a specially developed computer program AMS14 (Ancient Motif Search; Kormilets, 2014), we have identified the Khavinson peptide motifs in the primary structure of all the known proteins in the above-mentioned rodent species.

Results. The following Khavinson peptide motifs have been identified in proteins of the long- and short-lived rodents:
— Bronchogen (AEDL) in Fascin, Transcription factor AP-4, and Uncharacterized protein (human Nck-associated protein 5-like protein homolog);
— Epitalon (AEDG) in von Willebrand factor A domain-containing protein 3B (vWA3B) and Tubulin-specific chaperone A;
— Prostamax (KEDP) in MAP7 domain-containing protein 2;
— Cardiogen (AEDR) in Dehydrogenase/reductase SDR family member 4 (DHRS4);
— Testagen (KEDG) in General transcription factor IIF (TFIIF) subunit 2, Serrate RNA effector molecule-like protein, or Serrate RNA effector molecule homolog, or Arsenite-resistance protein 2 (SRRT, ARS2), and Lipoxygenase-like protein domain-containing protein 1, or Lipoxygenase homology domains 1 (LOXHD1).

Some proteins in the long-lived naked-mole rat contain motifs of the Khavinson peptides, missing in their counterparts in the short-lived brown rat and house mouse:
— Cardiogen (AEDR) in Eukaryotic translation initiation factor 3 subunit A (EIF3A);
— Testagen (KEDG) in Patatin-like phospholipase domain-containing protein 7 (PNPLA7), or NTE-related esterase (NRE).

**Conclusion.** The Khavinson short-chain peptides Cardiogen and Testagen are present in some proteins of the long-lived naked mole-rat *Heterocephalus glaber* and absent in the same proteins of the short-lived brown rat and house mouse, providing further argument for the application of these peptides in geriatrics.

**Reference:**
AGEING IN EMERGING MARKET ECONOMIES. THE SPECIAL CASE OF RUSSIA

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Relevance. This article briefly reviews the specific patterns of population ageing in emerging market economies paying special attention to the situation in Russia.

Results. The term ‘emerging markets’, or ‘emerging market economies’, refers to a group of rapidly developing countries. The composition of this group is inconstant and the criteria for inclusion country into the list of emerging economies are vaguely defined with the exception of speed of their economic growth. Among the emerging market economies are five BRICS countries: Brazil, Russia, India, China and South African Republic, as well as other representatives of Africa (e.g., Morocco), Asia (e.g., Indonesia), Eastern and Central Europe (e.g., Poland), and Latin America (e.g., Chili). Many of these countries have become significant players in regional or even global geopolitics due to their large populations, and considerable economies and markets. In spite of significant economic advancements, the emerging market economies are confronted with numerous challenges related to the speed and magnitude of population changes, including population ageing. While the global old-age dependency ratio is projected to triple by the end of current century, in more developed economies it will double, and in some emerging economies, including China, it might grow fivefold. If unattended properly and timely, population ageing may become critical constraint to growth and even social and political stability in emerging market economies. One of the major factors affecting the potential of emerging economies to deal with the challenges and opportunities of ageing of their populations is the weak national capacity, including lack of institutions and scarcity of strategic policy planning. At the same time, the demographic patterns of many emerging economies point to the opportunities of utilizing the ‘demographic dividends’ for preventing and overcoming problems associated with population ageing and utilizing its opportunities. Russia is the only ex-Soviet state which is undoubtedly recognized as the emerging market economy.
The country has also significantly advanced in demographic transition. By the proportion of persons at 60 and above years old in its population (around 20 per cent in 2015) Russia was among the top fifty countries of the world (within 201 ranked countries). Declining fertility is at play in furthering the demographic transition in Russia as elsewhere. Another contributing mechanism in Russia, as well as in several other ex-Soviet countries, is high mortality among the younger population. Mortality in ex-Soviet countries has been steadily declining in recent years: for Russian males at age 15 to 50 years old it has dropped from 266 deaths per 1000 in 2000-2005 to 192 deaths per 1000 in 2010-2015. Yet the ‘mortality crisis’ in Russia has not been overcome as this indicator is still five times higher than in Western European countries. High mortality among the working age population may not only accelerate the process of population ageing, but also, along with low fertility, trigger population decline, and thus potentiate ‘demographic deficit’. **Conclusion.** The Russian government views ageing as an issue of major concern, and high mortality as an unacceptable population trend. Since 1991, the year of dissolution of the USSR, Russia has developed two strategic policy documents on ageing: the Federal Programme ‘Older Generation’ (1997-2004); and the ‘Strategy of Actions to Benefit the Older Citizens’. The latter document currently under implementation was adopted by the government in 2016 and is designated to last until 2025. The Strategy aims at increasing longevity and quality of life of the older generation of Russia, and includes the following priority directions: provision of income security and stimulation of employment of older citizens; improvement of health care for older citizens and development of geriatric services, including professional training and retraining of specialists; provision of access of older citizens to information and education resources; enabling organization of leisure activities of older citizens; development of up-to-date forms of social services and of social service market; promotion of production of goods and services to meet the needs of older citizens; and development a society which encompasses the interests, needs and capabilities of older citizens. Advancing the priority directions of the Strategy does not envisage allocation of additional resources from the federal budget, thus its implementation will require well-coordinated efforts for mainstreaming issues of ageing into the existing national strategic initiatives.
COMBINING LIFE EXTENSION TREATMENTS: A PROPOSAL FOR HIGH-THROUGHPUT TESTING IN RODENTS

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Relevance. Life extension treatments have usually been tested separately, one at a time, at a single dosage or a few dosages. This is a reductionist approach, appropriate for building a foundation of understanding at the metabolic level. But if our goal is practical life extension in the near term, it may be more efficient to think as an inventor or engineer would. With high-throughput screening of candidate treatments, we might hope to identify the most promising treatments, the most effective dosages, and combinations that synergize. In this way, we sacrifice understanding, but maximize our probability of identifying a protocol of extraordinary effectiveness, given limited time and resources. There is a growing backlog of promising ideas which have yet to be tested in mammals. In addition, there are many effective treatments, already identified and tested singly but not in combination. Many interventions have been reported to extend lifespan in mammals by 5 - 10%. Could these all be combined to extend lifespan 50% or more? No, this would almost certainly fail. There is much redundancy among the mechanisms of action
of the known interventions, so the underlying metabolic pathways would become saturated. We thus expect that most combinations are likely to interact negatively, such that lifespan extension from simultaneous treatment with A and B will be less than the sum of A + B, and may be no better than A alone. But more rarely, we may find pairs of interactions that synergize; in other words, in a few cases we might expect that the mean life extension from two treatments A and B is equal to or even greater than the sum A + B of the benefits from the treatments separately.

Herein, I ask: What would be an appropriate experimental and statistical protocol for testing new treatments and combinations of known treatments, if our resources are limited and our goal is to identify the outliers that have extraordinary effectiveness?

**Methods.** As examples, I have explored two methodologies in numerical simulation. In one, a single treatment is tested in a range of dosage domains, and results are fitted to a parametrized dose-response template. In the second, combinations of three treatments are tested, each on a small number of test animals, and the results are deconvoluted using multilinear regression. For both these proposals, real laboratory data is not yet available, so I have analyzed computer-generated data to evaluate the effectiveness of the proposed methodology.

**Results I: Studies of a Single Treatment at Various Dosages**

The model begins with 80 (simulated) mice, each receiving a different dosage of a trial drug. Dosages range over a factor of 100, and are equally spaced on a logarithmic scale. Sample data are randomly generated, based on assumptions about the dose-response curve that are varied in each simulated case. Then data are analyzed, and an attempt is made to recover the input dose-response curve based on the random output for 80 mice.

