Ankara Üniversitesi Veteriner Fakültesi Dergisi/Ankara Univ Vet Fak Derg; 2026:73(1): 93-101
DOI: 10.33988/auvfd.1627124
Research Article

Levels of medium-molecular-weight peptides in the cerebral cortex of rats of different ages and nervous system types under high-protein and protein-free diets

Samira İBRAHİMOVA[a,✉]

Ministry of Science and Education of the Republic of Azerbaijan, Institute of Physiology named after Academician Abdulla Garayev, Baku, Azerbaijan.

[a]ORCID: 0000-0001-7548-6170.

ABSTRACT

The aim of this study was to investigate the effects of high-protein and protein-free diets on the levels of medium-molecular-weight peptides (MMPs) in specific brain regions of rats of different ages and nervous system types. The animals were divided into stress-resistant and stress-sensitive groups. A control was selected from each group and maintained under vivarium conditions. The experimental animals were divided into two subgroups and were fed a special diet for periods of 10, 20, or 30 days. In the first subgroup, the dietary components were completely preserved (protein-based diet), whereas in the second subgroup casein was excluded from the diet (protein-free diet). At the end of the specified feeding periods, levels of MMPs in tissue homogenates of the bilateral limbic and orbital cortices (left and right hemispheres) of control and experimental rats were determined. In 3-month-old animals, MMP levels increased on both high-protein and protein-free diets. In contrast, in 12-month-old animals, MMP levels decreased under both conditions. Also, with both protein-containing and protein-free nutrition, interhemispheric asymmetry was observed in the MMPs content of the limbic and orbital cortices of the left and right hemispheres in 3- and 12-month-old stress-resistant and stress-sensitive animals.

Keywords: Hemispheres, High protein diets, Medium-molecular-weight peptides, Protein free diets

✉**Corresponding author:** samiraibrahimova007@gmail.com

Received: 05.02.2025, **Accepted:** 09.11.2025, **Publication Date:** 01.01.2026

How to cite this article: İbrahimova S (2026): Levels of medium-molecular-weight peptides in the cerebral cortex of rats of different ages and nervous system types under high-protein and protein-free diets. Ankara Univ Vet Fak Derg, 73 (1), 93-101. DOI: 10.33988/auvfd.1627124.

Introduction

Stress and its effects on various physiological systems are among the current issues in medicine and biology. Stress, whether positive or negative, is analyzed through the activity of both cerebral hemispheres. Right-hemisphere activity is responsible for the emergence of negative emotional states, whereas left-hemisphere activity is responsible for the emergence of positive emotional states (3).

Experiments indicate that individual resistance to the adverse effects of stress varies among higher animals. It has been determined that the levels of proteins and endogenous peptides (such as substance P, delta-sleep peptide, β-endorphin, biogenic amines, and expressed S-phos protein) are altered in various neurons within the brain structures of such animals (21).

Proper nutrition, through the intake of macronutrients (e.g., essential fatty acids, carbohydrates, essential amino acids) and micronutrients (e.g., vitamins, essential minerals), serves not only as a primary source of energy but also ensures the normal growth and development of the body. It enhances the body's resistance to disease and improves its ability to adapt adequately to the environment (19).

Low-protein diets primarily disrupt protein balance (rates of protein synthesis and degradation), which negatively affects many bodily functions, including enzyme activity, hormone synthesis, immune function, and adaptability (8).

It has been determined that dietary protein deficiency severely slows the development of the central nervous system, impairs conditioned reflexes as well as learning

and memory, and weakens inhibitory and excitatory processes in the cerebral cortex. Conversely, excessive protein intake increases central nervous system excitability (12).

The study of medium-molecular peptides (MMPs) has gained significant attention in modern medical practice because these peptides are considered potential indicators of endogenous intoxication. These substances, with molecular weights ranging from 300 to 5,000 daltons (Da), are regarded as incomplete degradation products of blood and tissue proteins. The accumulation of MMPs in tissues and intercellular fluids can lead to serious complications, primarily functional disorders of the nervous system. MMPs, which exhibit neurotoxic activity, weaken protein biosynthesis, disrupt oxidation and phosphorylation processes, interfere with the regulation of adenine nucleotide synthesis, and alter ion transport across membranes, erythropoiesis, phagocytosis, and microcirculation. MMPs can bind to and block receptors on various cells, exerting adverse effects on their metabolism and functions (1, 2). According to some studies, the normal level of MMPs in the blood is 0.250–0.280 (17) relative units, but this value can increase to 0.7–1.4 relative units (15). MMPs at physiological concentrations are considered components of blood plasma (14).

