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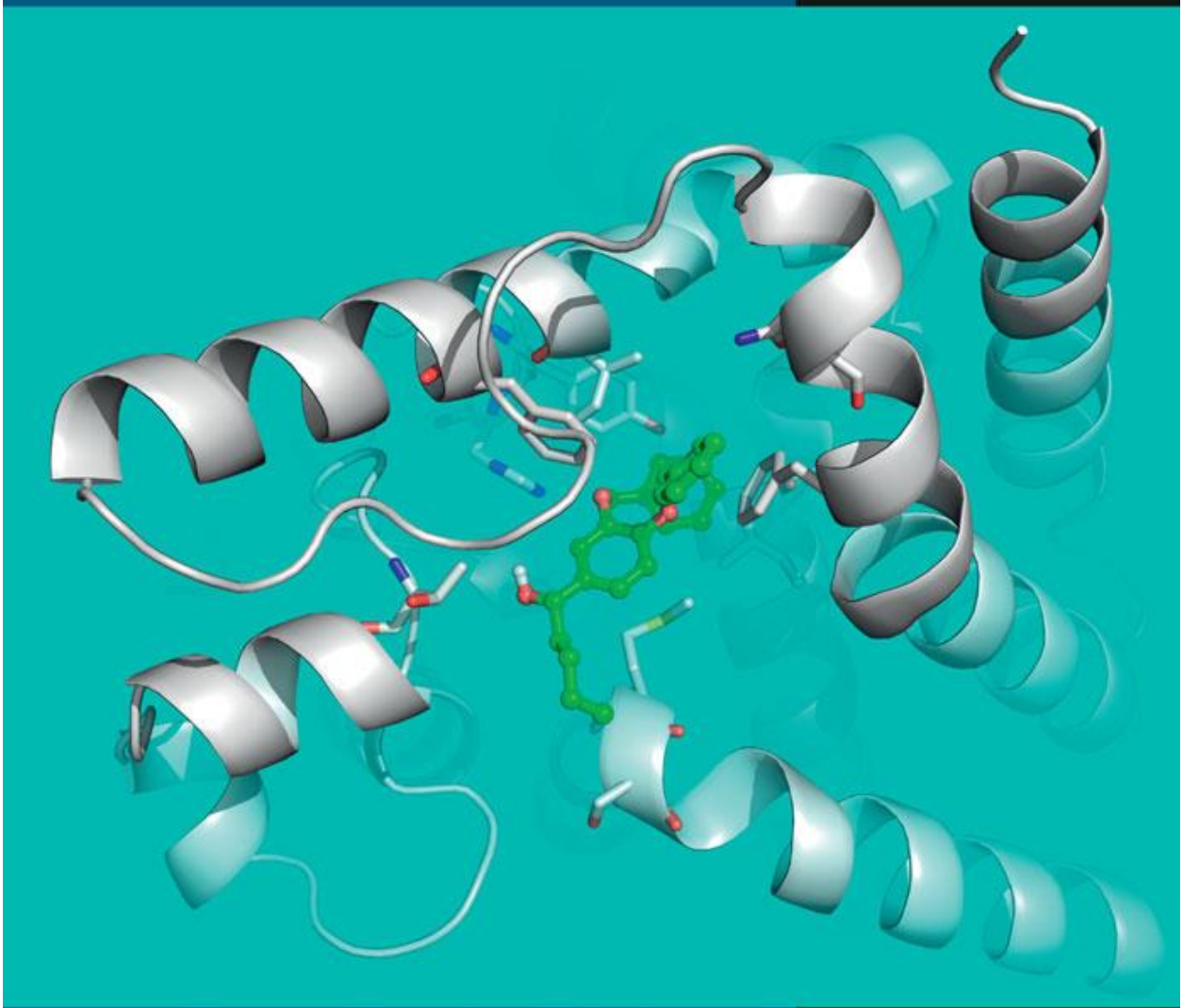

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The modern view on the role of glyprolines by metabolic syndrome

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Abstract

The analysis of the literature data and own experimental studies on the effect of glyproline peptides on fat, carbohydrate metabolism, and hemostasis system when modeling metabolic syndrome in animals (rats) was carried out. Violations of fat and carbohydrate metabolism are characterized by hypercoagulation, increased glucose levels, dyslipidemia, and decreased rheological properties of blood. In this condition, the arginine- and lysine-containing glyprolines (PGP, PRP, RPGP, KKRRPGP, RKKRPGP, and KRRKPGP) at multiple (7 days) intranasal introduction had a complex effect on the hemostatic parameters, increasing antiplatelet, anticoagulant, and fibrinolytic activity of blood plasma. All the studied drugs also showed normoglycemic and normolipidemic effects and led to a slowdown or decrease in body weight growth. The analysis of the presented material allows us to speak about the prospects of using hit compounds as protective therapeutic agents. In the case of violations of fat and carbohydrate metabolism, regulatory glyprolines protect the body by displaying antithrombotic, hypolipidemic, and hypoglycemic properties.

1 INTRODUCTION

Obesity and metabolic syndrome (MS) are the current issues of our time. MS is defined as abdominal obesity combined with hyperglycemia, dyslipidemia, insulin resistance, and hypertension.[1] These signs, as well as atherothrombosis, are key risk factors for type 2 diabetes mellitus (T2DM) and can lead to significant cardiovascular complications that should be considered as inflammatory.[2] At the same time, chronic overload of the body with free fatty acids and glucose causes an inflammatory process directly or through increased production of reactive oxygen species.[3] The first reaction of the body to MS is endothelial stress and increased platelet activity. Endothelial activation and insulin resistance cause the observed chronic metabolic inflammation, as well as metabolic and cardiovascular complications.[5]

Shown[6, 7] that regulatory peptides prevent thrombosis in atherosclerotic vascular changes, diabetes, and other conditions accompanied by hypercoagulation. Similar disorders are observed in the development of MS, which includes a number of risk factors that contribute to cardiovascular comorbidities. In this case, the body's compensatory-adaptive responses are disorganized.[8]

The correct MS therapeutic strategy has a corrective effect on fat metabolism. Antilipemic (i.e., fibrates and statins)[9] and antithrombotic drugs are used as therapeutic agents for atherogenic dyslipoproteidemia and cardiovascular risks in patients with MS.[10, 11] Some peptide compounds restore the body's homeostasis and contribute to the normal functioning of the hemostatic system, as well as lipid and carbohydrate metabolism in the body.[12-14] Studies of peptide bioregulators of glyproline nature[15] reveal their influence both on primary hemostasis and on various stages of plasma hemostasis.[13, 16] Regulatory glyprolines, which additionally contain arginine and lysine, are of great interest. Antiplatelet effects of taftsin, kyotorphin, and other arginine-containing peptides, including those involving the RGD (arginine-glycine-aspartic acid) sequence, are well known.[17] The addition of arginine to the Gly-Pro dipeptide increases the stability of the Gly-Pro-Arg tripeptide, which, like other short proline-containing peptides, is a product of hydrolysis of the collagen molecule.[18] Arginine in the body produces nitric oxide (NO), which is involved in vasodilation, reduces platelet aggregation, and improves the rheological properties of blood.[19] The abovementioned properties of arginine help reduce the risk of cardiovascular diseases and metabolic disorders, improve capillary growth in skeletal muscles, inhibit lipid peroxidation, and have an antioxidant effect.[20, 21]

Arginine and lysine have also been shown to increase fat metabolism by reducing low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG).[22] Lysine alone also metabolizes fat and simultaneously activates the fibrinolytic and anticoagulant potentials of the blood.[23, 24]

The purpose of this study is to summarize the literature data and our own experimental results on the complex study of lipid and carbohydrate metabolism, as well as the state of the hemostasis system under the action of regulatory peptides of the glyproline series, including those containing arginine and lysine, in experimental MS conditions.