1. Mean lifespan for each mouse is computed from a quadratic dependence on dosage, \( \ln(\text{LS}) = C + Bx - Ax^2 \).
2. Actual lifespan of each individual mouse is generated from a Gaussian distribution with a mean computed as above and a standard deviation equal to 20% of the mean. 20% is typical of standard deviation of lifespans of mice under identical treatment\(^1\).
3. If the base lifespan for no treatment is well-established ahead of time, then the parameter C in the quadratic formula is known, and similar accuracy can be obtained with only 40 mice, using a two-parameter model.
Results for each trial consist of ages at death for 80 mice, each of which received a different dosage. For analysis, the logarithms of the 80 lifespans are subject to bi-linear regression against dosage $x$ and $x^2$. This procedure aims to recover the original parameters $A$, $B$, and $C$, and I call the corresponding regression parameters $A'$, $B'$ and $C'$. Each such trial and analysis was repeated 10,000 times, simulating 10,000 replicates of the same experiment. The 10,000 runs constitute one “case”. I repeated the analysis for 100 cases, systematically exploring the 2-dimensional parameter space of $A$ and $B$ that determine the assumed dose-response curve. ($C$ is arbitrary.)

Overall, the computed values of life extension $B' - A'x^2$ tracked the input values of $Bx - Ax^2$ well, with a correlation $r^2=0.82$. $B'$ tracked $B$ well, and $A'$ tracked $A$ less well. The life extension at optimal dose was within 1% of the input values for an average 85% of all trials, and within 5% for 95% of all trials. The slope $B'$ was within 1% of the target coefficient $B$ for 80% of all trials, and within 5% for 82% of trials. (Where the slope $B'$ strayed from $B$, usually $A'$ varied in parallel, so that the errors mutually mitigated one another).

Results II: Studies of a Treatment Combinations, using All Possible Triples

Begin with 15 proven or promising treatments. There are $C(15,2)=105$ pairs which may potentially interact. It will be efficient to combine treatments in 3’s rather than 2’s, then use regression analysis to deduce the effects of individual treatments and also their pair interactions. There are $C(15,3)=455$ distinct triple combinations. Each triple is replicated in 3 mice, for a total of 1365 mice. Each treatment, then, is present in $3*C(14,2)=273$ mice, and each pair of treatments is present in $3*(15-2)=39$ mice. 39 replicates is a sufficiently large sample to extract information with confidence about each pair interaction. This is the mathematical economy of scale that makes this size study a sweet spot for testing the methodology.

Critical Assumptions

1. Pairwise but not 3-way interactions were included in this simulation. (Preliminary analysis suggests that including 3-way interactions will not change reliability of results.)

2. Most pairs are assumed to interact negatively, but the simulation allows for some positive synergies (and seeks to identify these).

3. For each treatment, only a single dosage is tested.
4. Parameters were chosen randomly such that there was always a combination of 3 that offered life extension greater than 50%.

Recipe for Analysis
1. 105 trivariate regressions, one for each pair of treatments. For example, the first regression would have three independent variables: A, B, and AxB, where AxB is a synergy term.
2. For each treatment, exclude the three strongest interactions, as determined in Step 1, and perform all 15 single variable regressions. That leaves 11 other cages (33 other mice).
3. Use the single regression coefficients from (step 2) and the triple regression coefficients from (step 1) to predict a lifespan for each combination of three treatments.
4. Compare these predicted lifespans with the “actual” average lifespans that were assumed in generating the model.

I conducted 20,000 trials, a single replicate of each trial. The “actual” best combination of 3 treatments was selected based on lifespan data 58% of the time. 81% of the time the best combination was among the top 3, and 90% of the time it was in the top 6 (out of 455).

Conclusion. If our interest is in identifying the most effective longevity treatments for potential human use, much more information can be extracted from each rodent than in customary lifespan protocols, which are optimized for basic scientific understanding. The two protocols analyzed in this paper are illustrative, and are not optimized; nevertheless, they point the way toward more efficient ways to use our time and our lab resources.

In single-treatment studies, information is maximized if every individual animal is given a different dosage. To cover a wide range of dosages, a logarithmic distribution of dosages is useful, and in lieu of zero-dose control animals, the distribution may be anchored at the low end with dosages an order of magnitude below the expected threshold of effectiveness.

In multi-treatment studies, three seems to be a manageable number of treatments to combine in each animal. Pairwise and three-way interactions can be inferred by regression analysis.

References:
PINEAL GLAND PEPTIDES REGULATE APOPTOSIS AND PROLIFERATION OF HUMAN SKIN FIBROBLASTS DURING AGING

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Relevance. Skin executes barrier function and is subjected the most to an adverse influence of the environment that determines the aging process among people older than 30 years. At molecular-cellular level skin aging appears in reduction of collagen, elastin and other signaling molecules synthesized by dermal fibroblasts. The search of new effective and safe low-molecular substances which stimulate skin regeneration is an actual problem of gerontocosmetology. The innovational method in regenerative medicine of using short peptides allows to slow down the rate of aging of various organs and tissues. AEDG peptide and polypeptide complex of epiphysis (PPE) contribute to a significant increase in life expectancy and slowing down of aging. AEDG peptide possesses the antioxidant activity, regulates the synthesis of melatonin, has the immunostimulatory properties, reduces the risk of tumor, regulates the functional activity of neuroimmunoendocrine system, increases telomere length in normal fibroblasts and contributes to overcome the Hayflick limit. PPE is a polypeptide drug isolated from the pineal gland of calves and is an effective agent for treatment of hormone-dependent tumors, dishormonal myocardiodystrophy and consequences of stress influences on the body; it restores the function of the immune and endocrine systems in case of their aging. The effects of AEDG peptide on the expression of 15 247 genes of the heart and brain of mice were researched due to DNA-microarray technology. As a proven fact AEDG peptide activates the expression of 194 genes, and inhibits the expression of 48 genes. GHK peptide which can be found in human plasma, saliva and urine, regulates the expression of 4000 genes including DNA repair genes, and stimulates the synthesis of collagen, dermatan sulfate, chondroitin sulfate, proteoglycans, decorin in the human dermal fibroblasts. Quantity of GHK peptide reduces in biological fluids with
Experts' opinion on current approaches in anti-ageing medicine and gerontology

Thus, in a basis of molecular mechanism of effect of a short peptide underlies their capability to epigenetically regulate the gene expression. Using cosmetics which contains GHK peptide improves wound healing, stimulates keratinocyte proliferation, increases skin elasticity and protects skin from photodamage. Perhaps, AEDG peptide and PPE may have similar effects. The purpose of research is an investigation of AEDG and PPE peptides effect on the expression of proteins-markers of functional activity of skin fibroblasts.

