Chapter 23
Role of Short Peptides as an Important Nutritional Element in Maintenance of Body Homeostasis

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Abstract This book chapter outlines the physiological and molecular mechanisms of metabolism of short peptides as well as its role in maintenance of homeostasis. Many of short peptides for oral use do not decompose in the gastrointestinal tract and absorb unchanged. Such peptides, which do not hydrolyze in a gastrointestinal tract, get to a blood plasma and then to various tissues and organs. Short peptides permeate through cytoplasmic membrane into the nucleus and nucleolus of a cell, where they able to bind to DNA an to regulate gene expression epigenetically. Interaction DNA-peptide probably is the earliest evolution form. It gives explanation of high biological activity and absolute safety of short peptides, and its' successful use in form of substance endowed with significant physiological effect.

Keywords Short peptides · Nutrition · Homeostasis · Gastrointestinal tract · Gene expression

23.1 Introduction

Numerous studies on regulatory peptides in recent decades have led to a re-examination of ideas about regulation mechanisms of physiological functions, maintenance of homeostasis processes and adaptation of the body's functional systems to the environment. Nutrition is one of the most important environmental factors that affect the human body throughout its life. Nutrients, being converted through metabolism, ensure the body's vital activity, affecting the quality and duration of life. Eating disorders always lead to some kind of negative consequences. Therefore, various pathological processes in the human body, as well as aging processes, can be considered from the point of view of disorders in the metabolism of nutrients at the organ, tissue and cellular level of the organization of living matter.

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In this regard, a rational diet adequate to age, professional activity, and health status is considered as the most important factor in the prevention of most age-associated human diseases.

Modern nutritional science considers two aspects: the epidemiology of nutrition (correlations between the characteristics of national nutrition and the presence of metabolic pathology in people from different countries) and the biochemical transformation of nutrients in the body (intake of biologically active substances with food, i.e. signaling molecules). From the point of view of molecular biology, information on the metabolic status of an organism is contained in the oldest (intracellular) and evolutionarily younger systems of regulation (nervous, immune, and endocrine), the functional activity of which is carried out through signaling molecules. These molecules carry out epigenetic regulation of gene expression to solve the general problems of the body's adaptation to changes in the supply of nutrients from the environment.

A large number of scientific works are devoted to the study of the pharmacokinetics of various biological substances when they are administered orally (Bai et al. 1995; Diao and Meibohm 2013; Vargas-Bello-Pérez et al. 2019). Among them, the study of their epigenetic activity has taken the leading place in recent years. Thus, according to a number of authors, it has been established that fatty acids and amino acids entering the blood and various tissues can interact with cellular targets (membrane and nuclear structures) and epigenetically regulate gene expression and protein synthesis (Ryan and Seeley 2013; Boyko et al. 2007). Among the signaling molecules necessary to maintain homeostasis in the body, a special group is made up of regulatory peptides of exogenous and endogenous origin (Anisimov and Khavinson 2010; Liu et al. 2016).

23.2 Biology of Endogenous Polypeptides

The functions of endogenous polypeptide molecules (growth factors, pancreatic polypeptide, calcitonin, angiotensin, peptide hormones of the hypothalamus, etc.) in the regulation of metabolism and neuro-immune-endocrine interactions are well studied. In recent years, more and more attention of scientists has been attracted to short peptides formed by the degradation of proteins that enter the body with food. A growing body of evidence indicates that regulatory oligopeptides are involved in the processes of growth, development, and regeneration (Khavinson et al. 2020; Sinjari et al. 2020; Khavinson et al. 2011a). Many of them are well-studied structures that regulate various physiological functions of the body. It is assumed that at the level of oligopeptides there is a unified system of regulation of both embryonic, growth, regeneration types and the functioning of the formed organism (Khavinson et al. 2019, 2021; Caputi et al. 2019). Thus, intensive studies of regulatory peptides have led to a radical re-examination of ideas about the regulation mechanisms of physiological functions and the principles of homeostasis process coordination in functional systems of the body.

It is known that the intestinal regulatory systems carry out the transport of peptides formed from proteins during digestion much faster than the absorption of a free amino acid mixture, to which the body did not adapt during evolution. The small intestine has di- and tripeptide transporters providing absorption of short peptides (Bai et al. 1995; Shen et al. 2001). The rate of transport of some dipeptides exceeds the rate of transport of those amino acids of which they are composed. Peptidases of the enterocyte brush border cleave a significant part (about 40–60%) of short peptides only to di- and tripeptides.