2 CHAPTER 1: SYSTEMS OF THE ORGANISM IN METABOLIC SYNDROME

2.1 Lipid metabolism

One of the main problems of modern society has become the obesity epidemic, primarily because of its clinical and social consequences.[2] The cause of overweight is an increase in body weight as a result of an increase in adipose tissue.[25] MS is a complex of clinical, hormonal, and metabolic disorders that play an important role in accelerating cardiovascular (CVD) and atherosclerotic diseases.[5] The main symptoms of MS can be three or more of the following disorders: abdominal obesity (the main feature), increased triglyceride levels, decreased high-density lipoprotein cholesterol (HDL-C), increased LDL-C, hypertension, hyperglycemia, insulin resistance, and impaired glucose tolerance.[26] The excessive consumption of animal fats containing saturated fatty acids leads to structural changes in cell membrane phospholipids and impaired expression of genes that control the development of insulin resistance.[4] Hypertriglyceridemia, often obtained in patients with the abdominal type of obesity, is accompanied by excessive deposition of lipids in the muscles, which also leads to insulin resistance.[5] Despite the fact that all components of MS are risk factors for CVD, individual signs of the syndrome within MS can only be considered if there is insulin resistance.[2]

Currently, it can be considered established that a key role in the development and progression of MS is played by obesity of the abdominal-visceral type, especially when it is combined with T2DM.[27] Therefore, the first step in treating MS should be to reduce the weight of abdominal visceral fat through diet therapy and increased physical activity.

Changes in lipid metabolism can occur as a result of violations of fat absorption in the intestine, the transition of fat from the blood to the tissue, fat deposition, and interdaily fat metabolism. Normal absorption of fat provides its emulsification, splitting into glycerol and fatty acids, and the formation of compounds with bile acids. Lack of lipase, which occurs in diseases of the pancreas, as well as a deficiency of bile acids are accompanied by a violation of fat absorption. Lipase, which is localized in the vascular endothelium and enters the blood under the influence of heparin, also cleaves chylomicrons, and the resulting nonesterified fatty acids are transported to organs and tissues. Fats entering the tissues are oxidized or deposited.[26]

2.2 The system of hemostasis

Many metabolic changes that are closely related to the development of MS have an impact on blood clotting and vascular-endothelial function, increasing the risk of both atherothrombotic cardiovascular events and venous thromboembolism.[25] The pathogenesis of atherothrombosis in MS is multifactorial, requiring a close relationship between the main components of MS, including not only insulin resistance, changes in the glycemic and lipid pattern, but also a violation of hemodynamics and the early appearance of endothelial dysfunction.[28, 29] Hemostatic changes (i.e., coagulation

balance, fibrinolysis, and platelet function), aimed at thrombogenicity of circulating blood, play an important role both in the progression of arterial wall damage and in acute vascular disorders.

Mechanisms that link abdominal obesity with prothrombotic changes in MS may be due to inflammation, oxidative stress, and dyslipidemia.[30] On the other hand, the MS comorbidities like procoagulant and hypofibrinolytic state is explained by the appearance of dysfunctions in the endocrine activity of adipose tissue, coagulological tendency, and chronic inflammation caused by the ability of adipocytes to produce and/or release cytokines and adipokines that affect blood clotting and fibrinolysis, platelet function, and pro-inflammatory state of the body.[31] The pathophysiology of MS-associated traits is complex. The effect of MS on platelet and plasma hemostasis of blood is the result of an inflammatory condition, dyslipidemia, and the accumulation of fat in the liver that accompany this pathology.[4, 28] Among hemostatic disorders, there is a significant increase in plasma levels of the type 1 plasminogen activator inhibitor (PAI-1), which is a known anomaly associated with oxidative stress and an inflammatory condition.[25] Endothelial dysfunction is also the main sign of MS development.[29] In addition, adipokines are now considered to have a direct modulating effect on vascular and circulating cells.[32]

Elevated platelet levels and their activating ability are well known as determining parameters for the physiological and pharmacological prediction of thrombotic events.[4] In preabdominal syndrome, a high level of basal-activated platelet mass, due to hyperlipidemia and damage livers, was detected. In this case, the activating ability of platelets was low, but increased with hyperglycemia and hyperlipidemia, which proved that high-calorie nutrition disrupted platelet function.[33]

It is also known that patients with MS and T2DM have a high risk of microcirculatory complications and microangiopathies.[28] An increase in thrombogenic risk associated with platelet hyperaggregation, hypercoagulability, and hypofibrinolysis. Factors leading to platelet activation in MS and T2D include insulin resistance, hyperglycemia, nonenzymatic glycosylation, oxidative stress, and inflammation.[27] In the regulation of platelet adhesion and aggregation processes, NO plays a significant role, which is synthesized in both endothelial cells, smooth muscle cells, macrophages, and platelets.[34] Modification of platelet NO-synthase (NOS) activity in MS patients may play a central role in the manifestation of platelet hyperactivation. Metabolic changes accompanying T2DM can lead to abnormal NOS expression and activity in platelets.[4] A decrease in the number and sensitivity of insulin receptors on platelets in T2DM causes platelet hyperactivity. Antiplatelet effects of insulin are mediated by a NO-induced increase in cGMP levels and increased regulation of cAMP and cGMP-dependent pathways. The ability of platelets to synthesize humoral factors that stimulate thrombogenesis, inflammation, T2DM, cardiovascular complications, development of dyslipidemia, and insulin resistance was established.[35]

When considering the relationship between MS and hemostasis, much attention is paid to endothelial function, platelet activity, blood clotting, fibrinolysis, and hemorheological markers.[28] Endothelium-dependent vasodilation is impaired in MS, which is mainly mediated by

reduced expression of vasodilators (i.e., nitric oxide and prostacyclin) with concomitant increases in vasoconstrictors (i.e., endothelin-1, angiotensin II, and thromboxane A2).[29] Cross-interaction between the activated endothelium and platelets leads to a prothrombotic vicious cycle. Increased coagulation along with impaired fibrinolysis is also present in MS. This is reflected in the high level of fibrinogen and PAI-1. Endothelial dysfunction associated with high concentrations of von Willebrand factor and tissue factor in the blood also contributes to this pathology. The viscosity of the whole blood and plasma increases with MS. Intervention in the lifestyle of patients with MS, namely weight loss and improving the composition of the diet, can improve the prothrombotic state in MS.[36]

Metabolic and genetic factors induce excessive expression of PAI-1 in the blood, and this contributes to a decrease in fibrinolysis, which leads to atherothrombosis. Patients with T2DM and MS have elevated levels of PAI-1 before clinical manifestations of atherothrombotic disease.[37]

2.3 The insulin system

Obesity has been shown to increase the risk of developing hypertension, T2DM, and also leads to vascular complications such as stroke or heart attack.[35] Adipose tissue, which is excessive in obesity, is the source of many hormone-active compounds (adipokines) that affect the body's homeostasis. The most famous among them are tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL6), leptin, adiponectin, resistin, omentin, visfatin, and ghrelin. In addition, adipokines can reduce tissue sensitivity to insulin and cause inflammatory processes, endothelial dysfunction, and atherosclerotic changes.[38]

Currently, much attention is paid to the definition of adipokines as modern markers of insulin resistance. It is assumed that changes in adipokine concentrations may be observed at least a few years earlier than the first symptoms of improper glucose metabolism.[39] Some researchers believe that the endocrine activity of adipose tissue, as well as the immunological status laid down genetically, play an important role in the pathogenesis of T2DM.[40] Diabetes is a complex endocrinological disease associated with metabolic disorders. MS can lead to insulin resistance through several pathways: an imbalance in the concentration of hormones (e.g., increased leptin, reduced adiponectin, and increased glucagon), increased concentrations of cytokines (TNF-alpha, IL6) and other pro-inflammatory factors, and possibly retinol-binding protein.[41] It is especially important that in obesity, increased release of nonesterified fatty acids from intra-abdominal adipose tissue increases the concentration of intracellular diacylglycerol and fatty acyl-CoA, which reduce the signal post-receptor of insulin.[25] Often during this period there are simultaneous changes in the function of β-cells, namely, there is compensatory hyperinsulinemia with abnormal secretory dynamics. As MS develops, the function of pancreatic β cells decreases, which cannot secrete enough insulin to overcome insulin resistance, and impaired glucose tolerance progresses to T2DM. Decreased function of pancreatic β cells may be associated with chronic hyperglycemia or glucotoxicity, chronic exposure to nonesterified fatty acids followed by lipotoxicity, oxidative stress, and inflammation.[42]

Glucose homeostasis is controlled by islet cells using two main glucoregulatory hormones, namely insulin and glucagon, secreted by the β- and α-cells of the pancreas, respectively. However, when carbohydrate metabolism is impaired, their secretion changes: insufficient insulin production is noted along with dysregulated glucagon secretion.[43]

To date, oral medications approved for the treatment of hyperglycemia in T2DM are a group of biguanides, glinides, and preparations of the second generation of sulfonylureas, glitazones, PPAR-γ agonists, α-glucosidase inhibitors, SGLT2 inhibitors, GLP-1 analogs, and dipeptidyl peptidase-4 inhibitors.[44] There is a choice of medications that facilitate the course of the disease, but it is not yet possible to prevent the progression of hyperglycemia.[45] Potentially new drug compounds are needed that can reduce blood glucose levels, maintain the number of pancreatic β cells with minimal risk of hypoglycemia, and a long period of effectiveness.