**Methods.** Materials of the research were primary rat skin fibroblast cultures and epidermis scrapes of women skin of different age. Skin fibroblasts were derived from skin of young rats (3 months) of Wistar line. Cells were cultivated in CO\textsubscript{2} incubator in a medium containing 10% FBS, 1% L-glutamine, 1.5% HEPES buffer, penicillin G, streptomycin and M199. We performed the immunocytochemical staining on the 3\textsuperscript{rd} passage («young» culture), and 14\textsuperscript{th} passage («old» culture) of skin fibroblasts cultures. The 3\textsuperscript{rd} cell passage was classified as «young» culture, and the 14\textsuperscript{th} cell passage - as «old» culture in compliance with recommendations of the International Association for Cultural studies (USA, San Francisco, 2007). All the cultures were divided into 3 groups: the 1\textsuperscript{st} group – control, the 2\textsuperscript{nd} group – with AEDG peptide at the concentration of 100 ng/ml, the 3\textsuperscript{rd} group – with PPE at the concentration of 1000 ng/ml. These concentrations were chosen as the most effective accordingly to the results of preliminary experiments. In research were used primary monoclonal antibodies to Caspase-3 ("Novocastra", 1:75), Ki67 ("Novocastra", 1:50), p53 ("Novocastra", 1:50), p16 ("Novocastra", 1:100) and the secondary antibodies conjugated with Alexa Fluor 488 or Alexa Fluor 647 (1:1000, Abcam). Caspase-3, Ki67, p53, p16 molecules are generously used as the markers for evaluating skin cells proliferation and apoptosis. The results of immunohistochemical staining were evaluated morphometrically by measuring the area of marker expression in the «Videotest Morphology 5.2» software. Measurement of p53 protein expression in the cells of the epidermis of 36 women aged 32 to 52 years was conducted by the enzyme-linked immunosorbent assay (ELISA). The 1\textsuperscript{st} group (control, n = 10) included women not taking the peptides, who have cosmetic procedures with machine cosmetology methods (RF technology (Sybaritic)). The 2\textsuperscript{nd} group included women taking pineal gland peptides in capsules (Endoluten, 1 capsule twice a day for 30 days) and having cosmetic procedures with machine cosmetology methods.
The epidermis scraping was made using scalpel under local anesthesia. We used p53 Human ELISA Kit (ab117995) for ELISA. We used the Bradford protein assay to measure the total protein concentration. Statistical analysis of the experimental data included the calculation of an arithmetic mean, a standard deviation from the mean, and a confidence interval for each sample. The statistical analysis was performed in the program «Statistica 8.0». Critical level of reliability of a zero hypothesis (about lack of distinctions) was accepted equal 0.05.

**Results.** Skin fibroblasts during their aging in culture exhibit increased expression of p53, p16, Caspase-3 proteins, which was 7.93, 8.70 and 1.83 times respectively higher than in the "old" control cultures; and decreased Ki67 expression, which was reduced by 45%. AEDG peptide reduced p53, p16 expression in the "young" fibroblasts cultures by 3.38 and 2.52 times respectively compared to the control. AEDG peptide reduced p53, p16, Caspase-3 expression in the "old" fibroblasts cultures by 4, 1.74 times and by 42% respectively, and increased Ki67 expression by 44% compared to the control. PPE reduced p53, p16, Caspase-3 expression in the "young" fibroblasts cultures by 3.60, 3.90, 2.31 times respectively compared to the control; and increased Ki67 expression by 16%. PPE reduced p53, p16, Caspase-3 expression in the "old" fibroblasts cultures by 7.64, 7.54, 2.31 times respectively; and increased Ki67 expression by 75% compared to the control. The study of the effects of PPE and machine cosmetology methods on the p53 expression in the skin epidermis from women of different age showed that in the control group (without application of pineal gland peptides) the amount of p53 protein before and after the cosmetic procedures did not differ significantly. Meanwhile after PPE peroral application in combination with machine cosmetology methods there was observed the reduction of p53 protein synthesis in the epidermis cells by 12%.

**Conclusion.** AEDG peptide and PPE can be an effective tool to enhance the functional activity of skin fibroblasts. AEDG peptide and PPE reduce apoptosis level (p53, p16, Caspase-3 proteins expression) and stimulate the expression of marker of proliferation Ki-67. During the PPE peroral application patients demonstrated the decrease in p53 protein synthesis in skin epidermis, what indicates on the highly informative results of geroprotective effect of peptides on skin fibroblasts isolated in cultures of rat’s cells. Thus, the usage of peptides for the purpose of prevention of
age-related skin changes can be a new direction in gerontocosmetology. AEDG peptide and PPE, which normalize the proliferation and apoptosis and slow down skin fibroblasts aging, in perspective may be considered as a component of cosmetics.

References:

SPECIFIC DISEASE OR GENERAL HEALTH-ORIENTED METRICS IN HEALTH AND AGING CLINICS: THE TIME TO CHOOSE

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Relevance. At present different specific disease-oriented biomarkers are used in clinics to assess treatment outcome, its progress, and efficiency. On the other hand, nonspecific health-oriented metrics have been developed to integrate biomarkers in indicators of biological aging (1). This challenges doctors in aging- and health-oriented clinics with the choice between two approaches to assess treatment efficiency.

Methods. This retrospective case study was conducted in GLMED Longevity & Beauty Residence to compare the sensitivity of outcome of separate biochemical and physiological indicators widely used in clinics (systolic blood pressure, body mass index, waist to hip ratio, carotid intima-media thickness, forced expiratory volume 1, forced vital capacity, erythrocyte sedimentation rate, white blood cells count, serum albumin level, blood creatinine level, blood urea nitrogen, lipoprotein(a), C-reactive protein, alkaline phosphatase, glycated hemoglobin, triglycerides, HDL LDL VLDL, total cholesterol, apoB-100 and apoA1, cytomegalovirus IgG) with the cumulative score obtained by their integration and suggested for assessing biological age (BioAge) (1). Ten subjects (one female) age 31-72 years were assessed before and after the regular residence health-orienting procedures (lasting for 45-229 days) with their post-hoc grouping in those who followed (N=5) and did not follow (N=5) disease-oriented diet recommendations.

Results. Repeated-measure multivariate analyses of covariance with bootstrap procedure to disregard the violation in parametric assumptions and the treatment duration and chronological age (ChronoAge) as covariates were conducted to assess the diet effect on separate and cumulative (BioAge) changes in the biomarkers. Creatinine and triglycerides levels significantly decreased in subjects who followed the
diet recommendations compared with those who did not (Pillai's Traces = .80 and .87, F = 19.65 and 19.97, p < .05, Partial Eta Squared = .80 and .87). In contrast, BioAge significantly increased in subjects who followed the diet recommendations compared with those who did not (Pillai's Trace = .48, F = 6.49, p < .05, Partial Eta Squared = .48).

Conclusion. Disease-oriented diet recommendations, while improving the condition of separate physiological systems (e.g., reducing the level of creatinine or triglycerides), may have a detriment effect on individual health in general as assessed, e.g., by a cumulative score integrating multiple physiological system conditions in biological age. Thus, specific disease- and general health-oriented approaches may contradict in effects and different treatment procedures should be reconsidered accordingly to take this mechanism into account. The data are preliminary as obtained on a small sample and should be confirmed in a larger population. The use of the bootstrap procedure ensures the reliability of these conclusions in this small group of subjects.

References:

EPITALON: FROM CELL TO BODY

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Relevance. Physiological fundamentals of ageing consist in gradual (during life course) accumulation of hazardous changes on molecular, cellular, tissular and organismal levels. All these changes produce pathologic influence on metabolic processes being exacerbated by unfavorable ecology and unhealthy life style. Changes developed and accumulated during aging decrease body's functionality, homeostasis and flexibility. As a result, its vulnerability increases, chronic diseases developed provoke premature mortality. Prevention and delaying changes which
cause ageing-associated pathologies are the main objectives of modern gerontology. Therefore, measures for prevention of ageing-associated pathologies and promotion of life quality are of utmost importance. Development of geroprotectors aimed at prevention of premature ageing and life span increase seems to be most instrumental for the purpose. The safety of geroprotectors is of primary importance especially bearing in mind their long-term application. Besides, it is important to understand the mechanism of action of such substances and methods which are capable to increase life expectancy. Therefore, comprehensive experimental studying the effects of such substances is currently relevant.