However, with age, in various parts of the digestive tract, there is a steady increase in involutional processes that contribute to the disruption of formation and absorption of short peptides, which ultimately leads to a violation of the adaptation processes and homeostasis of the functional systems of the entire organism.

All these data served as the basis for the development of biologically active food supplements based on short peptides. The most famous and "oldest" short peptide on the pharmaceutical market in the world is the dipeptide carnosine (β-Ala-His) (Mendelson 2008; Derave et al. 2019; Boldyrev et al. 2013). So, according to research, oral administration of carnosine has an antioxidant effect, affecting lipid peroxidation products, oxygen anions and other free radicals. It reduces their number to the level necessary for the full functioning of the signaling systems, which has a positive effect on the body's functions (Liu et al. 2016). An example of another mild peptide is the Asn-Leu-Pro-Arg (NLPR) peptide, which has neurotrophic effects. It was established that oral administration of the tetrapeptide Asn-Leu-Pro-Arg (NLPR) in rats with weakened memory is accompanied by an increase in susceptibility and presence of a behavioral response, and also enhances expression of nerve growth factor (NGF) in the brain. It is assumed that NLPR can improve memory by initiating the expression of NGF, i.e. is a potential drug candidate for treatment of memory impairment (Zhou et al. 1994).

A significant contribution to the study of the action mechanism of short peptides was made by studies carried out at the St. Petersburg Institute of Bioregulation and Gerontology, where various short peptides involved in maintaining the structural and functional homeostasis of cell population were synthesized and studied (Khavinson 2014). Among these peptides are short peptides Regevil (Vilon, Lys-Glu (KE)) and Epimental (Epitalon Ala-Glu-Asp-Gly (AEDG)) which have a normalizing effect on the function of the body's immune and endocrine systems (Anisimov and Khavinson 2010; Khavinson and Malinin 2005). Peptides KE and AEDG have a big spectrum of biological activity: they increase average and maximum life expectancy, reduce the incidence of malignant tumors, promote an increase in telomere length, overcome the Hayflick cell division limit, restore the functional activity of cells of the immune system and the endocrine system (Sevostianova et al. 2013; Khavinson et al. 2012a; Anisimov and Khavinson 2010; Khavinson and Malinin 2005). Peptide Lys-Glu-Asp-Ala (KEDA), which restores the intensity and rhythm of protein synthesis in the culture of hepatocytes of old rats, is also of interest (Timofeeva et al. 2005).

It should be noted that metabolism of short peptides obtained by enzymatic hydrolysis in the gastrointestinal tract of proteins that enter the body with food and their synthetic analogs seems to be uniform, therefore the general biological role of short

peptides in the regulation of homeostasis, regardless of their origin, is further examined. The metabolism of short peptides can be divided into 3 main stages: absorption into the blood in the gastrointestinal tract, interaction with targets of various organs and tissues, and elimination/resorption in the kidneys.

23.3 Metabolism of Short Peptides in the Gastrointestinal Tract and Their Absorption into the Bloodstream

Protein molecules that enter the body with food, under the action of enzymes of the gastric (pepsin, rennin, gastrixin) and intestinal (aminopeptidase, enteropeptidase) tract, are split mainly into di- and tripeptides. These short peptides are divided into two groups: resistant to hydrolysis and able to break down into amino acids. Hydrolysis of labile short peptides occurs on the surface of the brush border membranes of enterocytes (membrane digestion) or inside enterocytes using cytosolic peptidases (intracellular digestion) (Ugolev et al. 1975; Timofeeva et al. 2005, 2000; Shen et al. 2001).

Peptides resistant to hydrolysis (glycyl-glycine, proline-containing peptides, glycyl-sarcosine, carnosine, etc.) penetrate into the circulatory system in an unchanged form, which is confirmed by the data of various researchers (Matthews and Payne 1975). According to Addison et al. (1975), all dipeptides have the same transport mechanism from the gastrointestinal tract into the blood. It was established that the Ala-Gly-Gly (AGG) tripeptide and some dipeptides are not hydrolyzed in the small intestine and can enter the bloodstream unchanged. In addition, it has been shown that dipeptides, in particular the Gly-Pro (GP) peptide, can penetrate from the gastrointestinal tract not only into the bloodstream, but can also be transported even through the blood–brain barrier in an unchanged form (Boyko et al. 2000). In another study, it was shown that in addition to passive transfer in the alkaline border of enterocytes, there is a system of active transport of di- and tripeptides (Dyer et al. 1990).