2.4 The nervous system

Patients with MS are characterized by a relationship between melatonin hypersecretion, indicators of hyperactivity of the sympathetic nervous system, and vascular endothelial dysfunction.[46] These correlations are age-specific.

Regulation of fat metabolism in the body is carried out by the nervous and endocrine systems. On the one hand, this process of self-regulation, due to the relationship between the level of blood glucose and nonesterified fatty acids (NEFA): when excess glucose is part of NEFA is deposited in the fat depots, a deficiency of glucose reverses the process. On the other hand, fat metabolism is also regulated in a neurohumoral way.[47] With prolonged emotional stress, irritation of the sympathetic nerves, fat is mobilized from the fat depots and weight loss occurs, and irritation of the parasympathetic nerves is accompanied by fat deposition. Insulin inhibits the release of fat from the depot, promotes the conversion of carbohydrates into fats, and the accumulation of glycogen in the liver. Violation of this complex system of neurohumoral regulation is the basis of excess fat deposition in adipose tissue, which leads to obesity.

Lipids in the nervous system are represented by cholesterol and phospholipids as components of cell membranes and guarantee the physiological and psychological functions of the body. Violation of lipid homeostasis can lead to various pathological conditions.[48] Changes in cholesterol metabolism in the central nervous system lead to diseases, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and others. In the peripheral nervous system, changes in lipid metabolism are associated with the development of peripheral neuropathy, which can be caused by metabolic disorders, injuries, therapeutic, and autoimmune diseases. Transcription factors, such as liver x-receptors (LXR), regulate the metabolism of cholesterol and fatty acids in the nervous system. In the past few years, several studies have been conducted on the biology of LXR in the nervous system.

In recent years, among the factors contributing to the development of neurodegenerative diseases, MS has come out on top, having reached epidemic proportions. In the survival of neurons in MS, the importance of the sirtuin family is shown, which opens up opportunities for new pharmacological targets. It has been demonstrated that a high-fat diet used for the development of MS does not exacerbate the neurodegeneration caused by quinolinic acid, but it does worsen the sirtuin pathway that promotes fat metabolism.[49]

Other researchers indicate that many neurodegenerative diseases are associated with metabolic dysfunction, which is manifested by changes in lipid metabolism, since neurons have a heterogeneous lipid composition necessary for the development and functioning of the nervous system. The relationship between neurodegeneration and changes in the metabolism of phospholipids and sphingolipids, mitochondrial morphology, and membrane remodeling was established.[50]

To understand the pathogenesis of neurodegenerative disorders, the body's cellular response to danger, which is an evolutionarily preserved metabolic response, is of great importance. It protects the cell from chemical, physical, or biological influences that exceed its ability to homeostasis. The first wave of signals is the release of metabolic substances such as ATP and ADP, oxygen and reactive oxygen species, and is supported by purinergic receptors. After this, anti-inflammatory and regenerative pathways are activated, and then the cellular response stops. If the cellular response is not eliminated, the body's metabolism is disrupted, many systems and organs work, behavior changes, and chronic diseases occur. In MS, the memory of past stressful contacts is stored in the form of altered content of mitochondria and cellular macromolecules, which leads to an increase in functional reserve capabilities. The cellular response and purinergic response to life threats are directly controlled by the centers of the brain stem. Chemosensory integration of the entire body's metabolism also occurs in the brain stem and is a prerequisite for its normal development.[51]

The effects of the ethyl acetate fraction from Aruncus dioicus var. kamtschaticus (EFAD) for the treatment of neurodegenerative processes and cognitive disorders caused by a high-fat diet induced by obesity are also known. EFAD has been shown to inhibit lipid accumulation, reduce individual body weight, and prevent impaired glucose tolerance and insulin resistance.[52]

3 CHAPTER 2: PROTECTIVE (CORRECTIVE) ROLE OF GLYPROLINES BY MS

In this chapter, we present mainly our data on the effect of various glyproline peptides on the parameters of hemostasis, lipid metabolism, and body defenses with antithrombotic, hypolipidemic, and hypoglycemic properties in disorders of fat and carbohydrate metabolism during the MS.

The synthesis of the following peptides (Table **1**) was performed using classical peptide chemistry in solution. Both derivatives of L-amino acids and amino acids with free functional groups were used. The evaporation of solutions was carried out on a vacuum evaporator at 40°C. The identity of the

obtained compounds was checked using thin-layer chromatography (TLC) on plates with Silufol silica gel. The substances were detected in ultraviolet light (UV light), using ninhydrin, Barton, Pauli, and Reindel–Hoppe reagents, and o-tolidine in a chlorine medium. All the solvents used in the synthesis of peptides were appropriately purified.

Table 1. Chromatographic and mass spectrometric characteristics of the synthesized peptides

						Mass spectrometric characteristics (in positive ions)	
No.	**Peptide**	**Molecular weight**	**Molecular formula**	**Total purity, %**	**Retention time, min**	**$[M+H]^+$**	**Fragmentation of the m. peak**
1.	PGP	329.4	$C_{16}H_{25}N_3O_7$	98.15	5.42	270.1	173 (100), 155 (98), 116 (75)
2.	PRP	368.4	$C_{16}H_{29}N_7O_4$	97.5	6.76	369.2	254.1 (100), 272.2 (38), 116.1 (14)
3.	RPGP	425.5	$C_{18}H_{31}N_7O_5$	91.2	6.82	426.2	408 (100), 293 (54), 254 (24)
4.	KKRRPGP	838.0	$C_{36}H_{67}N_{15}O_8$	99.69	8.03	838.5	821.4 (100), 723.5 (25), 569.4 (42)
5.	RKKRPGP	838.0	$C_{36}H_{67}N_{15}O_8$	99.9	8.14	838.5	821.4 (100), 723.5 (68), 569.4 (32)
6.	KRRKPGP	838.0	$C_{36}H_{67}N_{15}O_8$	99.29	8.18	838.5	821.4 (100), 723.5 (55), 569.4 (31)

The synthesis of peptides performed using the method of mixed anhydrides, the carbodiimide method with the addition of 1-hydroxybenzotriazole (HOBT), and the method of activated esters. Both sequential build-up of the peptide chain and block build-up were used.

The homogeneity of the synthesized peptides was checked using high-performance liquid chromatography (HPLC) on a Milichrom-A02 chromatograph.

All the synthesized peptides were characterized using mass spectrometry on the LCQ Advantage Thermoelectron device, MAX.

Using the method of HPLC, chromatographic systems for purification of the synthesized peptides have been developed. The purity of all the synthesized peptides is shown in Table 1.

Figure 1 shows the design of the research.