Methods. Peptide preparations with various biological activity occupy a special place among substances revealing geroprotective properties. Numerous experimental studies showed an ability of peptide preparations not only to increase the life span in different animal species but also to prevent the development of tumors of different genesis. Unified mechanism of carcinogenesis in all mammals speaks for the importance of the experimental animal results obtained especially for prevention of neoplasia in human population. Longstanding study of chemical properties and amino-acid composition of polypeptide preparations has formed a basis for chemical synthesis of short peptides. Later on, short peptides were registered to reveal the same biological activity as natural polypeptides. For example, tetrapeptide Epitalon (Ala-Glu-Asp-Gly) in a dose 1000 times smaller than that of medicine Epithalamin, increased life span of Drosophila melanogaster on average by 35%. In a number of chronic studies on mice, Epitalon promoted life span increase, slowed down ageing of estrous function and even prevented spontaneous tumors. The effects of Epitalon and melatonin (pineal gland hormone) were compared in experimental studies related to disturbed light balance. Organism exposure to light at night time leads to suppression of pineal gland function and production of melatonin, causes disturbance of circadian rhythms, homeostasis and contributes to development of age-related diseases. Epitalon treatment stimulated night production of melatonin, produced normalizing effect on numerous hormone-mediated metabolic indices, prevented premature ageing and tumor incidence in rats. Administration of Epitalon to old monkeys (Macaca mulatta) contributed to a 3-fold increase in melatonin night level, while no similar effect was observed in young monkeys. The peptide administration to old monkeys led to restoration of
cortisol secretion daily rhythm and to restoration of glucose tolerance which falls down during aging. Experimental studies of Epitalon geroprotective effect mechanism emphasized its pronounced antioxidant activity. Epitalon contributed to effective suppression of oxygen active forms in blood serum and brain tissue of mice. In old rats, Epitalon suppressed formation of products of lipid peroxidation. Long administration of Epitalon to mice resulted in age-related decrease in chromosome aberrations in the bone marrow cells especially in mutant mice with increased production of active oxygen forms. Epitalon added to the culture of lung fibroblasts activated expression of telomerase gene which was accompanied by the increase in cell division by 43%. Anti-carcinogenic effect of Epitalon may be stipulated by the peptide specific influence at the level of regulation of gene expression. Thus, chronic administration of Epitalon to transgenic mice decreased HER-2/neu gene expression 3,7-fold in tumors of mammary glands as compared to control. DNA microchip technique helped to conclude that the level of some genes expression including those associated with carcinogenesis altered under the influence of Epitalon.

Research with fluorescent-marked Epitalon proved a possibility of short peptides penetration into a cell. The tetrapeptide was traced not only in a cytoplasm, but also in structures of karyon and karyosome. This fact is a proof of possible interaction of peptides with nucleic structures of DNA and/or RNA. It has been established later that Epitalon binds to single-stranded oligonucleotides which contain methylated cytosine. Comparison of Epitalon and other short peptides peculiarities of binding with oligonucleotides depicted its dependence on the nucleotide sequence of nucleic substrata. Thus, there are specific binding sites between peptide with a certain amino-acid sequence and oligonucleotide with a certain sequence of nucleotides. Epitalon appeared to bind preferably to CNG containing sequences which are target sites for DNA methylation in plants and animals. It is possible to assume that short peptides and Epitalon in particular, are capable to bind with CNG promoter binding site. It makes this binding site inaccessible for DNA methyl-transferases and, as a result, a promoter remains non-methylated which is the main issue for activation of the majority of genes.
**Conclusion.** Longstanding studies of short peptide effect proved their functional ability to suppress or contribute to various genetic or biochemical processes in cell.

Mean and maximum life span of experimental animals was increased due to the influence of short peptides. Short peptides are free from any allergic or toxic effects. Numerous experiments revealed the anti-cancerogenic activity of peptides, their ability to suppress the development of spontaneous, transplantable or induced tumors. Administration of peptides to elderly people resulted in melatonin level increase, normalized ageing-associated hormonal and metabolic changes and slowed down ageing processes in general. The results of studies in the mechanism of action of short peptides open new vistas for the latter as most appropriate geroprotectors.

**References:**
POTENTIAL BENEFITS OF DIETARY CALORIC RESTRICTION AND MIMETICS FOR HUMANS

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Relevance. The most robust and reproducible strategy for increasing the quality and quantity of lifespan in animals is dietary caloric restriction (CR), but a major question regarding this intervention has been its relevance to humans (1). Although some anecdotal CR experiments in humans have been reported, the overwhelming majority of such studies have been conducted with rodents and lower species. Over the last three decades, however, controlled investigations in non-human primates, as well as short term studies in humans, have been performed (1-4).

Methods. Dietary caloric intake has been reduced to varying extents, over various periods in the lifespan, with reductions of 40% in rodents, 30% in primates, and 25% in humans being the most common. However, a number of permutations, and manipulations of experimental variables have sometimes confounded interpretation of results. Additionally, the questions of what constitutes an “optimal” diet, not to mention an “optimal” lifespan, are the subjects of considerable debate, begging the question as to what conditions should be considered “control.” A continuing criticism of CR studies in rodents (and argument AGAINST relevance to humans) has been that the “control” animals are sedentary, overweight individuals, bearing little resemblance to their counterparts in the wild, and hence CR could ONLY be expected to improve their life situation.

Results. Although some exceptions exist (possibly due to certain confounding variables, as mentioned above), the overwhelming majority of CR studies report increased or longer maintenance of health (reduced and/or delayed disease) and function, as well as “healthy” lifespan. With the exception of the most recent human studies (not feasible to conduct over an entire lifetime), most also report absolute increases in median, and usually maximal lifespan. Because controlled, long term CR experiments in humans are not practical, lifetime studies in rhesus monkeys (genetically much closer to humans than rodents) were initiated some years ago and have now observed increased “healthy” lifespan, delayed loss of function,
and increased absolute lifespan under certain conditions. In addition, both CR monkeys AND humans exhibit certain short term markers of longevity, such as lower body temperature and insulin levels and/or insulin resistance, which also occur in CR rodents (1-4). It should also be noted that, in light of much greater population heterogeneity in humans and monkeys than in most rodent studies, a broader range of response to CR is observed for many endpoints.

**Conclusion.** Although controlled long term human studies are not practical, aggregate data from short term human studies and lifetime studies in monkeys and other mammals suggest that CR can increase the quality and quantity of life, but the degree and timing of restriction may need to be adjusted individually. Furthermore, irrespective of the possible benefits of CR for humans, many would be unwilling or unable to undertake this regimen on a long term basis. For this reason, we have introduced the strategy of caloric restriction mimetics....ways to obtain the benefits of reduced caloric intake, but WITHOUT dieting (4).

**References:**

COMPLEX ADMINISTRATION OF PEPTIDE GEROPROTECTORS FOR PREVENTION OF PREMATURE AGEING

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Relevance. Indicative sign of aging is organism decreased adaptation followed by decreased function of cells, systems and organs. Attention of scientists is paid to the changes in the main systems of the body arising during aging – immune, cardiovascular, central nervous, endocrine systems. Mechanism of interaction of various organs and systems comes to disequilibrium due to aging thus weakening the control over intrinsic environment, decreasing immune response and self-regulation [1]. It is necessary to emphasize that usage of geriatric methods of physiological stimulation for the aging body is scientifically-motivated by modern understanding in the mechanisms of aging. It means there is a basic opportunity to influence processes of human aging. Search of the new effective remedies influencing an aging body, research of the most rational combinations of geriatric means is an important task. Exploration of measures affecting deep mechanisms of aging becomes more and more popular among gerontologists.