In experimental animals, increases in indicators of oxidative modification of proteins and in the levels of medium-molecular-weight compounds in cerebellar tissue are observed under conditions of prolonged hypodynamia. Lobanov et al (2016) suggest that changes in the levels of medium-molecular-weight substances in different hemispheres of the cerebellum under long-term hypodynamia may be regarded as one of the regulatory mechanisms of compensatory responses to stress, and these changes may reflect the manifestation of their antioxidant effects (14). Our previous studies showed that under both protein-rich and protein-deficient dietary conditions, the levels of MMPs in the sensorimotor cortex and hypothalamus of stress-resistant and stress-sensitive rats were increased at 3 months of age and decreased at 12 months of age (7).

The limbic, orbital, and sensorimotor cortical areas, as well as the hypothalamus, are higher integrative centers of the brain that regulate adaptive-compensatory responses and food intake behavior, and play a significant role in modulating protein plasticity and enhancing the synthesis of oligopeptides from MMPs in the central nervous system (10). Based on these findings, the present study aims to determine the levels of MMPs in the limbic and orbital cortical regions of rats of different ages and with different nervous-system types under high-protein and protein-free dietary conditions.

Materials and Methods

In total, 280 male Wistar albino rats were included in the study. Prior to the experiment, all rats were subjected to a preliminary test to evaluate their behavioral response to a stress-inducing sound stimulus. The stimulus was an electric bell (90–120 dB) presented in a sound-isolated chamber measuring 0.4 × 0.3 × 0.5 meters, with a maximum exposure duration of 120 seconds (11). Based on their behavioral reactions, the animals were categorized into two groups.

Rats that responded to the intense auditory stimulus with pronounced locomotor agitation (e.g., running around the arena), occasionally escalating to convulsive, epileptic-like seizures accompanied by vocalizations, were classified as stress-sensitive. In contrast, rats that showed little or no change in behavior in response to the same stimulus were classified as stress-resistant. Following this classification, animals were further divided by age into two subgroups: 3-month-old and 12-month-old animals. As a result, four experimental groups were formed, consisting of 3-month-old stress-sensitive rats (n = 70), 3-month-old stress-resistant rats (n = 70), 12-month-old stress-sensitive rats (n = 70), and 12-month-old stress-resistant rats (n = 70). All animals were kept under standard vivarium conditions at a room temperature of 22–24 °C. Five rats were housed in each plastic cage (0.3 m height × 0.4 m width × 0.6 m length). The animals were fed once daily and water was provided ad libitum.

For the experiments, 10 animals from each stress-resistant and stress-sensitive group aged 3 and 12 months were selected as control animals and maintained under vivarium conditions. The remaining 60 animals from each group were divided according to the study's purpose and fed diets prepared using a special recipe (16) for periods of 10, 20, and 30 days, with 10 animals in each subgroup. The recipe included starch (65.0 g), vegetable oil (5.0×10^{-3} m³), fiber (5.0 g), a vitamin mixture (1.0 g), salt (0.4 g), and low-fat cooked minced meat. Meat was used as a substitute for casein. A total of 85.0 g of cooked beef, providing approximately 23.0 g of protein, was used (24).

In the first subgroup, the original composition of the recipe was preserved, while in the second subgroup, all protein was removed from the formulation. Each prepared feed ration was calculated as half the body weight of the experimental animal. Casein in the recipe was replaced with low-fat minced meat, which is rich in essential amino acids, vitamins, enzymes, and minerals (6).

Intact animals were not used in this study because comparing animals unexposed to any stimulus with those subjected to audiogenic sound stress would be inappropriate. At the end of the specified feeding periods— namely on the 10th, 20th, and 30th days of the

protein-containing and protein-free diets— the animals were decapitated, the brains were extracted, and corresponding regions of the left and right hemispheres (specifically the limbic and orbital cortices) were dissected according to the atlas (22). Levels of MMPs in tissue homogenates were determined by a spectrophotometric method (9). The method was based on determining MMP levels in the supernatant obtained by deproteinizing blood serum and tissue samples with a trichloroacetic acid solution, followed by centrifugation. The absorbance was measured at a wavelength of 254 nm.