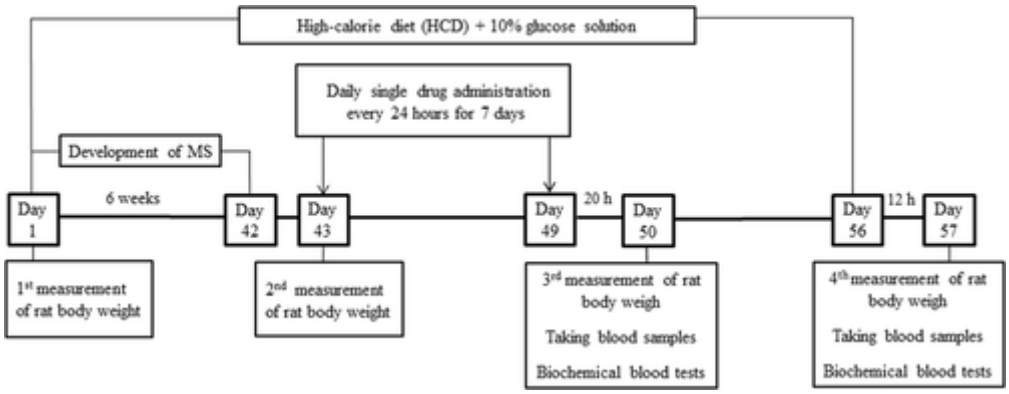

Figure 1
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Study design

Experimental MS was caused by feeding the rats the high-calorie diet (HCD), which included lard (15%), semolina with milk (30%), wheat flour and bread (15%), sugar (5%), animal fats (i.e., margarine with hydrogenated fats, mayonnaise, and cheese; 25%), standard granulated feed (10%). The HCD energy value was at least 3500 kcal/kg (135% of the standard diet). As a drink, the animals received a 10% glucose solution.[14]

At the start of experiments, a total of 80 Wistar male rats (body weight: 300–350 g) were randomly divided into one of the five experimental groups: Group 1 (MS rats without treatment), Group 2 (MS rats treated with PGP), Group 3 (MS rats treated with PRP), Group 4 (MS rats treated with RPGP), Group 5 (MS rats treated with KKRRPGP), Group 6 (MS rats treated with RKKRPGP), Group 7 (MS rats treated with KRRKPGP), and Group 8 (health rats, normal control). To develop experimental MS rats of 17 groups were fed HCD for 6 weeks (from 1st to 42th day of the diet period. Then, from the 43rd to 49th day animals of 27 groups were given HCD and were simultaneously administered the solutions of peptides PGP, PRP, and RPGP (at doses of 50 μg/kg body weight, 20 μl), KKRRPGP, RKKRPGP, and KRRKPGP (at doses 100 μg/kg body weight, 20 μl), respectively, by the intranasal way once a day for 7 days. Rats of Group 1 (MS rats without treatment) were fed the HCD and intranasally administered 20 μl of 0.85% saline per rat for 7 days. Group 8 (health rats) were given standard laboratory feed during all period of the experiment and did not receive any drugs.

After 20 h (at the 50th day) and again 7 days (at the 57th day) after discontinuation of treatment blood samples were obtained from each animal for biochemical analyses.

The content of rats for 6 weeks on a HCD led to the development of MS, as evidenced by established hypercoagulation and a decrease in fibrinolysis: in the blood of animals, coagulation

according to activated partial thromboplastin time (APTT) data significantly increased by 12%, FXIIIa activity (an increase of 17%), summary fibrinolytic activity decreased by 100%, fibrindepolymerization activity (FDPA) by 145% and fermentative fibrinolytic activity by 256%, at increased more than two times compared to the corresponding normal values. It is known that the development of MS in rats also increases the concentration of cholesterol, triglycerides, and glucose control animals contained in the HCD significantly increased the concentration of total cholesterol (TC; by 18%–20%), TG (by 75%), and LDL-C (by 45%) and reduced the concentration of HDL-C (by 17%) compared to normal healthy rats.[53] The data obtained indicated the development of MS in the body of rats that were subsequently treated with peptides.

3.1 Changes in the hemostatic system

Fibrindepolymerization or nonenzymatic fibrinolytic activity of animal blood plasma characterizes its ability to prevent the processes of beginning fibrin or thrombosis.

After 20 h after sevenfold administration of the studied peptides to rats with MS, fibrinolysis increased in blood plasma: FDPA—by 97% (PGP), 140% (PRP), 133% (RPGP), 70% (KKRRPGP), 206% (RKKRPGP), 150% (KRRKPGP) and activity of tissue plasminogen activator (t-PA)—by 16% (PGP), 70% (PRP), 19% (RPGP), and 24%–26% when active RKKRPGP and KRRKPGP. At the same time, anticoagulant activity increased by 10% (PGP), 16% (PRP), 22% (RPGP), 15% (KKRRPGP), 25% (RKKRPGP), 21% (KRRKPGP), and ADP-induced platelet activity decreased by 31.5% (PGP), 68.5% (PRP), 52% (RPGP), 35.5% (RKKRPGP), and 21.7% (KRRKPGP; Table 2).

Table 2. Changes in hemostasis parameters after sevenfold administration of peptides against the background of the development of metabolic syndrome in rats (%); M ± m

Experimental conditions	Platelet aggregation	APTT	FDPA	t-PA
PRP	31.5 ± 6.2**	116 ± 8.4*	240 ± 7.6**	170 ± 10.2**
PGP	68.5 ± 9.7*	110 ± 2.9	197 ± 3.8**	116 ± 9.1**
RPGP	48.2 ± 5.3 **	122 ± 8.7**	233 ± 5.7**	119 ± 11.3**
KKRRPGP	103.0 ± 5.3	115 ± 4.1*	170 ± 9.0**	98 ± 5.7
RKKRPGP	64.5 ± 6.2*	125 ± 2.7**	306 ± 18.0**	124 ± 12.3*
KRRKPGP	78.3 ± 9.2*	121 ± 2.1**	250 ± 7.0**	126 ± 9.3*

- *Note*: Statistical indicators are calculated relative to the corresponding control samples taken for 100%. Comparison of experience with control: *$p < .05$, **$p < .01$.

In earlier studies, the antifibrin-stabilizing effect of short proline-containing peptides (PG, PGP) was established under in vitro conditions that inhibited the activity of factor XIIIa (FXIIIa) in blood plasma.[6] In this study, the effects of three lysine-containing peptides KKRRPGP, RKKRPGP, and KRRKPGP in vivo were also determined by their antifibrin-stabilizing effect. Thus, 20 h after sevenfold administration of peptides in the blood plasma of rats, the activity of FXIIIa decreased by 33%, 41%, and 55%, respectively. After 7 days (i.e., 168 h) after the discontinuation of the peptide administration, the antifibrin-stabilizing activity was maintained in the rat blood during the continued feeding of rats in accordance with the HCD, although to a lesser extent than 20 h after the last administration of the peptides.

So, 20 h after the end of the introduction of peptides, the greatest anticoagulating effect in the blood was observed when using PRP and RPGP, as well as RKKRPGP and KRRKPGP.

Seven days after the discontinuation of the use of peptides against the background of continuing HCD, the preservation of increased anticoagulant-fibrinolytic and antiplatelet activity of the blood was noted.

3.2 Changes in the lipid profile

A study on the effect of PGP, PRP and RPGP peptides on lipid profile parameters in animals with MS showed that after sevenfold intranasal use of PGP and PRP at a dose of 50 mcg/kg, there was a slight decrease in the concentration of LDL-C In the blood of animals, which was 92% and 83% compared to the control group (rats with MS without treatment; Figure **2A**). Treatment of rats with MS peptide RPGP resulted in a more pronounced decrease in the concentration of LDL-C (69% relative to control). As seen in Figure **2B**, there was a significant decrease in the concentration of triglycerides: the level of TG was 51% (PGP), 60% (PRP), and 52% (RPGP) relative to the control values ($p < .01$).