This approach is based on the system of bioregulation existing in the body. This system is operated by cellular mediators represented by peptides whose function is to transfer selectively the information with interaction of cells of the immune, nervous, endocrine and other systems. The process of information transfer is disturbed in case of body pathologies. Production and accumulation of endogenic compounds including peptides transferring distorted information disturb normal work of the body regulating systems [5]. Thus intake of physiologically active peptides contributes to restoration of self-regulation of organs and systems. It is recommended to apply these substances as a part of complex therapy at any age to maintain normal level of metabolic processes, prevention and treatment of various diseases, rehabilitation after serious illnesses, injuries, surgical operations and to slow down all processes of aging in the body. A new concept of complex administration of peptide bioregulators has been developed at the St. Petersburg Institute of
Bioregulation and Gerontology. This concept is based on low-molecular peptides of natural and synthetic origin and demonstrates high efficiency in long-term clinical trials. [1, 2]. It should be noted that short synthetic peptides (Cytogens) reveal faster and targeted action which is stipulated by their structure. Natural peptides (Cytomaxes) manifest their effect later and act milder due to biologically bound microenvironment of the peptide components. Most rational seems to administer the peptides indicated in a consecutive order: a complex of Cytogens should be followed by a complex of Cytomaxes due to the difference in mechanisms of their action.[3, 4]. For example, correction and maintenance of the central nervous system functions appeared most effective when the complexes of Cytogens and Cytomaxes were prescribed. The complexes of peptides produced normalizing effect on brain cells, vessels feeding brain, and liver permitting to maintain normal blood lipid spectrum. So far, a complex usage of short peptides (Pinealon, Vesugen and Ovagen) is recommended to be followed by natural peptides (Cerluten, Ventfort and Svetinorm), both influencing the cells of brain, vessels and liver. Results of clinical studies showed that complex administration of Vesugen (short peptide) and Ventfort (natural peptide), both restoring metabolism in vessels endothelium and improving blood supply to myocardium [2], in combination with natural peptide Chelohart optimizing functional activity of myocardium cells is the most expedient. Application of Ovagen short peptide and Svetinorn natural peptide, both contributing to the improvement of lipid exchange, increases therapeutic effect. Normal function of immune system performs a very important role in slowing down aging. Consecutive administration of Crystagen short peptide and Vladonix natural peptide influencing immune system cells followed by Vesugen and Ventfort improving blood microcirculation in various organs and tissues also contributed to normalization of immune functions. Malfunction of the loco-motor apparatus deteriorated the quality of life of old and elderly people. Degenerative and dystrophic diseases of joints and spinal cord are a widespread problem. During recent years osteoporosis which affects women especially in a post-menopausal period appeared to be an urgent problem. Only complex approach may help in solution of these problems. The results of conducted clinical studies testify to the necessity of long-term (not less than 2 months) administration of Cytogens and Cytomaxes complexes: Cartalax short peptide and Sigumir natural peptide both
amending metabolism in cells of cartilaginous and bone tissues in combination with Vesugen short peptide and Ventfort natural peptide which evidenced to restore blood microcirculation and improve blood supply of cartilaginous and bone tissues supplemented with Crystagen short peptide and Vladonix natural peptide influencing immune link of pathogenesis of this group of diseases. It is important to notice that Sigumir contains mineral substances, including calcium and phosphor in bio-related form and in optimal balance thus providing for its complete digestibility. Therefore, Sigumir is a necessary component in a complex treatment and prevention of osteoporosis. Disturbance in lipid exchange leads to development of the most widespread age-related pathology – to atherosclerosis of vessels of elderly people. This complicated problem demands complex approach when except the diet therapy is compulsory to use medicines providing normalization of digestive system functions [5]. The results of clinical studies justified application of Ovagen and Vesugen short peptides to restore metabolism in liver cells and optimize processes of parietal lipid exchange in vessels to be followed by Svetinorm and Ventfort natural peptides for the same purposes. In addition, Suprefort was added to the scheme to normalize secretory function of pancreas. Application of these peptides allows to influence different mechanisms underlying development of disturbance of lipid exchange and to prevent rapid progression of atherosclerotic processes in various vessels. The same complex of peptides contributes to correction of malfunction and maintenance of functions of gastrointestinal tract as it provides normalization of secretory function of liver and pancreas. A widespread diseases of bronchopulmonary system, especially among elderly people, dictates the necessity of maintenance of these functions in order to prevent respiratory diseases. In clinical studies Chonluten short peptide and Taxorest natural peptide influencing different structures of epithelium of bronchial mucosa being administered in a consecutive order (interval of 3-6 months) contributed to a decreased incidence of respiratory infections bronchitis, thus improving respiratory functions especially in the elderly. Longstanding experience of a clinical usage of preparations which are built up on short and natural peptides testifies to necessity of regular course prescription of various complexes of peptide bioregulators with an interval of 3-6 months for maintenance of the reached metabolic level in cells of organs and tissues. This is confirmed by the results obtained from a
longitudinal clinical study conducted at the State Institute of Gerontology (AMS), Ukraine [2]. Authors give results of long-term administration of peptide bioregulators of immune system (Thymalin) and neuroendocrine system (Epithalamin) in elderly patients by courses 2 times a year. It demonstrates that bioregulation therapy contributes to maintenance of functional condition of various organs and systems at optimal level for the corresponding age.

**Conclusion.** Thus, there is an opportunity for wide usage of natural peptides in medical practice for the purpose of regulation of various body functions. The most important area is preventive administration of peptide bioregulators to increase body resistance against influence of destabilizing factors. It allows to slow down aging, decrease risk of development of age-related pathology and promotes the period of active longevity and life quality of elderly people.

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GLOBAL AGEING: CHALLENGES AND PERSPECTIVES

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2017 marks the fifteenth anniversary of the Second World Assembly on Ageing. The Assembly adopted the Madrid International Plan of Action on Ageing which has been serving as the principal framework for designing international, regional and national policy responses to challenges and opportunities of population and individual ageing.

Relevance. The review article is devoted to the major challenges and opportunities of population ageing and corresponding policy responses.

Results. Emerging and continuing challenges and opportunities of population ageing. During the recent fifteen years the world has continued the process of demographic transition manifested in population ageing and caused by decline in mortality and fertility. Since 2002, the world population has increased from 6 billion to 7.5 billion, an increase by 25%. For the same period of time, the population of people aged 60 and above years has grown from 630 million in 2002 to 956 million in 2017, which is equal of 52%. The 60+ population is projected to grow to 1.4 billion in 2030, the target date for the Sustainable Development Goals (SDGs), and to 2.1 billion by 2050. Population ageing has advanced in all world regions, with Asia clearly leading the global process with 56% of all world older people (at 60 and above years old), followed by Europe with 19.6%. Demographic ageing is not simultaneous: while some countries are advancing in demographic transition, others remain demographically ‘young’. Moreover, several European countries, including countries of the former Soviet Union, are experiencing both population ageing and population decline leading to ‘demographic deficit’.

According to the United Nations, ten years are supposed to be added to the average lifespan (i.e., life expectancy at birth) by the middle of the current century. From 2000 through 2015, humanity has already gained 3.5 years, and it is projected to add another year or more by 2020. The largest gain in longevity has occurred in Eastern Africa – 12 years, and the lowest, 3 years, has happened in economically and socially advanced
Western Europe. It is projected that the female life expectancy advantage over men is likely to shrink by 2030 in several industrialized counties, while there is more than a 50% probability that by 2030, national female life expectancy will break the 90 year barrier in Korea. While continued increase in longevity is postulated by various authors, there have been alarming findings coming from Europe, where decrease in healthy life expectancy during the first decade of the current century was reported, and from the USA, where the age-adjusted death rate had increased, possibly because of rising premature mortality in certain ethnic groups. International migration has emerged in recent years as the major demographic, as well as social and economic challenge manifesting itself in the refugee crisis. The effects of mass migration on population age structure and the corresponding demographic, social and economic consequences in both sending and receiving countries are being assessed and analysed. The issues of older persons in conflict and emergency situations have recently risen to the prominence owing to the violent conflicts and humanitarian crises in various parts of the world, and most notably in Western Asia.