Statistical Analysis: The average quantitative indicator and its standard error were calculated for each group. After determining the minimum and maximum values, the reliability of intergroup differences was assessed using the Student's t-test (5). In the present study, age was not included as a factor in the statistical analysis. The 3- and 12-month-old animals were analyzed together in a single statistical model. Therefore, only two independent variables — stress phenotype and dietary type — were considered. Differences between groups were considered significant at $P<0.05$.

Results

According to our findings, the levels of MMPs in the symmetrical limbic and orbital cortices in the left and right hemispheres of the brains of stress-resistant and stress-sensitive rats of different ages varied depending on whether the rats were fed high-protein or protein-free diets.

Figures 1–4 show percentage MMP levels in the limbic and orbital cortices of 3- and 12-month-old stress-resistant and stress-sensitive rats fed a high-protein diet, compared with the control group (Table 1).

On the 10th day, against a background of high-protein nutrition, levels of MMPs in the symmetrical limbic cortex of the left and right hemispheres of 3-month-old animals in the stress-resistant group were 118% ($P<0.01$) and 142% ($P<0.001$), respectively; in the orbital cortex, levels were 191% ($P<0.01$) and 150% ($P<0.001$), respectively. On day 20, in the limbic cortex the values were 112% and 105%; in the orbital cortex they were 155% ($P<0.01$) and 106%. On the 30th day, levels in the limbic cortex were 176% ($P<0.001$) and 179% ($P<0.001$), and levels in the orbital cortex were 227% ($P<0.001$) and 150% ($P<0.001$) (Figure 1).

In 12-month-old animals in this group, the symmetrical limbic cortex in the left and right hemispheres showed 100% and 103%, respectively, on the 10th day of high-protein nutrition; in the orbital cortex, the values were 86% and 92%. On the 20th day, in the limbic cortex, the values were 103% and 109%, while in the orbital cortex, they were 92% and 97%. Finally, on day 30, the levels in the limbic cortex were 97% and 112%, and in the orbital cortex were 75% and 81%, compared with control animals (Figure 2).

Table 1. The effect of a high-protein diet on the levels of MMPs in the brain structures of 3- and 12-month-old animals from the stress-resistant and stress-sensitive groups.

Experimental conditions	Structures			
	Limbic cortex		Orbital cortex	
	Left hemisphere	Right hemisphere	Left hemisphere	Right hemisphere
3-month-old stress-resistant rats				
10 days	118% **	142% ***	191% ***	150% ***
20 days	112%	105%	155% ***	106%
30 days	176% ***	179% ***	227% ***	150% ***
12-month-old stress-resistant rats				
10 days	100%	103%	86%	92%
20 days	103%	109%	92%	97%
30 days	97%	112%	75%	81%
3-month-old stress-sensitive rats				
10 days	200% ***	200% ***	193% ***	188% **
20 days	153% **	141% **	140% **	163% ***
30 days	167% ***	188% ***	167% ***	169% ***
12-month-old stress-sensitive rats				
10 days	82%	76%	79%	76%
20 days	115%***	107%	105%	112% *
30 days	72%	71%	76%	76%

*; $P<0.05$; **; $P<0.01$; ***; $P<0.001$

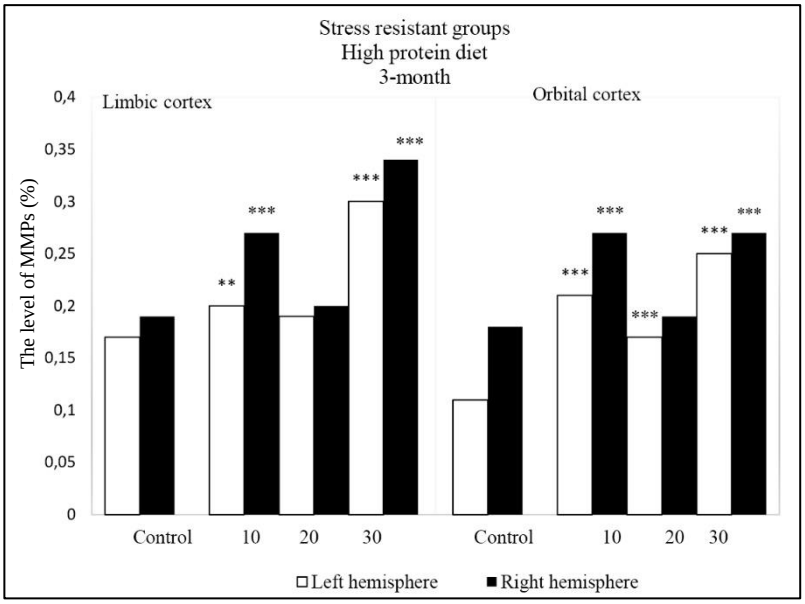

Figure 1. The effect of a high-protein diet on the levels of medium-molecular-weight peptides in the brain structures of 3-month-old animals from the stress-resistant group.
*; P<0.05; **; P<0.01; ***; P<0.001.