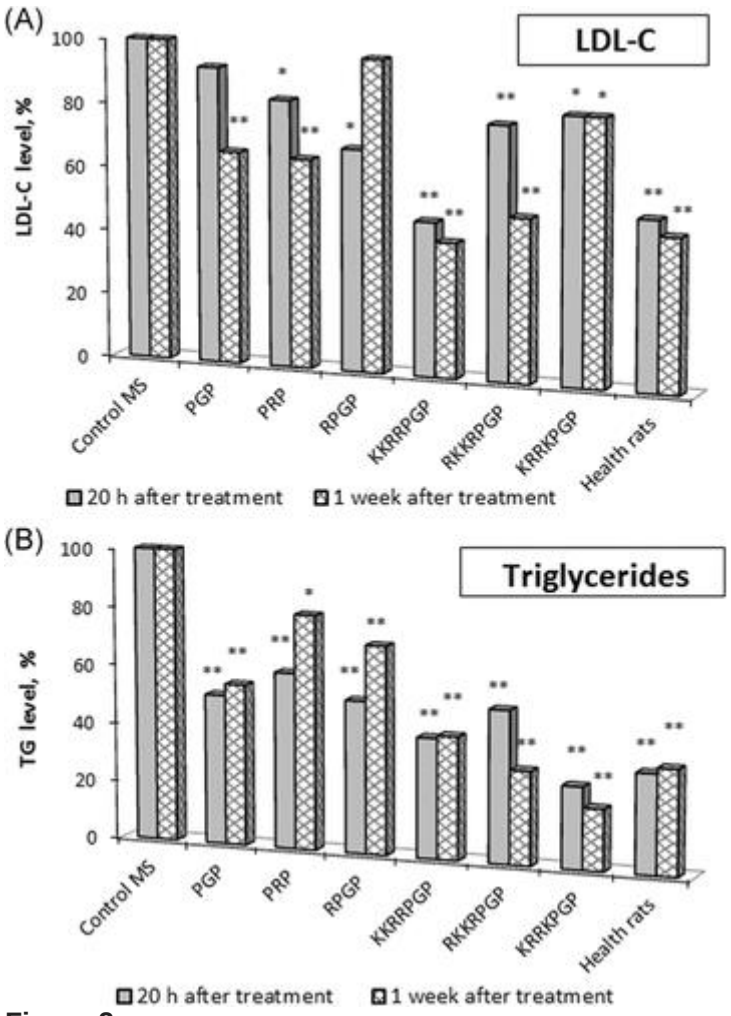

Figure 2

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The effect of peptides on lipid profile parameters of rats with metabolic syndrome (MS). Showed the level of LDL-C (A) and triglycerides (B) in rat (with MS) blood plasma after 20 h and 7 days after sevenfold intranasal treatment with peptides (a dose of 50 mcg/kg). *$p < .05$, **$p < .01$, the accuracy of the differences relative to the corresponding samples of the “MS Control” group, taken as 100%

Seven days after discontinuation of treatment with PGP and PRP peptides, the level of LDL-C continued to drop, since this indicator was 65% relative to the control in both cases, and the TG concentration remained the same. The effect on the lipid profile of the RPGP peptide decreased slightly a week after discontinuation of treatment, since there were no significant differences from the control of LDL-C levels. The level of TG in these conditions increased, but was significantly lower than the control values and was 71% compared to MS without treatment.

Sevenfold intranasal administration of peptides KKRRPGP, RKKRPGP, and KRRKPGP at a dose of 100 mcg/kg resulted in changes in the lipid profile. Thus, the use of RKKRPGP and KRRKPGP peptides caused a significant reduction in LDL-C to 79% and 83% relative to MS in untreated rats. An even greater decrease in this indicator was due to treatment with the KKRRPGP peptide (48% compared to the control). Also, treatment with these peptides had a significant drop (two to three times) in the level of TG in the blood of animals: KKRRPGP–up to 41%, RKKRPGP—up to 52%, and KRRKPGP—up to 28% relative to control.

A week after the discontinuation of the KKRRPGP and KRRKPGP peptides, the concentration of LDL-C in the blood of animals remained at the same level (42% and 83% relative to control, respectively), and after treatment, RKKRPGP continued to decrease and amounted to 51% relative to MS of rats without treatment. At the same time, the TG concentration also maintained reduced values (KKRRPGP: 42%, KRRKPGP: 21% relative to the control), and in the case of the RKKRPGP peptide, this indicator continued to decrease and amounted to 32% relative to the control (Figure **2**).

Thus, after treatment with peptides PGP, PRP, RPGP, KKRRPGP, RKKRPGP, and KRRKPGP, the parameters of lipid metabolism (LDL-C and TG) in different degrees approached the values corresponding to the lipid profile of healthy animals.

3.3 Changes in blood glucose levels

Given that the MS is accompanied by hyperglycemia, determining the concentration of glucose in the blood of animals with this pathology after the introduction of the studied peptides is of particular interest. The changes in the above parameter after the introduction of various glyprolines were studied. Thus, the administration of PGP and PRP tripeptides, as well as PGP peptide at a dose of 50 mcg/kg, only slightly reduced the glucose concentration. Peptides containing the amino acid lysine (at a dose of 100 mcg/kg) had a hypoglycemic effect. The maximum effect was found after the intranasal administration of KKRRPGP (4.96 ± 0.5 mmol/L; Figure **3**). This may be due to the presence of lysine, which facilitates fibrinolytic processes in the bloodstream and promotes normal blood supply to tissues.[23]

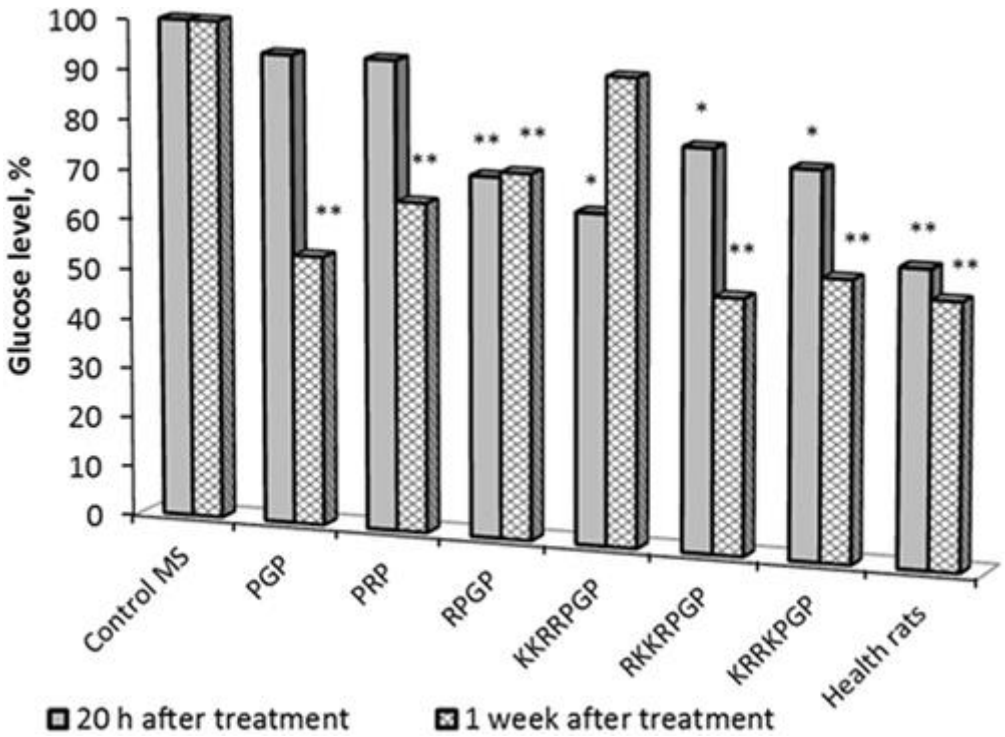

Figure 3
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The effect of peptides on blood glucose concentration in MS rats. Showed the blood glucose level after 20 h and 7 days after sevenfold intranasal administration of different peptides to rats with MS.

*$p < .05$, **$p < .01$, the accuracy of the differences relative to the corresponding samples of the "Control MS" group, taken as 100%. MS, metabolic syndrome

Seven days after the discontinuation of peptides, the glucose concentration increased only in the group of animals with MS, who were previously been administered with a peptide KKRRPGP intranasally. For all other peptides, a significant decrease in glucose concentration was observed on average by 39%, relative to the values in control animals.

3.4 Changes in body weight

Weighing animals before the introduction of peptides (day 43 of the experiment), 20 h after their last introduction (day 50), and 7 days after treatment (day 57) showed that the use of studied glyprolines against the background of HCD led to a slowdown in body weight gain in all experimental groups (Figure 4).