**Policy responses**

The Madrid Plan of Action provides recommendation for addressing ageing issues in three policy directions: older persons and development; advancing health and well-being into old age; and ensuring enabling and supportive environment. Within the next ten months international community will be conducting the third cycle of the review and appraisal of the Plan’s implementation. The preliminary assessment undertaken by the United Nations has revealed prevalent and emerging issues and related policy advancements around the world.

**Social protection** remains one of the major areas of public concern and policy interventions. The continuing challenges in this area include low level of coverage by pension schemes, particularly in developing regions of the world. In many countries, pension benefits are insufficient to meet basic needs and prevent impoverishment. While low-income countries are struggling to mobilize resources for providing social protection to their citizens of different ages, many high and middle-income countries are facing the declining number of working-age tax payers and thus the shrinking budget capacity to provide for support mechanisms for older persons. The ongoing global economic crisis has prompted many
developed and developing countries to reduce budgetary expenditures. In spite of the above challenges, there have been important advancements in many countries aimed at introducing pension benefits; improving already existing pension benefits (e.g., through indexing and/or adding pension supplement); expanding pension coverage, including to informal sector workers; and strengthening sustainability of social protection system.

*Health and social care* have universally focused on prevention and treatment of non-communicable diseases and rehabilitation of older persons suffering from such diseases in order to prevent frailty and disability in older age. Dementia, including Alzheimer’s disease, has emerged in recent decades as the major challenge for ageing individuals and formal and informal care systems. The prevailing policy content in health and social care has been policies of active and healthy ageing inclusive of promoting preventive measures throughout the life course; facilitating various forms of participation of older persons; and safeguarding their dignity and security, particularly in later stages of individual life. In more concrete terms, policy measures have included promotion of ‘ageing in place’; de-institutionalization of care provision; expanding community and in-home care models; and introduction (improvement) of regulation of care services and care institutions.

*Human Rights of older persons* have become major policy priority at various levels: national, regional and global. Efforts of civil society to draw public attention to the issues of age discrimination in labour market, as well as neglect and abuse of older persons, particularly older women, in the family and in institutions, have urged policy makers to introduce new and amend existing national legislation to attend to the human, social and economic rights of older persons. At the regional level, two legally binding documents were adopted recently: the Inter-American Convention on Protecting the Human Rights of Older Persons, by the Organization of American States in June 2015; and the Protocol to the African Charter on Human and Peoples’ Rights on the Rights of Older Persons in Africa, by the African Union in January 2016. At the global level, the United Nations Working Group on Ageing was established in 2010 ‘with the purpose of strengthening the protection of the human rights of older persons’. In 2012, the Working Group was requested by the United Nations General Assembly “to present to the General Assembly… a proposal containing… the main elements that should be included in an international legal instrument to
promote and protect the rights and dignity of older persons...” Until now, however, the international community has not reached a consensus on the desirability of elaborating an international legally binding document on the rights of older persons.

**Conclusion.** Most of the above challenges and opportunities and corresponding policy responses are traditional for international ageing. The SDGs adopted in 2015 provide an opportunity for mainstreaming the issues of ageing into global process of promoting sustainable development in ageing societies.

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MOLECULAR GENETIC TESTING AS A METHOD OF PREVENTION OF AGE-RELATED PATHOLOGY

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Relevance. It is well-known that any disease is much easier to prevent than to treat. Prevention of age-related pathology, increasing the period of active longevity and improving the quality of human life are among the most important goals of public health service. That is why preventive medicine plays a leading role in health maintaining of a modern human. The risk of developing of any pathology is conditioned by both genetic and epigenetic factors.

Methods. The current level of scientific achievements, especially in the field of molecular genetic research, allows us to offer the patient the personalized diagnostics. As is known, the outcome of the international Scientific Program «Human Genome Project» was the complete decoding of the nucleotide sequences of all 23 pairs of human chromosomes. Due to such large-scale studies, it became possible to conduct precise molecular diagnostics and determine a person predisposition not only to chromosomal diseases (Down syndrome, Patau syndrome, Edwards syndrome), but also to multifactorial diseases such as coronary heart disease, arterial hypertension, diabetes mellitus, Alzheimer disease and some cancer types. Therefore, molecular genetic testing makes it possible to find out hereditary variants (polymorphisms) of genes that are compatible with life, however, coupled with unfavorable external and endogenic factors (medicines, food, bad habits, environmental pollution, infections) may cause various pathological conditions and diseases. Information about DNA features of a particular person is the basis for creation of an individual “Genetic passport” of this person. Therefore, the genetic passport of health contains information about the features of the DNA structure, individual predispositions to a number of diseases, as well as recommendations for the patient and the doctor on the prevention of these diseases.

Results. St. Petersburg Institute of Bioregulation and Gerontology has twenty years of experience in using molecular genetic testing in clinical practice. Since 1995, more than 3000 patients have undergone molecular
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genetic testing (“Genetic Passport”) in the medical center of the Institute. For many years, these patients were under the constant control of the medical center doctors, which made it possible to assess the influence of the ongoing medical and preventive measures on their health, taking into account their genetic predispositions to the development of pathology. The medical and preventive measures, provided considering the results of genetic testing, contained diagnostic and dietary recommendations, recommendations on the regime of physical activity, and bioregulating therapy. An outstanding feature of bioregulating therapy is its physiological bioregulating effect on metabolic processes in cells that are known to be disrupted in case of a diseases and while aging. By affecting the gene expression, peptides stimulate the synthesis of protein in the cells of the body and regulate the functional activity of organs and systems. The effect of peptide bioregulators on the DNA prevents chromosomal aberrations, providing genetic stability throughout life. Therefore, as a result of regulating processes, an influence of pathogenetic factors, DNA damage, mutations and pathological transformations are precluded or reduced, and the restoration of cellular homeostasis is enhanced. Peptide bioregulators do not exert any side effects and do not cause allergic reactions because they consist of natural amino acids that are normally present in our body.

**Conclusion.** Long-term experimental and clinical studies have shown that individual selection of peptide bioregulators (depending on the identified genetic predisposition to a particular disease) allows to effectively prevent various diseases and significantly improve the patients' quality of life. It was proved that when patient was taking peptide bioregulators considering the results of molecular genetic testing, the risk of disease development reduced (by 1.5-3 times) compared to patients who did not take peptide preparations, but who had a predisposition to a genetically determined disease.

Therefore, using of molecular genetic testing to prevent age-related pathology is promising from both a scientific and practical points of view.

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SHORT PEPTIDES UPREGULATE GROWTH AND NEURONAL DIFFERENTIATION IN HUMAN PERIODONTAL LIGAMENT STEM CELLS

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Relevance. Stem cells from periodontal ligament are worldwide used for in vitro model of several neurodegenerative disease. Our results shown an improvement of cell growth and neuronal differentiation after treatment with Epitalon, Vilon, Cartalax, Vesugen and a mix of them.