When studying the morpho-physiological characteristics of the transport of the cyclic nonapeptide Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly (CYFQNCPRG) through the intestinal epithelium, its partial absorption also occurred while maintaining physiological activity. The localization of the label to this nonapeptide in the cytoplasm of enterocytes and the intercellular space in the basal region of the intestinal epithelium of rats and frogs has been shown using the methods of electron and fluorescence confocal microscopy (Natochin et al. 2004; Prutskova and Seliverstova 2012).

These data are consistent with the results of studies of short peptides developed under the guidance of prof. V.Kh. Khavinson. Numerous studies have shown that short peptides KE, AEDG, KEDA are not hydrolyzed in the stomach, duodenum, jejunum and ileum, and are only slightly hydrolyzed in the colon and liver. The degree of hydrolysis of these peptides was determined by cleavage by L-amino acid

oxidase. In addition, it was shown that the KEDA peptide is not subject to hydrolysis in blood plasma, which confirms the assumption about the possibility of transport of peptides in the bloodstream to specific target organs for them (Tutelyan et al. 2003; Timofeeva et al. 2005).

The results showing that the peptides KE, AEDG, KEDA are able to regulate the activity of digestive enzymes in old animals are important. It was found that oral administration of the KEDA peptide in old rats for 2 weeks increased the activity of sucrase and maltase in the duodenum by 60%, maltase in the jejunum and colon by 3 times, and aminopeptidase M and glycyl-L-leucinepeptidase by 2 times (Timofeeva et al. 2005). The KE peptide in a similar experiment increased the activity of maltase (by 1.2 times in the duodenum, by 1.5 times in the jejunum, by 1.3 times in the ileum) and alkaline phosphatase (by 2.2 times in the jejunum). and the ileum and 1, 8 times—in the duodenum) (Khavinson et al. 2001). It is important to note that the peptides KEDA and KE promoted an increase in the activity of gastrointestinal enzymes in old animals to values typical for young animals. In addition, oral administration of the KE and AEDG peptides for 1 month in aged rats improved the absorption of glucose and glycine in the medial region under the action of the KE peptide, and in the proximal and distal parts of the small intestine under the influence of the AEDG peptide (Khavinson et al. 2002).

23.4 Tissue-Specific and Gene-Specific Interaction of Short Peptides with Target Cells in Various Organs

Through the bloodstream, short peptides penetrate into various organs and tissues. Probably, short peptides, in accordance with their structure, have an affinity for certain organs and tissues in which they accumulate and exhibit the greatest biological activity. It was found that short peptides KE and AEDG have a pronounced tissue-specific effect on the thymus and pineal gland (Anisimov and Khavinson 2010, Khavinson et al. 2011a). It is assumed that the interaction of short peptides with target tissues is based on their ability to penetrate the cytoplasmic and nuclear membrane into the nucleus and nucleolus and interact with DNA, epigenetically regulating the expression of genes encoding a number of signaling molecules and protein markers of cell functional activity (differentiation factors, proliferation, apoptosis, transcription) (Khavinson et al. 2012a; Fedoreeva et al. 2011; Tünnemann et al. 2006; Khavinson et al. 2011b).

It was found that short peptides synthesized on the basis of the Tat-protein (activator of transcription of the viral genome of human immunodeficiency HIV-1) are able to penetrate into the cell. These peptides have been combined into the cell-penetrating peptides (CPP) group (Tünnemann et al. 2006). Penetration into the cell through the membrane is most often characteristic of alkaline peptides containing an excess of positively charged amino acid residues in the structure. The advantage of

these peptides is that they easily overcome the acidic glycocalyx layer that is adjacent to the cell membrane (Futaki et al. 2003; Duchardt et al. 2007). For synthetic alkaline and amphiphilic peptides containing several lysine residues in the structure, the ability not only to penetrate into the cell, but also to form complexes with DNA and RNA was noted. It was found that the binding of these peptides to DNA leads to strengthening of its double helix (Kubo et al. 2012).