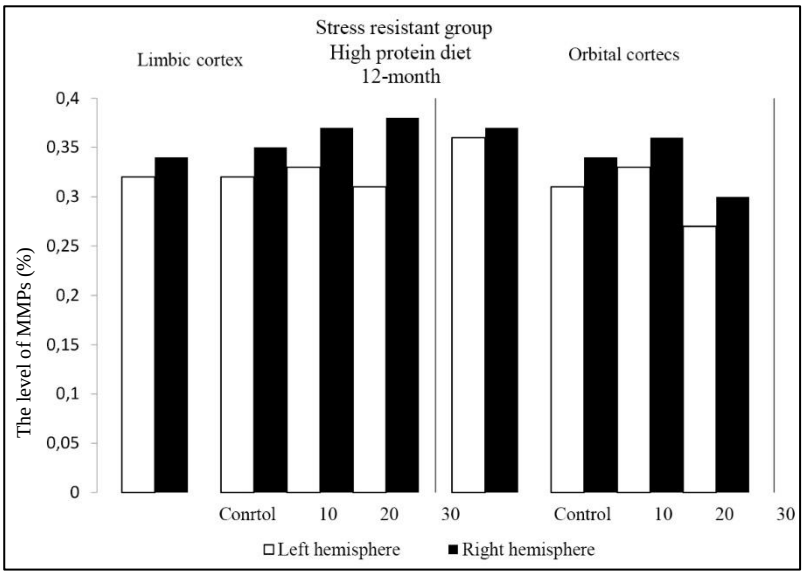

Figure 2. The effect of a high-protein diet on the levels of medium-molecular-weight peptides in the brain structures of 12-month-old animals from the stress-resistant group.
*; P<0.05; **; P<0.01; ***; P<0.001.

The level of MMPs in the bilateral limbic cortex (left and right hemispheres) of 3-month-old animals in the stress-sensitive group on the 10th day of high-protein feeding was 200% (P<0.001, P<0.001). In the orbital cortex, it was 193% (P<0.001) and 188% (P<0.01). On the 20th day, levels were 153% (P<0.01) and 141% (P<0.01) in the limbic cortex, and 140% (P<0.01) and 163% (P<0.001) in the orbital cortex. On day 30, values in the limbic cortex were 167% (P<0.001) and 188% (P<0.001), and values in the orbital cortex were 167% (P<0.001) and 169% (P<0.001) (Figure 3).

In 12-month-old animals in this group, on day 10 of the diet, limbic cortex symmetry in the left and right hemispheres was 82% and 76%, respectively; orbital cortex symmetry was 79% and 76%. On the 20th day, levels in the limbic cortex were 115% (P<0.001) and 107%, and in the orbital cortex were 105% and 112% (P<0.05). Finally, on day 30, values in the limbic cortex were 72% and 71% (left and right hemispheres, respectively), and values in the orbital cortex were 76% in both hemispheres (Figure 4).

Figures 5–8 show MMP levels (expressed as percentages) in the limbic and orbital cortices of 3- and 12-month-old stress-resistant and stress-susceptible rats fed a protein-free diet, compared with the control (Table 2).

Table 2. The effect of a protein-free diet on the levels of MMPs in the brain structures of 3- and 12-month-old animals from the stress-resistant and stress- sensitive groups

Experimental conditions	Structures			
	Limbic cortex		Orbital cortex	
	Left hemisphere	Right hemisphere	Left hemisphere	Right hemisphere
3-month-old stress-resistant rats				
10 days	159% ***	158% ***	227% ***	161% ***
20 days	147% **	147% **	218% ***	144% **
30 days	170% **	173% ***	272% ***	200% ***
12-month-old stress-resistant rats				
10 days	113%	112%	92%	110% *
20 days	103%	106%	108%	114% *
30 days	97%	103%	86%	92%
3-month-old stress-sensitive rats				
10 days	120%	112%	93%	94%
20 days	147% *	171% ***	147% **	181% **
30 days	193% **	194% **	227% **	231% **
12-month-old stress-sensitive rats				
10 days	87%	84%	79%	76%
20 days	103%	98%	105%	112%
30 days	108%	113%	76%	76%