Methods. Human periodontal ligament stem cells (hPDLSCs) were collected after signed informed consent was obtained from ten healthy patients. In this study we enrolled 5 male and 5 female patients (range age 20-40 years old). All volunteers were exempt from systemic and oral diseases. Biopsies were obtained from the alveolar crest and horizontal fibres of the PDL by scraping the roots of non-carious third molar teeth with a Gracey's curettes. hPDLSCs were cultured in xeno-free medium without animal derived molecules, Mesenchymal Stem Cell Growth Medium-Chemically Defined (MSCGM-CD) as previously described to Trubiani et al. (1). hPDLSCs were seeded and maintained for 10 days in Neurobasal-A Medium (Gibco®, Life Technologies, Monza, MB, Italy) containing B27 (2%) (Life Technologies), L-glutamine (2 mM) (Life Technologies), penicillin (100 U/ml) (Life Technologies), streptomycin (100 mg/ml) (Life Technologies) and amphotericin B (5 mg/ml) (Life Technologies) (neuroinductive medium) and supplemented with basic FGF (20 ng/ml) (TemaRicerca, Milan, Italy). The medium will be changed every 3 days, as previously described by Trubiani et al. 2016 (2). Experimental design of the study divided samples in six different groups: hPDLSCs cultured without peptides (ctrl@group); hPDLSCs cultured with Epitalon (test1@group); hPDLSCs cultured with Vilon (test2@group); hPDLSCs cultured with Cartalax (test3@group); hPDLSCs cultured with Vesugen (test4@group); hPDLSCs cultured with a mix of all abovementioned peptides together (test5@group). All the peptides were diluted in PBS at a concentration
0.01µg/ml and were added to cell medium and replaced every 3 days. The cells were placed at 37°C in a humidified 5% CO₂ incubator. Cells maintained in MSCGM-CD were used as control cells. After 10 days of induction, differentiated and undifferentiated cells treated or not with peptides were collected for subsequent analysis. MTT assay, confocal laser scanning microscopy analysis and western blot analysis were used to evaluate cell viability and neuronal differentiation capacity, respectively. In particular cells were labelled with neurogenic fluorescence antibodies: Nestin, Tyrosine Hydroxylase (TH) and GAP-43 and observed at confocal system LSM10META (Zeiss, Jena, Germany) in order to evaluate the neurogenic commitment. Cytoskeleton actin were labeled in green fluorescence, nuclei using a blue fluorescence and neurogenic markers appear in red fluorescence. GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA) was used for statistical data analysis. The factor under investigation was the time elapsed for MTT assay. Data were expressed as means and standard deviation of the recorded dependent variables: the optical density (MTT assay). The differences among the levels of the factor under investigation were evaluated performing distinct two-way-ANOVA tests. Tukey tests were applied for pairwise comparisons. A value of p<0.05 was considered statistically significant in all tests. All the peptides used in the present study were kindly granted by Khavinson Vladimir and Trofimova Svetlana, Saint Petersburg Institute of Bioregulation and Gerontology, Russia.

Results. MTT assay showed cells proliferation trend without any significant differences among test1@group, test2@group, test3@group and test4@group when compared to the untreated cells (ctrl@group), while test5@group showed a high proliferation rate when compared to ctrl@group. All differentiated samples showed a characteristic cytoskeleton actin rearrangement on immunofluorescence analysis. Cells after induction period change their morphology from fibroblast-like to circular or ovoidal shape. hPDLSCs in undifferentiated conditions express low levels of neurogenic markers, as Nestin and GAP-43. An over-expression of TH, Nestin and GAP-43 was present after 10 days of induction process. Cells treated with Epitalon, Vilon and all of peptides together express high levels of TH, Nestin and GAP-43. In particular best results were obtained in cells of test5@group, showing a higher commitment process in terms of neurogenic proteins expression and morphological changes. Considering
immunostaining results, western blot analysis carried out on cells of test5@group and ctrl@group to in order to validate the protein expression. Nestin and GAP-43 specific bands were overexpressed in test5@group and ctrl@group after neurogenic differentiation induction.

**Conclusion.** Considering the limitations of the present study, we retain intriguing the possibility to enhance cell proliferation and neurogenic commitment in hPDLSCs using small biological molecules, in order to be used in the next future to limit the senescence process and to promote tissue regeneration *in vivo*. Working in progress in our laboratory are addressed to better understand the role of these peptides on molecular signaling pathway regulating cell growth and commitment.

**References:**


SHORT PEPTIDES MODULATE DNA METHYLATION, GENE EXPRESSION AND CELL DIFFERENTIATION IN ANIMAL AND PLANT CELLS

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Relevance. As far as short peptides increase lifespan and improve physiological functions of various organisms [1], the main goal of this work was to investigate the molecular mechanisms of their action in animal and plant cells.

Methods. Gene expression was measured by real-time polymerase chain reaction (PCR-RT). RNA purification was done using RNA Protect Cell Reagent and RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's recommendations. The obtained RNA samples were used to synthesize the first strand of complementary DNA using oligo (dT)18 (Sintol, Moscow) and reverse transcription kit–Omniscript RT Kit (Qiagen, Germany) according to the kit manufacturer’s recommendations. Quantitative PCR with the fluorescent dye SYBR Green I was performed by means of QuantiFast SYBR Green PCR Kit (Qiagen, Germany) and thermocycler CFX96 Real-Time PCR Detection System (BioRad Laboratories, Inc., USA). Construction of oligonucleotide primers was performed by means of online service NCBI Primer-Blast. For our experiments, we used oligonucleotides synthesized by Sintol (Moscow, Russia). Interaction of fluorescence-labeled peptides with different compounds was measured by spectrofluorimeter.

Results. Short peptides can penetrate into animal cell, its nucleus and nucleolus [2]. Therefore, in principle, they may interact with various components of cytoplasm and nucleus including DNA, different RNA, proteins, and chromatin histones, in particular. In fact, in vitro peptides interact site specifically with oligonucleotides, DNA, H1 and core histones [2]. In the human bronchial epithelium cells the peptide bronchojen (Ala-Glu-Asp-Leu) activates synthesis of anti-inflammatory proteins and cell
regeneration factors (CD79a, Ki67, Mcl-1, p53), regulates expression of genes coding for differentiation factors (FoxA1, FoxA2, Nkx2.1, SCGB1A1, SCGB3A2) and other functional proteins (MUC4, MUC5AC, SftpA1), it changes CpG methylation status of promoters in respective genes [3]. Peptide pancragen (Lys-Glu-Asp-Trp) modulates transcription of genes coding for cell differentiation factors in the carcinoma cell culture from human pancreas. In particular, pancragen increased expression of differentiation factor Pax6 and others in aging cells of human pancreas. It induces full CpG demethylation of Pax6 gene promoter.

Thus, short peptides modulate DNA methylation and, therefore, they may control all genetic functions including transcription, DNA replication and repair. The peptide activation of transcription is often associated with CpG demethylation of gene promoters. We have suggested the most possible mechanism of such peptide regulation of transcription [4]: the peptide binding to gene promoter seems to protect CG or CNG site against methylation and these sites are left to be unmethylated that is crucial for activation of most genes. Specific peptide binding with histones may influence the various enzymatic histone modifications and it can be another control mechanism of genetic functions. It is not ruled out that specific peptide binding with miRNA may be involved in control for RNA-directed DNA methylations and the miRNA gene silencing. Anyway, short peptides may modulate practically all known epigenetic mechanisms (DNA methylation, histone code, RNA gene silencing). Therefore, there is no doubt that peptide regulatory functions have mainly epigenetic nature and signaling character. Short biologically active peptides Epitalon (Ala-Glu-Asp-Gly), Bronchogen (Ala-Glu-Asp-Leu) and Vilon (Lys-Glu) in concentrations 10^{-7} - 10^{-9} M influence essentially growth, development and differentiation of tobacco (Nicotiana tabacum) callus cultures [5]. In tobacco cells peptides investigated modulate expression of many genes including genes responsible for tissue formation and cell differentiation. These peptides differently modulate expression of the CLE family genes coding for known endogenous regulatory peptides, the KNOX1 genes (transcription factor genes) and GRF (growth regulatory factor) genes [5] coding for respective DNA binding proteins such as topoisomerases, nucleases and others. Peptides studied may be related to a new generation of plant growth regulators. Such short peptides acting gene specifically both in animal and plant cells seem to be evolutionally early ones and common for eukaryotes.
Conclusion. Short peptides represent an efficient signaling system of epigenetic control of cell physiology. They control all genetic processes and seem to be common in animals and plants. Peptides investigated belong to a new generation of plant growth regulators. The further investigation of peptide interactions with DNA, various RNA and chromatin, in particular, is very important for deciphering of mechanisms of gene functioning, cell differentiation and evolution. The search for and the design of new short biologically active peptides is a key promising way to the origin and production of a new generation of drugs that are gene addressed and strongly needed to prevent premature aging, to treat cancer and other diseases.