The direct interaction of the peptide with the membrane is determined by the electrostatic interaction of positively charged side groups of amino acid residues of arginine and lysine with negative carboxyl groups of phosphatidylserine, exposed on the outer side of the cytoplasmic membrane (Denisov et al. 1998). For negatively charged (carboxyl) side groups of peptides, the binding sites are positively charged groups of phosphatidylcholine and phosphatidylethanolamine. Thus, pinocytosis may be the main mechanism for the penetration of short peptides through the cytoplasmic membrane.

In addition, it was shown that FITC-labeled short peptides AEDG, Glu-Asp-Arg (EDR), Lys-Glu-Asp-Gly (KEDG) penetrate the nucleus and nucleolus of HeLa cells. HeLa cells were incubated with FITC-labeled peptides for 12 h (Fedoreeva et al. 2011). It is known that the nucleus of euariotic cells has a system of transport pores (nucleopores) formed by protein complexes nucleoporins. The inner diameter of nucleopores is about 50 nm; therefore, they are permeable to freely diffusing low molecular weight substances with a molecular weight of up to 3.5 kDa, which include short peptides (Ohno et al. 1998). The results obtained make it possible to consider the possibility of direct interaction of short peptides with DNA. In recent years, the method of molecular modeling has been increasingly used to analyze nanostructures, which include short peptides (Sokolova et al. 2012).

Comparison of the spatial arrangement of functional groups on the surface of the large groove of double-stranded DNA and side groups of regulatory peptides showed that the AEDG peptide can bind to the complementary DNA site on the promoter region of the gene, causing local strand separation, and thereby initiating the process of RNA gene transcription by polymerase II (Khavinson et al. 2012a, b Anisimov and Khavinson 2010).

The ATTTS sequence complementary to the AEDG peptide was found in the promoter portions of the Ki67, P53, IL-2, MMP2 and Tram1 genes and telomerase. It was experimentally proved that the addition of the AEDG peptide to the culture of human lung fibroblasts induces the expression of the telomerase gene, telomerase activity, and promotes the elongation of telomeres by 2.4 times (Khavinson and Malinin 2005).

Activation of gene expression is accompanied by an increase in the number of cell divisions by 42.5%, which demonstrates overcoming the Hayflick limit of cell division (Anisimov and Khavinson 2010). In addition, in the pineal gland cell culture of young and old animals under the influence of the tetrapeptide, an increase in the synthesis of MMP2 and Ki67 proteins and a decrease in the synthesis of the proapoptotic protein p53 were observed (Khavinson et al. 2012a, b).

Epigenetics postulates the tissue, subcellular, and age specificity of DNA methylation and indicates that the character of DNA methylation in cancer cells is different

than in normal cells (Fedoreeva et al. 2011). Taking these facts into account, it can be assumed that the same biologically active short peptide can bind to DNA depending on the nature of its methylation and will have different effects on gene functions in different tissues (cells), in the nucleus and mitochondria, in young and old cells, in normal and malignant cells. Thus, specific (complementary) peptide-DNA interactions can epigenetically control the genetic functions of a cell, and this mechanism probably played an important role at the earliest stages of life and evolution (Vanyushin and Khavinson 2016).

23.5 Elimination and Resorption of CP in the Kidneys

The question of whether short peptides are excreted in the urine or resorbed in the kidneys is still controversial (Bumbaca et al. 2019; Litvin et al. 2019; Shen et al. 2001). According to some researchers, short peptides resistant to hydrolysis by gastrointestinal tract enzymes are eliminated in the urine (Timofeeva et al. 2005). In another work, using confocal microscopy, it was shown that the introduction of a fluorescently labeled polypeptide into the intestines of rats led to its accumulation in the epithelium vesicles of the proximal renal tubule, which indicates the role of the kidneys in the metabolism of not only endogenous, but also exogenous peptides and proteins (Natochin et al. 2005). These data are consistent with the study, which indicates that the metabolism of peptides with a molecular weight of less than 60 kDa occurs in the proximal renal tubules (Diao and Meibohm 2013).