*; P<0.05; **; P<0.01; ***; P<0.001

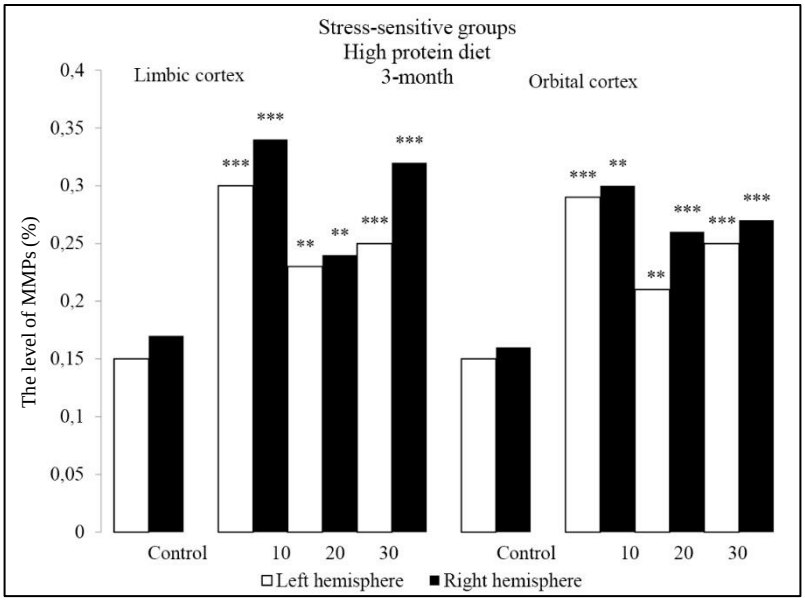

Figure 3. The effect of a high-protein diet on the levels of medium-molecular-weight peptides in the brain structures of 3-month-old animals from the stress-sensitive group.

*; P<0.05; **; P<0.01; ***; P<0.001.

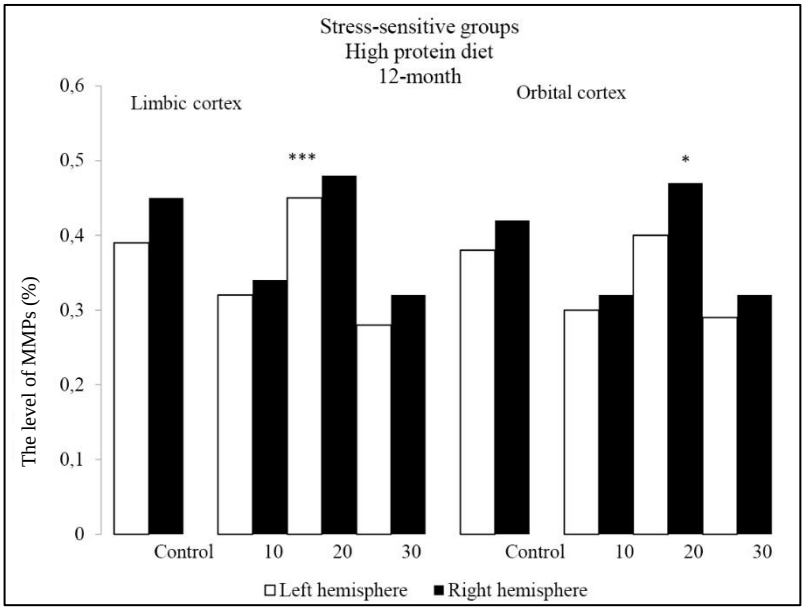

Figure 4. The effect of a high-protein diet on the levels of medium-molecular-weight peptides in the brain structures of 12-month-old animals from the stress-sensitive group.

*; P<0.05; **; P<0.01; ***; P<0.001.