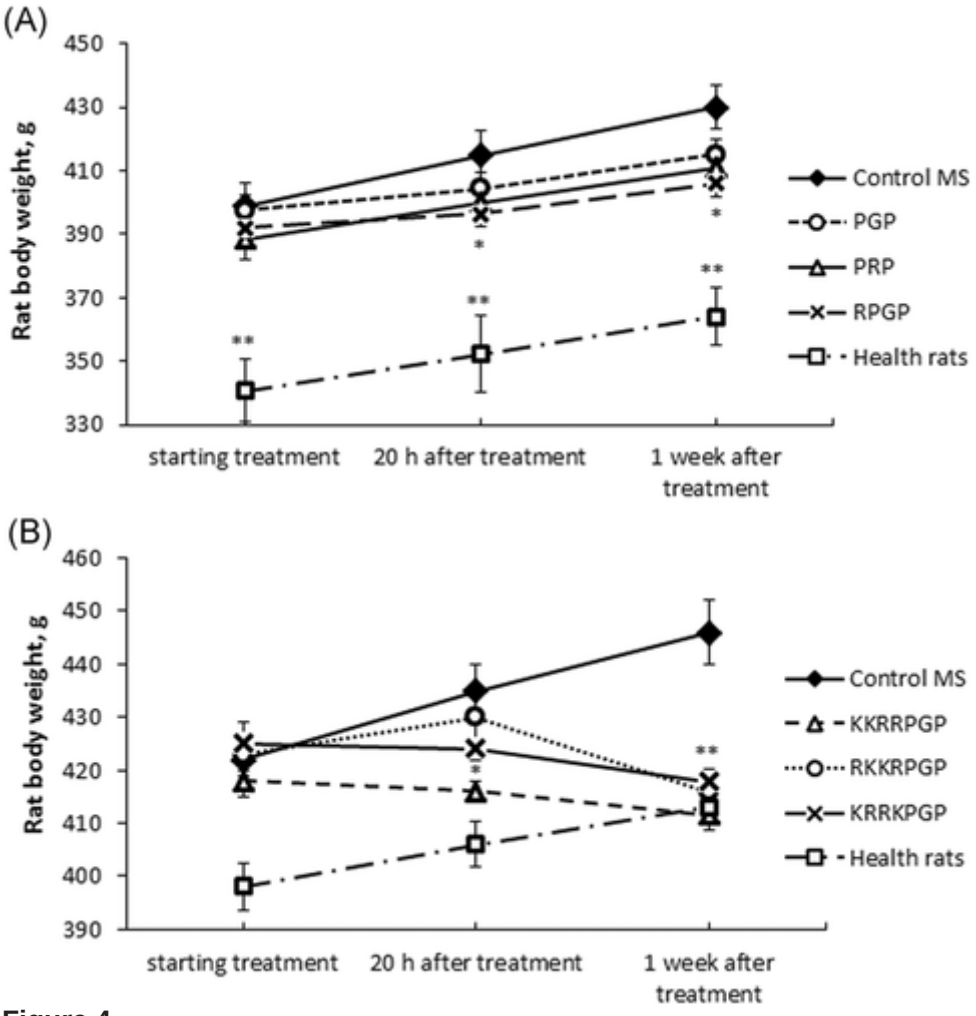

Figure 4
Open in figure viewerPowerPoint
Changes in rat body weight. Showed the weight of animals before the intranasal introduction of peptides (day 43 of the experiment), 20 h after their last introduction (day 50), and 7 days after the treatment (day 57). (A) The figure represents the effect of peptides PGP, PRP, and RPGP. The effects of peptides KKRRPGP, RKKRPGP, and KRRKPGP are shown in (B). The "Health" group corresponds to healthy animals

Studies have shown that after the introduction of PGP, PRP, and RPGP (Figure 4A), the increase in body weight in animals of these groups (weight gain) was 7.0 ± 1.7, 11.3 ± 0.8, and 4.3 ± 0.6 g, which was 56%, 28%, and 72% less than in rats with MS without treatment (control, 15.7 ± 0.8 g). After 1 week of discontinuation of treatment in rats of the experimental groups, the slowdown in body weight growth remained, since the total weight gain (from the 43rd to the 57th day of the experiment) was 62% (PGP), 72% (PRP), and 48% (RPGP), although it did not reach the values of the Normal group.

As one can see in Figure 4B, the use of peptides KKRRPGP, RKKRPGP, and KRRKPGP against MS led to either a slowdown in body weight growth (RKKRPGP) or a decrease in this indicator (KKRRPGP and KRRKPGP). The weight gain on the 50th day of the experiment after treatment with RKKRPGP was +6.8 ± 1.9 g (38% less than in the control), and after treatment with KKRRPGP and KRRKPGP, the rats lost weight by 9% and 34% (gain −1.0 ± 1.5 and −3.7 ± 1.1 g, respectively) relative to the values of body weight in the control (gain +11.0 ± 2.2 g). After 1 week of discontinuation of treatment with these peptides, the weight loss of the experimental group of animals continued and reached values corresponding to healthy animals of the Normal group.

4 CHAPTER 3: POSSIBLE MECHANISMS OF GLYPROLINES BY MS

This review describes the original properties of glyprolines and defines the main provisions of their regulatory role in the body with MS. Analysis of the results of our own experiments in comparison with the literature data indicates both specific neuromodulatory and universal (lipidemic, hypoglycemic, and anticlotting) properties of glyprolines. The peptides simultaneously showed anticoagulant, fibrindepolymerization, antiplatelet, and antifibrin-stabilizing effects in the bloodstream in metabolic disorders.[21] The mechanism of their effects is due to direct and indirect action.

Experiments were conducted on animals that received HCD enriched with fats, carbohydrates, and cholesterol, which led to the development of MS and prothrombotic changes in the hemostasis system. At the same time, blood clotting increased and the functions of all parts of the anticoagulant system decreased, which is consistent with the data of other researchers. It is possible that cytokines responsible for fatty degeneration of the arterial wall play a role in these reactions.[12]

After repeated daily use of each of the arginine- and lysine-containing peptides for 7 days, after 20 h, the normal functioning of the hemostatic system in the blood plasma of animals with developed MS was restored: the APTT indicators were extended, the activity of t-PA and FDPA increased, and the platelet aggregation and FXIIIa activity decreased compared to the control. This anticlotting action of peptides in relation to the state of the hemostatic system was observed in animals with MS and 7 days after the withdrawal of the administration of the studied peptides.

Therefore, according to hemostasiological studies, arginine- and lysine-containing peptides showed anticoagulant activity and inhibited blood clotting factors, including the main clotting enzymes thrombin and FXIIIa, in comparison with the control. This is confirmed by data on the effect of other

proline-containing peptides on inhibition of the activity of the thrombin enzyme.[54] Blockage of thrombin activity by arginine-containing peptides may be due to the presence of the amino acid arginine in their structure, which contributes to the release of nitric oxide from the vascular endothelium,[55, 56] which leads not only to anticoagulant, but also antiplatelet action of these peptides in the body.[57] At the same time, there is a change in the degree and rate of formation of a fibrin clot in the bloodstream, the influence of FXIIIa on the processes of polymerization and stabilization of blood fibrin in the direction of normalization of the function of the hemostasis system. In addition, the release of t-PA from the vascular endothelium into the bloodstream under the action of the studied peptides contributes to an increase in the fibrinolytic potential of the blood. A decrease in hypercoagulation shifts and an increase in fibrinolysis may be due to increased gene expression for antithrombin III, protein C, and t-PA.[58]

The decrease in t-PA under the influence of the peptides may also be due to their interaction with platelet glycoprotein receptors, followed by inhibition of platelet binding to fibrinogen.[59] It is known that RGD-, RGDS-, RPD-amino acid sequences with the additional inclusion of proline exhibit a high antiplatelet effect due to the interruption of the final stage of fibrinogen binding to the GP IIb/IIIa of activated platelets.[60] This fact is noteworthy because peptides with this sequence of amino acids can be used for future designs of bifunctional antithrombotic agents.[61]

So, based on our results and available literature data, we can assume that the antiplatelet effects of proline peptides we studied, expressed in the suppression of ADP-dependent platelet aggregation, are due either to their interaction with fibrinogen or inhibition of platelet receptors. It is known that both thrombin inhibitors and platelet activation inhibitors, which play a key role in the formation of a blood clot, are used to suppress pathological thrombosis. Natural RGD-containing peptides had not only an antiplatelet effect, but also a significant anticoagulant effect.[54] The peptides we studied had a similar effect. Their anticoagulant activity is probably related to the inhibition of blood clotting factors (Xa and prothrombin complex factors), as evidenced by the increase in APTT activity that we have established.