23.6 Clinical Efficiency of Oral Administration of Dietary Supplements Based on Short Peptides

Long-term experimental studies have shown that under the influence of unfavorable environmental factors, emotional stress, development of age-related pathology, the process of the body's main systems' self-regulation is disturbed, the correction of which is possible with the help of specific short peptides. In addition, the data presented in the review that short peptides may be resistant to hydrolysis in the gastrointestinal tract and blood, as well as epigenetically regulate gene expression, thereby normalizing metabolism, served as the basis for the development of drugs and biologically active food supplements based on short peptides (Khavinson 2014; Khavinson et al. 2019).

Based on the short peptides Ala-Glu-Asp-Gly (AEDG) and Lys-Glu (KE), biologically active food supplements Epimental® (Epitalon) and Regevil® (vilon) have been developed.

Epimental® (Epitalon) was developed based on the results of an experimental study of the tetrapeptide Ala-Glu-Asp-Gly. Long-term experimental studies have

shown that Ala-Glu-Asp-Gly regulates metabolic processes in the cells of the neuroendocrine system, activates antioxidant defense processes, stimulates pineal and extrapineal melatonin synthesis in EC cells of the stomach and intestines, leading to optimization of the biorhythms of cortisol secretion and other hormones (Anisimov et al. 2003; Khavinson and Malinin 2005; Khavinson et al. 2012a; Khavinson 2014; Khavinson et al. 2011c).

The safety of dietary supplements Ala-Glu-Asp-Gly was confirmed by the results of a study of its general toxic effect. In the study of acute toxicity, it was found that a single administration of an Ala-Glu-Asp-Gly solution to animals at a dose 5000 times higher than the therapeutic one recommended for clinical use does not cause toxic reactions. The study of the subacute and chronic toxicity of Ala-Glu-Asp-Gly indicates the absence of side effects during its long-term use in doses exceeding the therapeutic one by 100–1000 times. When assessing the general state of animals, morphological and biochemical parameters of peripheral blood, morphological state of internal organs, state of the cardiovascular and respiratory systems, liver and kidney function, pathological changes in the body were not found. Thus, the absence of a general toxic effect indicates the safety of using Ala-Glu-Asp-Gly as a biologically active food supplement in order to maintain the function of the neuroendocrine system.

Clinical study on the effectiveness of a biologically active food supplement Epimental® (Epitalon) showed its high efficacy in subjects with conditions caused by chronic stress factors (Trofimova et al. 2021).

The study involved 560 subjects aged 35 to 68 years (260 men and 300 women) with conditions after prolonged exposure to occupational or psychoemotional stress, including those caused by frequent changes in time zones.

Stress is a pathological process, which consists in the formation of a complex of nonspecific protective, compensatory and pathological reactions of the body that arise in response to the action of extreme or pathological stimuli that threaten homeostasis: pain, hypoxia, hunger, psycho-emotional overstrain and other emergency factors that lead to changes of the same type in the lymphoid tissue, including the thymus gland, blood composition, adrenal glands, leading to a change in the biorhythm of hormone secretion. There are close links between stress and occurrence of physical illness. Modern experimental and clinical data, based on observations of people and animals, confirm the results obtained by Hans Selye, the classic of the theory of stress, and reveal the psychological processes by which emotional reactions to stress can make a person susceptible to this or another disease.

Recently, more and more often in clinical practice, the cause of disturbances in the biorhythm of hormone synthesis and related disorders on the part of the autonomic nervous system (insomnia, emotional lability, apathy, etc.) is jet lag disorder, a mismatch between the human biorhythm and the daytime rhythm, due to the frequent change of time belts.

The subjects were randomly divided into two groups: tmain and control. The subjects of the main group (270 people) received dietary supplements Epimental® (Epitalon), 1 capsule per day with meals for 20 days. The control group included 290 subjects with similar conditions who received a placebo in a similar manner. An

informed consent was signed with each study participant in accordance with protocol No. 7 dated March 5, 2018, approved by the ethics committee of the St. Petersburg Institute of Bioregulation and Gerontology.

All the subjects were under occupational or psychoemotional stress for a long time, including 142 people exposed to jet lag, 430 people under occupational stress caused by extreme psychoemotional stress at work, lack of rest during long time. The subjects complained of an asthenic state: general weakness, decreased appetite, headaches, sleep disturbances, increased irritability, apathy, emotional lability, rapid fatigue, decreased performance, decreased memory and attention, dizziness, increased sweating, changes in blood pressure.