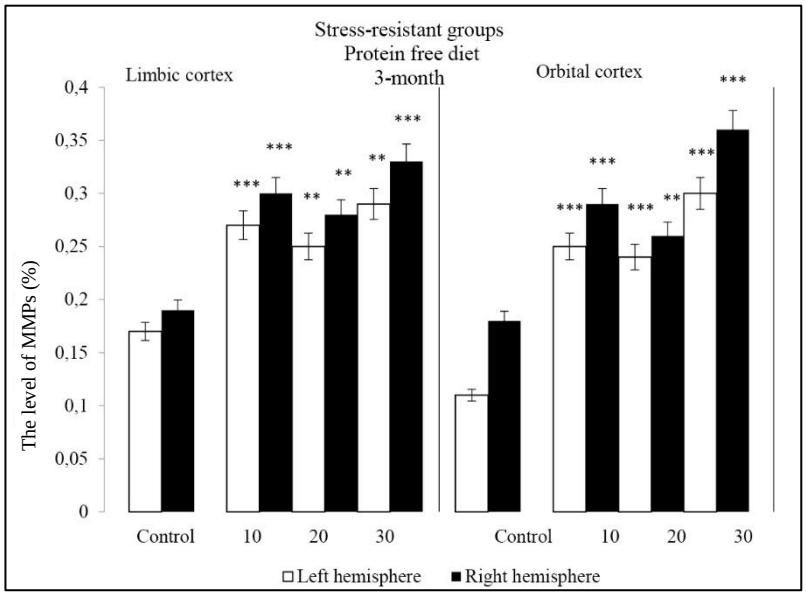

Figure 5. The effect of a protein-free diet on the levels of medium-molecular-weight peptides in the brain structures of 3-month-old animals from the stress - resistant group.
*; P<0.05; **; P<0.01; ***; P<0.001.

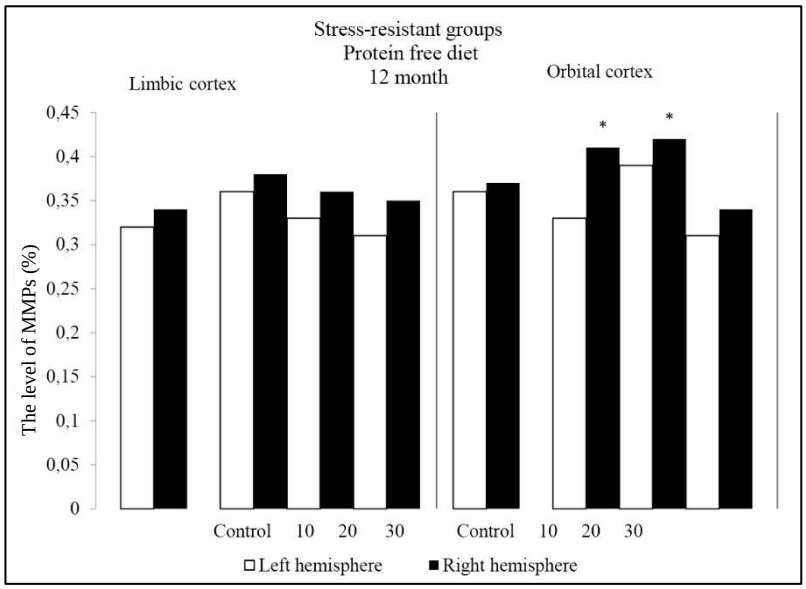

Figure 6. The effect of a protein-free diet on the levels of medium-molecular-weight peptides in the brain structures of 12-month-old animals from the stress – resistant group.
*; P<0.05; **; P<0.01; ***; P<0.001.

On the 10th day of the protein-free diet, the levels of MMPs in the limbic cortices of the left and right hemispheres in three-month-old stress-resistant animals were 159% (P<0.001) and 158% (P<0.001), respectively, while in the orbital cortices they reached 227% (P<0.001) and 161% (P<0.001). On the 20th day, the level was 147% (P<0.01) in each limbic cortex, and 218% (P<0.001) and 144% (P<0.01) in the orbital cortex. On day 30, values in the limbic cortex were 170% (P<0.01) and 173% (P<0.001); values in the orbital cortex were 272% (P<0.001) and 200% (P<0.001) (Figure 5).

In twelve-month-old animals in this group, on day 10 the symmetrical limbic cortices of the left and right hemispheres showed 113% and 112%, respectively; the orbital cortices showed 92% and 110% (P<0.05). On day 20, the levels were 103% and 106% in the limbic cortex and 108% and 114% (P<0.05) in the orbital cortex. Finally, on day 30, the values were 97% and 103% in the limbic cortex and 86% and 92% in the orbital cortex, compared with the control (Figure 6).