References:
EFFECT OF PEPTIDES ON THE FUNCTIONAL STATE OF SKIN CELLS

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Relevance. Aging is a process of complicated dynamic reconstruction and adaptation of the body to time-dependent conditions of its existence. Age-related changes of skin are demonstration of biological aging process and are going according to fundamental gerontology. Significant changes occur inevitably: mitotic activity of skin cells decreases, synthetically processes are gradually broken, the modified proteins are getting accumulated, defect of membranes of cells, DNA and enzymes occur as a result of aging program in skin cells and in entire body cells either. Maintenance of biological wholeness in body is regulated at the cellular level by signals which allow keeping an equilibrium condition between the basic physiological processes (proliferation) and apoptosis. Peptides take part in a signal transduction between various types of cells. Comparative study of synthetic peptides effect in culture of skin tissue at young and old animals will allow to find out the potential mechanism of regulation of skin cellular processes, to determine possibilities of synthesis of new peptide bioregulators for geriatric practice and gerontocosmetology.

Methods. As objects for testing the biological activity of synthetic peptides in culture of skin cells for young and old animals were used: organotypic cultures of skin from lower part of abdomen of young animals (3 months old) and old animals (24 months old), rats of Wistar line (2500 explants in total); dissociated cultures of fibroblast cells. Subcultivation was done on the 3rd and 4th day, cultivation was done up to 14th passage with reaching the monolayer. Di - tri - and tetra-peptides (AV-17 (KE, H-Lys (N-Glu-OH) - OH, T-38 (KED, Lys-Glu-Asp), Epitalon (Ala-Glu-Asp-Gly), synthesized at the St. Petersburg Institute of Bioregulation and Gerontology were researched in cultures of cells. Growth of skin explants in the organotypic cultures was studied during life by the phase-contrast microscope (the Alpha Telecom series 10, MTN-13, Russia) in 3 days after the beginning of cultivation, effective concentration of peptides were 0,05-10,0 ng/ml, quantitative specification of explants growth was made by the software package of PhotoM 1.2. Protein expression of proapoptotic p53
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and PCNA proliferation marker were defined in a peripherical zone of growth on the 3rd day. Immunocytochemistry research was conducted with monoclonal mouse antibodies to protein r53 and to PCNA marker (1:75, Novocastra), the morphometric research was conducted by computer analyzing system of microscopic images (NikonEclipse E400, Nikon DXM1200, IntelPentium 4) and the software "Videotest-Morfology 4.0". 10 visual fields with zoom ×400 were analyzed. Results of researches were processed by the computer programs EXCEL and STATISTICA 5.0 (Statsoft). In the dissociated cultures of cells: cultivated concentration was 50 thousand cells by ml, passaging was conducted in 3 days, cultivation was carried out up to 14th passage, immunocytochemical staining was done. Peptides were used in concentration of 10 ng/ml. Detection of the marking molecules was carried out by an immunofluorescence method using primary antibodies: anti-collagen I type (monoclonal, 1:75, Dako), anti-sirtuin-SIRT6 (monoclonal, 1:25, Dako). Confocal microscope Olympus Fluo View 1000 (Japan) and the software of "OlympusFluoViewer 3.1b" were used for analyzing the results. 10 visual fields were analyzed in each case (zoom 200). Measurement of the relative area of expression (%) and average brightness were taken. Statistical data processing was conducted with the software Statistica 6.0.

Results. In case of administration of effective concentration of AV-17 dipeptide to cultural medium index of the area increased by 22±3% in the skin explants of young animals, more expressed processes of stimulation of cellular proliferation were observed in skin explants of old animals and index of the area increased by 44±8% in comparison with index of the area of control explants. In case of administration of effective concentration of T-38 to cultural medium index of the area increased by 62±9% in the skin explants of young animals, in the skin explants of old animals index of the area increased by 54±5% in comparison with index of the area of control explants. In case of administration of effective concentrations of Epitalon tetrapeptide to cultural medium index of the area in skin explants of young animals increased by 40±11% in comparison with index of the area of control explants while in skin explants of old animals index of the area did not change and remained at the level of control values. The expression of proliferation marker (PCNA) is in a positive correlation and the expression of proapoptotic protein r53 is in a negative correlation with an increasing index of the area of zone of growth
in skin explants of young and old animals by action of di-tri- and tetra-peptides. In culture of cells of fibroblasts the collagen-1 expression by the influence of T-38 peptide was authentically increased by 2 times in "young" cultures and by 4 times in "old" cultures in comparison with control. The expression of sirtuin-6 increased by two times in case of administration of T-38 peptide to "young" culture of fibroblasts and to "old" culture increased by 4 times in comparison with control. T-38 peptide provides geroprotective properties toward the skin fibroblasts. Collagen-1 and sirtuin-6 are molecular targets of biological effect of T-38 peptide in culture of fibroblasts.

**Conclusion.** The synthesized di-tri- and tetra-peptides regulate the basic cellular processes - proliferation and skin apoptosis. Strengthening of cellular proliferation is implemented due to decrease in intensity of processes of apoptosis. Efficiency of action of small concentration of the researched short peptides - 0,05 ng/ml approaches to action of ultra-small doses of biologically active agents that opens prospects of application of small doses of the researched peptides in gerontocosmetological practice for the correction of age-related skin changes. The proliferative activity toward skin cells (revealed at the synthesized di-tri- and tetra-peptides) is the significant fact which allows to carry out the correction for slowing down the process of skin aging. The established stimulating effect of di-tri- and tetra-peptides toward cellular proliferation in skin tissues creates base for synthesis of short peptides in purpose of their application at geriatric practice and gerontocosmetology.

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ANTI-INFLAMMATORY DRUGS FOR LIFESPAN AND HEALTHSPAN EXTENSION*

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The new vision of GEROSCIENCE stress that aging is the major risk factor for age-associated diseases (AAD) and that a small number of basic, highly networked mechanisms are responsible for the aging process and are shared by AAD. Low grade, chronic, sterile inflammation, dubbed INFLAMMAGING, is one of these aging pillars and indeed most if not all AAD have a strong inflammatory component. I recently argued that the major source of inflammatory stimuli fuelling Inflammaging is the continuous production of MOLECULAR GARBAGE (cell debris, altered an misplaced proteins, among others). Moreover, age-associated changes of the gut microbiota and accumulation of senescent cells in the different organs of the body contribute to Inflammaging and aging. Within this scenario AAD and geriatric syndromes such as FRAILTY can be conceptualized as conditions of accelerated inflammaging/aging. Accordingly, I'll review the data in animal model and humans which suggest that anti-aging, anti-inflammatory strategies (calorie restriction, Mediterranean diet, physical activity, senolytic drugs and interventions) reduce inflammaging and extend healthspan. Finally I'll argue that the extension of lifespan observed in humans in the last 50-100 years can be largely attributed to a reduction of inflammaging due to improved nutrition and hygiene, which reduce the production have the capability to reduce the production of inflammatory garbage by our body.

*abstract submitted after deadline
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