The effectiveness of the use of dietary supplements Epimental® (Epitalon) was assessed subjectively, by studying the dynamics of the subjects' complaints, and by objective indicators, including the determination of cortisol, adrenocorticotropic hormone (ACTH) and melatonin levels in the blood serum. The content of melatonin in the blood was measured twice: in the morning at 9:00 and in the evening at 21:00.

It was found that the use of Epimental® (Epitalon) improved the general condition of patients in the study group. The subjects who received Epimental® (Epitalon) noted improvements concerning all the following indicators: apathy, emotional lability, sleep disturbance, rapid fatiguability, decreased performance, and decreased attention focusing.

While apathy, fatigue, decreased performance were noted during the initial examination in all patients of both groups, then after correction with the Epimental® (Epitalon) dietary supplement, these complaints decreased in 60–70% of the cases in patients of the main group. While in the control group, there were no significant changes, in addition, complaints of emotional lability and decreased concentration of attention even increased. It should be noted that the regulation of the function of the neuroendocrine system using Epimental® (Epitalon) contributed to the restoration of sleep in more than half of the subjects. If during the initial examination 67,4% of respondents mentioned this complaint, then at the secondary examination there were 32,2% of patients complaining of the sleep disturbance. In the control group, no significant changes were recorded.

It must be mentioned that upon repeated examination, all subjective indicators in patients of the main group significantly differ from those in patients in the control group. Thus, the complaints of patients, characterizing the state after prolonged exposure to stress factors, had a positive trend only in the subjects of the main group.

As can be seen from above, the use of Epimental® (Epitalon) in subjects exposed to prolonged occupational or psycho-emotional stress, including those caused by frequent changes in time zones, contributed to an improvement in subjective indicators that significantly differed from those before the use of dietary supplements and from indicators in patients of the control group.

During the initial study of the level of melatonin in the blood of patients of both groups, it was found that the level of melatonin in the morning (9:00) was decreased by 1.3 times, the level of melatonin in the evening (21:00) was decreased by 2.3 times compared to the lower limit normal values. After application of Epimental® (Epitalon), there was a significant increase in the level of melatonin in the morning

(from 6.9 ± 1.1 up to 18.2 ± 1.4 pg/ml) and in the evening (from 35.1 ± 1.3 up to 72.3 ± 3.6 pg/ml) to the lower limit of the reference values. At the same time, in the subjects of the control group, the indicators of the level of melatonin in the morning (from 6.3 ± 0.9 up to 7.8 ± 1.1 pg/ml) and in the evening (from 31.4 ± 1.2 up to 37.2 ± 1.9 pg/ml) increased slightly and did not reach the lower limit of the norm.

The use of Epimental® (Epitalon) in patients exposed to prolonged occupational or psycho-emotional stress contributed to the stabilization of the hormonal status, which indicates the leveling of maladjustment disorders and catabolic reactions.

The content of cortisol and adrenocorticotropic hormone (ACTH) before the examination was noted at the lower limit of the norm, which indicated the depletion of the reserves of the adrenal cortex. The level of cortisol in the main group was 239.1 ± 18.9 nmol/L, while in control group it was 232.7 ± 20.1 nmol/L; the level of ACTH is the main group was 12.6 ± 1.0 pg/ml, while in control group—10.9 ± 0.9 pg/ml. Significant difference between these indicators in both groups were not recorded. After complex treatment with Epimental® (Epitalon), the level of cortisol and ACTH in the blood plasma returned to normal and was detected in the middle region of the reference values of these indicators, which is extremely important for optimizing the body's response to stress factors. These changes correlated with improvements in subjective scores. The lack of positive dynamics of the content of cortisol and ACTH in the blood of the subjects of the control group is noticeable.

Thus, the results of the studies have shown that the use of Epimental® (Epitalon) dietary supplements to food contributed to the improvement of the neuroendocrine system of the body, which explains the improvement in the general condition in patients of the main group. Against the background of the use of dietary supplements Epimental® (Epitalon), a decrease in complaints of sleep disturbances, headaches, dizziness, apathy, weakness, rapid fatigueability, decreased performance, emotional lability, decreased memory and attention, increased sweating, decreased appetite was noted.

During the use of Epimental® (Epitalon), no side effects, complications and drug dependence were identified.