Against the background of a protein-free diet, on day 10, levels of MMPs in the limbic cortex of the left and right hemispheres of 3-month-old stress-sensitive animals were 120% and 112%, respectively; in the orbital cortex, levels were 93% and 94%, respectively. On day 20, levels were 147% (P<0.05) and 171% (P<0.001) in the limbic cortex, and 147% (P<0.01) and 181% (P<0.01) in the orbital cortex. On day 30, values in the limbic cortex were 193% and 194% (both P<0.01), and in the orbital cortex were 227% and 231% (both P<0.01) (Figure 7).

In 12-month-old animals, on the 10th day of protein starvation, levels were 87% and 84% in the limbic cortex and 79% and 76% in the orbital cortex. On day 20, the values were 103% and 98% in the limbic cortex and 105%

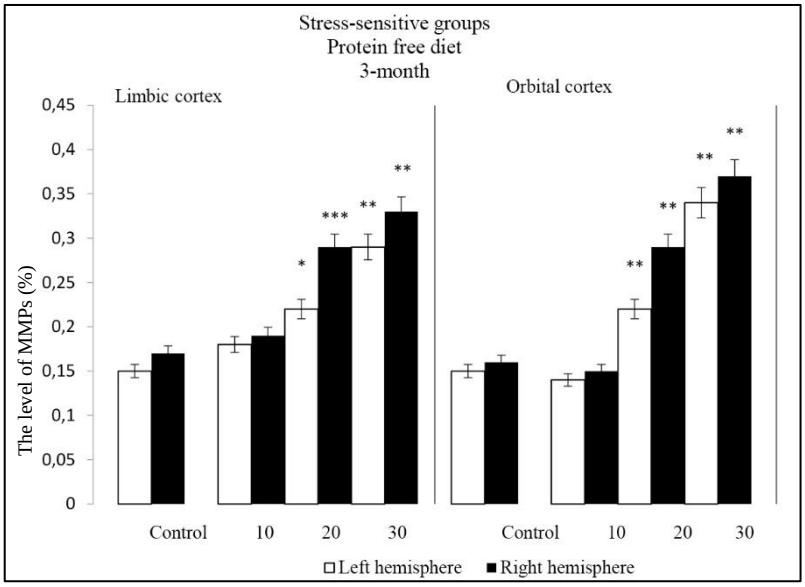

Figure 7. The effect of a protein-free diet on the levels of medium-molecular-weight peptides in the brain structures of 3-month-old animals from the stress-sensitive group.
*; P<0.05; **; P<0.01; ***; P<0.001.

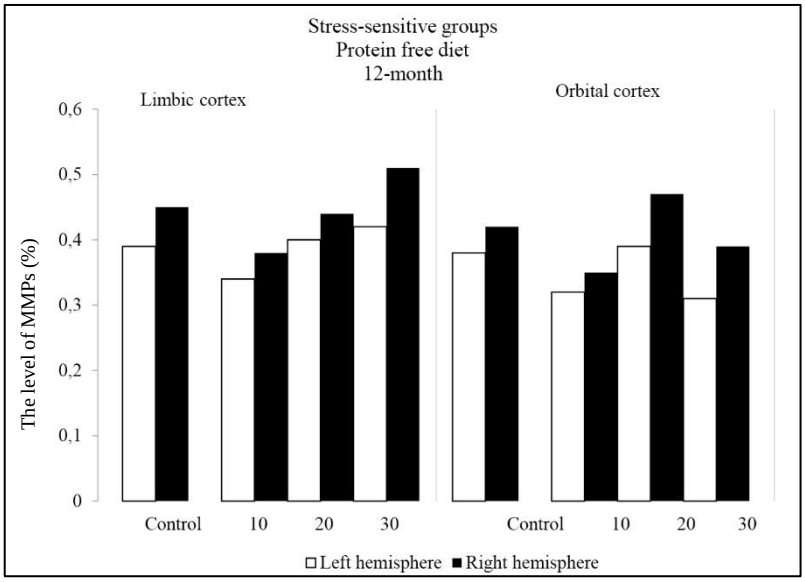

Figure 8. The effect of a protein-free diet on the levels of medium-molecular-weight peptides in the brain structures of 12-month-old animals from the stress-sensitive group.
*; P<0.05; **; P<0.01; ***; P<0.001.

and 112% in the orbital cortex. Finally, on day 30, levels were 108% and 113% in the limbic cortex and 76% in both the left and right orbital cortices, compared with the control (Figure 8).