To explain the mechanism fibrin-depolymerization effect of the above peptides is possible as a direct effect of peptides, because they have both FDPA and indirect effect. Peptides inhibit the conversion of fibrinogen to fibrin mainly at the "self-assembly" stage of fibrin, as described in Byshevsky et al.[62] The mechanism of antifibrin-stabilizing action of arginine- and lysine-containing peptides is probably their ability to inhibit thrombin involved in the activation of FXIIIa.[59]

Previously, it was shown that the regulatory peptide PGP in intranasal multiple administration to animals with hypercholesterolemia, along with fibrin-depolymerization and anticoagulant activity in the bloodstream, has a normocholesterolemic effect.[13] In the present review described the combined effect of arginine- and lysine-containing glyprolines as the process of blood clotting and lipid and carbohydrate metabolism in the body.

The studied glyprolines affected lipid and carbohydrate metabolism, reducing LDL-C and triglycerides in the blood, as well as the level of blood glucose, which is due to their structural features and due to the additional action of individual amino acids included in their composition.[63] In addition, the effects may be related to the property of glyproline peptides like heparin to reduce the atherogenic properties of blood through activation of blood lipoprotein lipase.[64] The results obtained indicate the potential of the studied compounds to influence homeostatic processes, change the metabolism and functions of cells, participate in the regulation of metabolism, and maintain metabolic balance. These results provide new factual material that characterizes the features of structural and functional interaction that underlie the ability of the studied regulatory peptides to influence the body's homeostasis. It is becoming increasingly obvious that low-molecular-weight peptide compounds play a significant role in regulating the body's metabolic processes.

5 CONCLUSION

Based on the presented literature data and experimental studies, it can be concluded that glyproline lysine- and arginine-containing peptides have a unique combined effect on the parameters of fat and carbohydrate metabolism and indicators of the hemostatic system, showing antithrombotic, hypocholesterolemic, and hypoglycemic properties in the blood plasma.

The results obtained indicate the potential of arginine- and lysine-containing peptides to influence the homeostatic processes, to change the metabolism of organs and tissues, and to participate in the regulation of metabolism, as well as to maintain the metabolic and hemostatic balance of the body.

Under the conditions of modeling MS in rats, the studied peptides had a complex effect on the hemostatic system parameters, increasing the antiplatelet, anticoagulant, fibrindepolymerization, and antifibrin-stabilizing properties of blood plasma. When violations of fat metabolism, accompanied by increased blood clotting and a decrease in its rheological properties, arginine- and lysine-containing peptides have a combined effect not only on primary and plasma hemostasis, but also on the function of the vascular endothelium, expressing a tissue activator of plasminogen in the bloodstream. As for the lipid-lowering effect of peptides, after treatment with peptides PGP, PRP, RPGP, KKRRPGP, RKKRPGP, and KRRKPGP, the parameters of lipid metabolism (LDL-C and TG) in different degrees approached the values corresponding to the lipid profile of healthy animals. All the studied peptides led to a slowdown in body weight growth, and KKRRPGP and KRRKPGP even led to a decrease in this indicator.

According to our data changes in the peptide, molecule may lead to altering of these peptides effect.[6, 13-15] Modification of the amino acid sequence in molecules of KRRKPGP, KKRRPGP, and RKKRPGP leads to significant changes in the peptide properties. We assume that this largely depends on the location of arginine and lysine in the peptide molecules, since there is evidence that confirms the importance of the participation of arginine- and lysine-containing polypeptides in blood clotting processes.

The results indicate the prospects of using regulatory with antithrombotic, hypolipidemic, and hypoglycemic properties to protect the body in cases of violations of fat and carbohydrate metabolism. Because the presence of a regulatory continuum of glyproline peptides, the body creates a continuity of processes of regulation and implementation of response physiological reactions.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

Biographies

- **Nikolai F. Myasoedov** graduated from Radiochemistry Department, Mendeleev University of Chemical Technology of Russia in 1960. He is a Professor of Chemistry (1982) at the Institute of Molecular Genetics, Russian Academy of Sciences. Since 1988, he is chair of Department of Chemistry of Physiologically Active Compounds of the same institute; full member of the Russian Academy of Sciences (2003); head of the scientific school “Synthesis and Studies of Pharmacologically Important Peptides, including Isotopically Modified Peptides.” He is the author of more than 770 peer-reviewed publications, including 5 monographs and 220 patents. The list of journals includes Frontiers in Pharmacology, BMC Genomics, Molecular Immunology, Journal of Molecular Neuroscience, Restorative Neurology and Neuroscience, Journal of Labelled Compounds and Radiopharmaceuticals, and others; Member of Editorial Board of “Radiochemistry,” “Bioorganic Chemistry,” “Molecular Genetics, Virology and Microbiology,” “Journal of Labelled Compounds and Radiopharmaceutical,” and “Stroke.” He was awarded by USSR State Prize in 1983 and Prize of the Government of the Russian Federation in 2001. He has got a gold medal and diploma at the 50th World Exhibition of Innovations, Investments and New Technologies (2001, Brussels), diploma at the 93th international Salon of Inventions “Concours-Lepine” (2002, Paris), gold medal and diploma at the International Salon of Investments (2007, Geneva), and gold medal at the US-Russian Business Community (ARBU). The main area of his study is chemistry of physiologically active substances, including peptides, bioorganic chemistry, and biotechnology.
- **Lyudmila A. Lyapina** graduated from the Department of Biochemistry of the Biological faculty of Lomonosov Moscow State University in 1963. She is a Professor of Physiology (1997) at the Lomonosov Moscow State University. Since 1993, she is head of the Laboratory of Blood protective systems named after Prof. B. A. Kudrjashov at the Department of Human and Animals Physiology of Faculty of Biology, Lomonosov Moscow State University. She leads a research group that studies the regulatory relationship between the body's clotting and anticoagulant systems in normal and pathological conditions, including metabolic syndrome, diabetes, and thrombosis. She is author of more than 300 peer-reviewed scientific papers, including 12 monographs and 51 patents. The list of journals include Pathophysiology, Bulletin of Experimental Biology and Medicine, Thrombosis and hemostasis, Biology Bulletin, Thrombosis Research, Haematologica, Doklady Biochemistry and Biophysics, and others. She has two discoveries—a new phenomenon of nonenzymatic fibrinolysis (1981) and the phenomenon of blockade of the function of the insulin system by a diabetogenic factor (1992); two Diplomas of the exhibition of innovative projects: (1) Discovery and synthesis of new biological blood anticoagulants (2009) and (2) Development of a new biologically active complex heparin methods of chemical thermodynamics and coagulation analysis (2014); the gold medal at the American-Russian Business Community (ARBU; 2010). She is a member of the all-Russian Association of Thrombosis, Hemorrhages, and Vascular Pathology named after Schmidt-Kudrjashov. The main activity of her study is the role of physiologically active substances, including heparin, its complexes and regulatory peptides in the hemostatic and associated systems.