The results of a clinical study of Epimental® (Epitalon) dietary supplements allow us to conclude that Epimental® food supplements have a regulatory effect on the neuroendocrine system, which allows us to recommend its use in people exposed to prolonged occupational or psycho-emotional stress, including jet-lag syndrome caused by frequent change of time zones (Trofimova et al. 2021).

Regevil® (vilon) Lys-Glu (KE) was developed based on the results of an experimental study of the Lys-Glu dipeptide (KE). Long-term experimental studies have shown that peptides have a tissue-specific effect on the cells of those tissues for which they are specific. According to experimental studies, the Lys-Glu (KE) dipeptide regulates metabolic processes in the cells of the immune system, improves the indicators of cellular and tissue homeostasis in the cells of the immune system, restores impaired immunological reactivity, activates antioxidant defense processes, and stimulates tissue regeneration in the event of their suppression. These properties of the Lys-Glu (KE) dipeptide are the mechanism of its immunomodulatory and

anti-stress action. (Anisimov and Khavinson 2010; Khavinson and Malinin 2005; Khavinson 2014; Khavinson et al. 2011d).

The safety of Lys-Glu (KE) dipeptide was confirmed by the results of a study of its general toxic effect. In the study of acute toxicity, it was found that a single administration of a solution of Lys-Glu (KE) dipeptide to animals in a dose 5000 times higher than the therapeutic one recommended for clinical use does not cause toxic reactions. The study of the subacute and chronic toxicity of the Lys-Glu (KE) dipeptide indicates the absence of side effects with its long-term use in doses exceeding the therapeutic one by 100–1000 times. When assessing the general state of animals, morphological and biochemical parameters of peripheral blood, morphological state of internal organs, state of the cardiovascular and respiratory systems, liver and kidney function, pathological changes in the body were not detected. Thus, the absence of a general toxic effect indicates the safety of using Lys-Glu (KE) dipeptide as a biologically active food supplement in order to maintain the function of the immune system.

A clinical study of the effectiveness of the dietary supplement Regevil® (vilon) has shown its high efficiency for the complex restoration of the functions of the immune system in pathological conditions of various origins, including for accelerating tissue regeneration after various injuries, when exposed to extreme environmental factors, as well as in elderly and senile people to maintain the functions of the immune system (Trofimova et al. 2021).

The study involved 520 people aged 43 to 76 years (268 men and 252 women) during the period of convalescence after suffering acute respiratory, bacterial or viral diseases.

It is known that various factors of a physical, chemical and biological nature, depending on the duration or intensity of their impact on the human body, can lead to depletion of adaptive and compensatory mechanisms and cause profound disturbances in various links of the immune system.

Pathological disorders in the immune system, as a rule, contribute to a protracted course of the underlying disease with a tendency to relapse, a decrease in the body's resistance to infection and the development of severe complications.

The subjects were randomly divided into two groups: the main and the control. The subjects of the main group (276 people) received dietary supplements for food Regevil® (vilon), 1 capsule per day with meals for 20 days. The control group included 244 people with similar conditions who received a placebo in a similar manner. An informed consent was signed with each study participant in accordance with Protocol No. 2 dated January 24, 2018, approved by the Ethics Committee of the St. Petersburg Institute of Bioregulation and Gerontology.

The effectiveness of the use of dietary supplements to food Regevil® (vilon) was assessed by the dynamics of complaints of the examined and by a number of objective indicators: general clinical examination of blood and urine, immunological examination of peripheral blood (the number of T- and B-lymphocytes was determined by the method of immunofluorescence with monoclonal antibodies obtained to differentiation antigens of lymphocytes CD3, CD4, CD8, CD20; the content of immunoglobulins of various classes was determined by the method of radial immunodiffusion in gel according to Mancini; functional activity of T-lymphocytes was determined by

the lymphocyte migration test (LMIT) with ConA). Subjects complained of asthenic state: general weakness, loss of appetite, headaches, sleep disturbance, increased irritability, apathy, emotional lability, rapid fatiguability, reduced performance.