Discussion and Conclusion

Results revealed that in the limbic and orbital cortices of both hemispheres of 3-month-old stress-resistant and stress-sensitive animals, the level of MMPs increased after one month on a high-protein diet, whereas in 12-month-old animals, it decreased. The increase in MMP levels in the limbic and orbital cortices of both hemispheres of 3-month-old animals from both stress-resistant and stress-sensitive groups fed a high-protein diet can be attributed to the diet's high protein content and to accelerated breakdown of exogenous proteins by specific proteolytic dipeptidases. The long-term consumption of high-protein food induces changes in metabolic processes. When proteins are oxidized, they undergo proteolysis mediated by specific proteases, resulting in the formation of MMPs. In the literature, MMP formation has been associated with systemic proteolysis (4). The decrease in MMP levels in the brains of 12-month-old animals can be attributed to the beneficial effects of the high-protein diet. Protein-rich foods enhance the antioxidant defenses of vital organs and reduce oxidative stress. Food-derived antioxidant peptides reduce the production of reactive oxygen species (ROS) and activate endogenous antioxidant defense systems in both cellular and animal models (25).

The observed increase in 3-month-old animals can be explained by the long-term intake of exogenous protein through their diet, while the decrease in 12-month-old animals may be attributed to the antioxidant effects of such food. Several studies have shown that five 2-

oxoimidazole-containing dipeptides (2-oxo-IDPs) are present in beef and that these dipeptides exhibit potent antioxidant activity in vivo (22).

As the duration of protein-free feeding increased, the elevated concentrations of MMPs observed in the limbic and orbital cortices in both hemispheres of 3-month-old animals, compared with the control group, were likely attributable to the stress effects of this diet. Under conditions of protein deficiency, the body meets its protein requirements by mobilizing its internal reserves (4). This indicates that processes related to the organism's adaptation to starvation and the transition to endogenous nutrition are triggered. When dietary protein intake is low, the body activates its internal reserves. During this period, to maintain brain function and meet its demands, structural proteins from various organs and tissues (such as blood serum, muscule, bone and liver) are broken down into free amino acids (13, 23).

Following such feeding, MMP levels decreased in studied cortical areas of both the left and right hemispheres of 12-month-old animals. The restriction of dietary protein leads to a decrease in the concentrations of essential amino acids, which are crucial for metabolic adaptation to a protein-deficient diet (18).

Under extreme conditions, the brain structures studied serve as centers responsible for maintaining the body's internal metabolic homeostasis and supporting adaptive behavior. As shown in all figures, interhemispheric differences in MMP levels in the limbic and orbital cortices between the left and right hemispheres were observed in 3- and 12-month-old control and experimental animals from both stress-resistant and stress-sensitive groups after one month on high-protein or protein-free diets. Protein deficiency is one of the most significant stressors. The body's adaptive-compensatory reactions in stressful situations are primarily mediated by the right hemisphere, resulting in increased functional activity of this hemisphere.

During emotion formation, the functional activity of the brain's hemispheres changes: the right hemisphere becomes more active during negative emotions, while the left hemisphere becomes more active during positive emotions (20). All MMP indicators are at the limits of physiological tolerance. Exceeding these limits is known to lead to various pathologies. It can be inferred that this borderline state of MMPs may contribute to the development of a negative emotional state, which in turn increases the body's readiness to respond to new stressors. Consequently, innate self-preservation mechanisms are enhanced.

Thus, under unfavorable nutritional conditions the mechanisms of protein synthesis, renewal, and detoxification remain stable compared with those of sexually mature adult animals. Under both high-protein and protein-free diets, interhemispheric differences in the levels of MMPs were observed in the brain structures of stress-resistant and stress-sensitive groups.

Acknowledgements

This study was derived from the PhD thesis.

Financial support

This research received no grant from any funding agency or sector.

Ethical Statement

The experimental protocol was approved by the Local Ethics Committee of the Institute of Physiology named after Academician Abdulla Garayev (Protocol No. 01, dated 30.01.2024) and was subsequently confirmed by the National Committee on Bioethics, Ethics of Science and Technology of the Republic of Azerbaijan (Decision No. 1/25, dated 04.03.2025). All procedures were performed in compliance with approved ethical standards.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

Conducting experiments, statistical calculations, collecting materials on the topic and writing articles belongs to SI.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to the ARRIVE Guidelines to protect animals used for scientific purposes.

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