- **Lyudmila A. Andreeva** graduated from the Mendeleev Russian University of Chemical Technology in 1979. Since 1997, she has been the head of the Sector of Regulatory Peptides in the Department of Chemistry of Physiologically Active Substances at the Institute of Molecular Genetics of the Russian Academy of Sciences. She is a highly qualified specialist in the field of peptide chemistry, who knows many methods of synthesis of peptides. She is author of more than 250 peer-reviewed scientific papers, including 2 monographs and 42 patents. The list of journals include Peptides, Amino acids, Journal of Molecular Recognition, Journal of Labelled Compounds and Radiopharmaceuticals, Reports of the Academy of Sciences, Journal of Neuroscience, International Journal of Peptide Research and Therapeutics, Doklady Biochemistry and Biophysics, Russian Journal of Bioorganic Chemistry, Pathophysiology, and Neurochemistry. the list of awards includes Winner of the Russian Government Award in the field of science and technology for the work “Development, Organization of Production and Implementation of a New Drug Semax—0.1% solution” in 2001; gold medal at the 48th and 50th World Exhibition of Innovations, Investments and New Technologies in Brussels, in 1999, 2001; Grand Prix at the V International Salon of industrial property “Archimedes-2002”; gold medal at the International Investment Salon (Geneva, 2007); gold medal at the American-Russian business community (ARBU; 2010) for the patent RU no. 2378005 (2010). The main scientific interests of her studies include synthesis and structure-function studies of various peptides of its analogs and fragments, study of the mechanism of their action, and development of innovative peptide-based drugs.
- **Marina E. Grigorjeva** graduated from Biological Faculty, Lomonosov Moscow State University with a degree in physiology (Russia) in 1986. After graduation, she is currently working at the same university at the Department of Human and Animal Physiology in the Laboratory of Blood Protective Systems named after Prof. B. A. Kudrjashov. Since 2014, she is leading researcher of the same department. In 1992, she defended her PhD thesis in physiology. She is the author of more than 200 peer-reviewed publications, including 7 patents, 3 monographs (Russia), and separate chapter in 5 monographs (USA). The list of journals include Journal of Thrombosis and Haemostasis, Haematologica, Pathophysiology, Thrombosis Research, Biology Bulletin, Bulletin of Experimental Biology and Medicine, Doklady Biochemistry and Biophysics, Biochemistry, Moscow University Biological Sciences Bulletin, and others. She was awarded a diploma by the Ministry of education and science of the Russian Federation (2013). She is a member of the all-Russian Association of Thrombosis, Hemorrhages and Vascular Pathology named after Schmidt-Kudrjashov. The main area of her study is protective effect of regulatory peptides and their complexes with heparin in normal and pathological conditions accompanied by increased blood clotting (i.e., thrombosis, stress, and metabolic disorders). These experimental studies are related to medical practice and are of fundamental and practical importance.
- **Tamara Y. Obergan** graduated from Department of Chemistry and Technology of Biologically Active Compounds, Lomonosov University of Fine Chemical Technology of Russia in 1993. She is a junior researcher (since 1993), a research associate (since 2003), and a senior researcher (since 2007) at the Laboratory of Blood Protective Systems named after Prof. B. A. Kudrjashov, Department of Human and Animal Physiology, Biological Faculty, Lomonosov Moscow State University, Russia. In 2004, she defended her PhD thesis “Glyprolines and their complex compounds with heparin as physiological modulators of the body's anticlotting system function.” She is the author of more than 170 peer-reviewed publications, including 3 monographs and 12 patents. The list of journals include Pathophysiology, Journal of Thrombosis and Haemostasis, Thrombosis Research, Haematologica, Biology Bulletin, Bulletin of Experimental Biology and Medicine, and others. She has got a gold medal at the American-Russian Business Community (ARBU) for the patent RU no. 2378005 (2010). She was awarded for her participation in the innovative project “Development of a new biologically active complex of high-molecular heparin with aspartic acid by methods of chemical thermodynamics, molecular dynamics and coagulation analysis in vitro” (2014, Moscow). She is a member of the all-Russian Association of Thrombosis, Hemorrhages and Vascular Pathology named after Schmidt-Kudrjashov. The main area of her study is to study the role of physiologically active substances (including heparin, its complex compounds and regulatory peptides) in the hemostatic system in normal and pathological conditions (i.e., disorders of carbohydrate and glucose metabolism, blood clotting disorders, etc.).
- **Tatiana A. Shubina** graduated from the Department of Human and Animal Physiology of the Biological Faculty of Lomonosov Moscow State University in 1997. In 1999–2001, she completed the work, written as a requirement for a PhD, on “Local and general fibrinolysinolysis in inflammatory processes induced by a surgical trauma.” In 2001–2003, she worked in the scientific laboratory of clinical problems of atherothrombosis of the Institute of Cardiology named after Prof. A. L. Myasnikov. She is the author of 89 peer-reviewed scientific papers, including 1 monograph and 6 patents. The list of journals include Pathophysiology, Bulletin of Experimental Biology and Medicine, Thrombosis and Hemostasis, Biological Bulletin, and Thrombosis Research. She received the gold medal at the American-Russian

Business Community (ARBU) for patent development (2010). She is a member of the Russian Association for the study of Thrombosis, Hemorrhages and Vascular Wall Pathology named after Schmidt-Kudrjashov and a member of the National Association of Specialists in Thrombosis, Clinical Hemostasiology and Hemorheorogy (NATH). Currently, she works in the Laboratory of Blood protective systems named after prof. B. A. Kudrjashov of the Biological Faculty of Lomonosov Moscow State University and studies the functional relationship between the hemostasis and the insulin system of the body in normal and in the development of various disorders of carbohydrate metabolism (experimental diabetes type 1 and 2, metabolic syndrome). Her research work is aimed at studying ways of preventing and correcting these diseases using various substances of peptide and nonpeptide nature.

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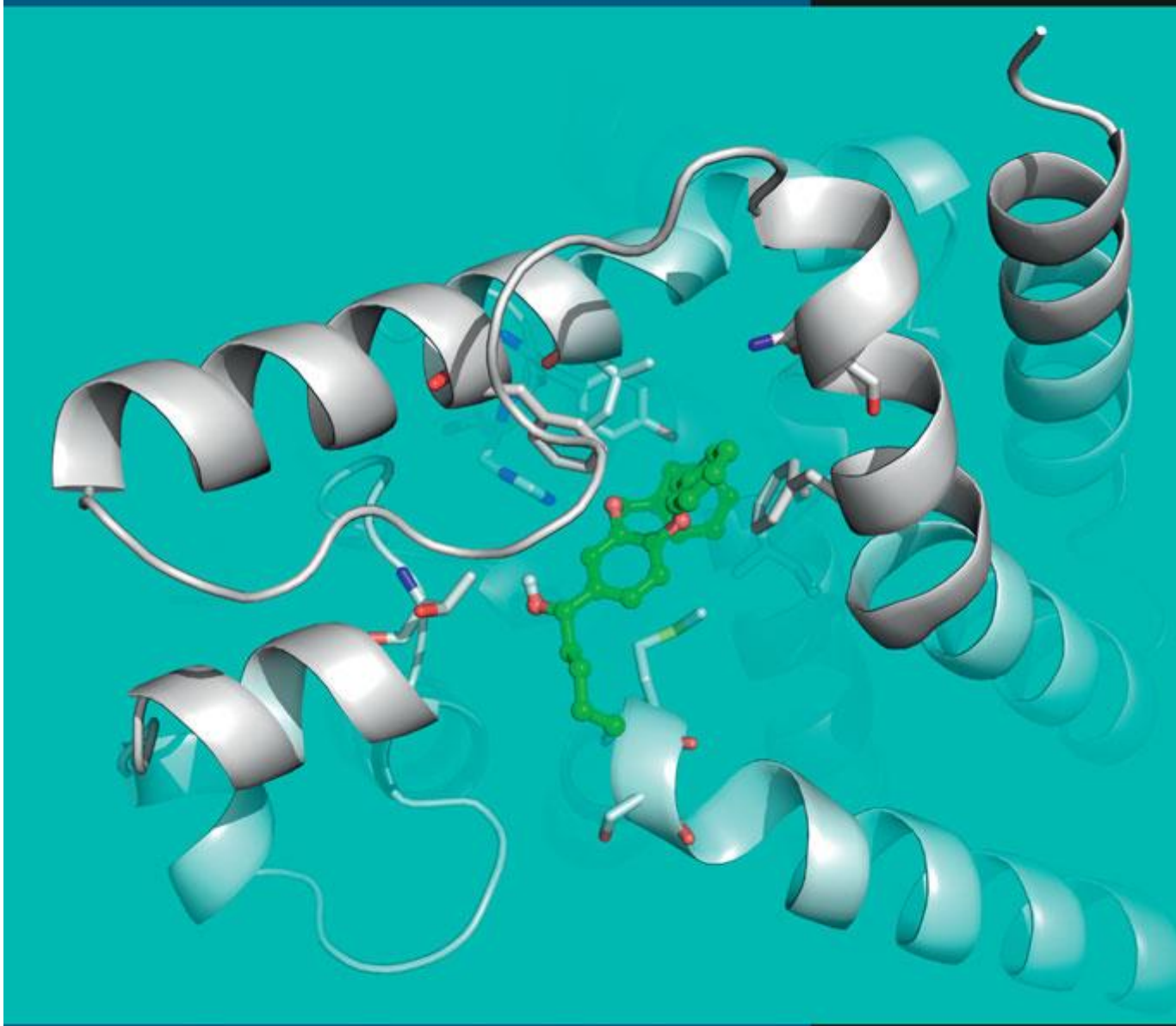

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