The studies have shown that the majority of the surveyed who are in the recovery period, regardless of the etiology of the disease (viral as a complication after influenza, acute respiratory infections or pneumococcal), have disorders in the immune status, manifested in a decrease in the number of CD3+, CD4+ cells with a slight increase in the number of lymphocytes with the CD8+ phenotype, which indicates a decrease in the level of immunoreactivity (CD4+/CD8+). The results of LMIT with ConA characterize a decrease in the functional activity of T-lymphocytes (mainly CD8+, i.e., T-suppressors/killers). The content of CD20+-cells, representing a subpopulation of B-lymphocytes, did not significantly differ from normal values, but, at the same time, an increase in the amount of immunoglobulins M and G in blood serum was observed.

It should be noted that the quantitative indicators of the content of CD3+ and CD4+ cells are characteristic of the lower limits of physiological fluctuations in their number in persons of a given age, which may indicate a depletion of their immune system. As a rule, persons with a secondary immunodeficiency state had a pronounced asthenic syndrome and significant changes in the cardiovascular system.

The results of the conducted studies indicate that Regevil® (vilon) is an effective solution for the correction of secondary immunodeficiencies developing in response to exposure to extreme factors. The use of Regevil® (vilon) allowed to normalize the impaired parameters of the immune system in 86% of cases.

The greatest effect from the use of Regevil® (vilon) was observed in relation to subpopulations of T-lymphocytes and their functional activity: there was a significant increase in the content of CD3+ and CD4+ lymphocytes to the level of normal values, with their initial significant decrease, normalization of the CD4+/CD8+ ratio, a significant decrease in the LMIT index. A less distinct reaction was observed on the part of the B-system of immunity (CD20+), probably due to its greater conservatism and insufficient duration of the drug intake, although a tendency to an increase in the content of B-lymphocytes up to the lower limit of the norm was noted. Attention is also drawn to a significant increase in the leukocyte content compared with the indicator before the use of the peptide: initially this indicator was at the lower limit of the norm ($4.6 \pm 0.6 \times 10^9$/l), after the course of using the drug, the indicator increased to optimal values ($6.5 \pm 0.2 \times 10^9$/l), which indicates a more rapid reversal of the inflammatory process in the body than in patients of the control group.

The positive dynamics of laboratory indicators was accompanied by a pronounced improvement in subjective indicators. So, after the course with the use of Regevil® (vilon), the subjects who had pneumonia noted a significant improvement in their general condition, leveling of residual effects of bronchial and pulmonary dysfunction (reduction of cough and shortness of breath) and a decrease in the severity of asthenic syndrome, always accompanying secondary immunodeficiencies caused an infectious disease. Thus, the obtained results of the study indicate not only the immunomodulatory effect of the drug, but also its ability to accelerate tissue regeneration due to the immunostimulating effect.

No side effects, complications, contraindications, drug dependence with the use of Regevil® (vilon) were identified during the clinical study.

Thus, the clinical study carried out has shown that Regevil® (vilon) promotes the normalization of cellular immunity indicators, stimulates the processes of tissue regeneration in case of their suppression, does not cause side effects, complications and drug dependence. The dietary supplement for food Regevil® (vilon) is recommended to be used to accelerate the restoration of the functions of the immune system after inflammatory diseases of infectious and non-infectious genesis (including after pneumonia of viral and bacterial etiology), it is also recommended for the older people to maintain the functional activity of the immune system (Trofimova et al. 2021).

Thus, oral administration of the dietary supplements Epimental® (epitalon) and Regevil® (vilon), created on the basis of the short peptides Ala-Glu-Asp-Gly (AEDG) and Lys-Glu (KE), turned out to be effective in clinical research aimed at maintaining the functional activity of the immune neuroendocrine systems of the body (Trofimova et al. 2021).

23.7 Conclusion

Experimentally and clinically demonstrated possibility of oral administration of drugs based on short peptides is the indication for their preventive and therapeutic uses. Some of the main characteristics of these short peptides are: their resistance to the action of enzymes of the gastrointestinal tract and blood plasma; their ability to activate absorption of various biological substances in the gastrointestinal tract; and their ability to penetrate into the cytoplasm, nucleus and nucleolus of target cells of various tissues. Furthermore, interaction of short peptides with DNA and their effects on the epigenetic regulation of gene expression provides an explanation for their high biological activity and successful use as substances with physiologically adequate and potentially geroprotective actions.

Compliance with Ethical Standards

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Conflict of Interest All authors declare they have no conflict of interest.

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