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RESEARCH ARTICLE

Effects of Swimming Exercise and Protein Intake on Growth Factors and Stress Hormones in Mice

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ABSTRACT

This study investigates the combined effects of protein intake and swimming exercise on the expression of key growth factors (BDNF, IGF-1, VEGF) and stress-related hormones (CRHBP, cortisol, epinephrine). Six-week-old ICR male mice were divided into four groups based on dietary and exercise interventions: Group 1 received a protein diet with exercise (PEG), Group 2 received a protein diet without exercise (PNEG), Group 3 was provided with a protein-free diet with exercise (NPEG) and Group 4 was given a protein-free diet without exercise (NPNEG). After a 6-week experimental period, BDNF, IGF-1, and VEGF mRNA expression levels were analyzed using the Real-Time Loop-Mediated Isothermal Amplification (RT-LAMP) method. In contrast, the levels of stress-related hormones were measured through ELISA. The results reveal significant impacts of dietary protein intake and swimming exercise on the expression of these critical markers. BDNF and IGF-1 levels were highest in PEG, highlighting the beneficial effects of combining protein intake with swimming exercise. VEGF expression also showed significant differences, particularly between PNEG and NPNEG, and PNEG and PEG. CRHBP, cortisol, and epinephrine levels varied significantly across groups, especially between those subjected to different dietary and exercise regimens. This suggests a strong interaction between protein intake and exercise in modulating stress responses.

In conclusion, the study demonstrates that protein intake and swimming exercise synergistically influence the expression of growth factors and stress hormones in mice. These findings provide valuable insights into the physiological adaptations to diet and exercise in mammals, which could inform future research on optimizing dietary and exercise interventions for health and stress management.

Key words: Swimming exercise, protein intake, growth factor, stress hormone, mouse

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Introduction

Protein intake and physical exercise are critical factors that significantly influence mammals' physiological development and stress responses. Among various physical activities, swimming is a full-body workout that offers unique benefits, particularly for rodents, due to their innate

swimming abilities. Previous studies have demonstrated that swimming can enhance cardiovascular fitness, improve metabolic health, and induce positive changes in brain function (Contarteze et al., 2008; Liu et al., 2018). Additionally, dietary protein is essential for synthesizing growth factors and stress-related hormones, which are vital for the overall health and development of organisms (Iemitsu et al., 2004).

While numerous animal studies have investigated the relationship between exercise and dietary intake (Kido et al., 2024; Lugilde et al., 2022), a comprehensive understanding of how these factors interact to influence the expression of growth factors and stress hormones in mice remains limited. Growth factors such as Brain-Derived Neurotrophic Factor (BDNF), Insulin-like Growth Factor-1 (IGF-1), and Vascular Endothelial Growth Factor (VEGF) play crucial roles in brain development, neuroplasticity, and overall growth (Contarteze et al., 2008). For example, a 16-week aquatic exercise program has been shown to induce significant changes in BDNF and IGF-1 levels and cognitive function in elderly women (Kang et al., 2020). Conversely, stress hormones such as cortisol and epinephrine are key mediators of the body's response to stress and have significant implications for physical and mental health (Liu et al., 2018; de Punder et al., 2019).

Swimming in rodents is considered advantageous over other forms of exercise, such as running, as it is a non-weight-bearing activity that minimizes the risk of injury while providing a controlled environment for assessing the physiological effects of exercise (Freitas et al., 2024). Additionally, swimming induces less mechanical stress than running, making it a favorable modality for studying stress-related responses (Iemitsu et al., 2004).

Protein intake influences the synthesis of growth factors and the modulation of stress responses. Adequate protein levels are essential for maintaining muscle mass, supporting immune function, and promoting the synthesis of various biomolecules (Iemitsu et al., 2004). In contrast, protein deficiency can lead to impaired growth, weakened immune responses, and altered stress hormone levels, which may have long-term effects on health and development (Contarteze et al., 2008).

This study aims to investigate the combined effects of swimming exercise and protein intake on the expression of growth factors and stress hormones in mice.

Materials and Methods

Animals

In this study, six-week-old Institute of Cancer Research (ICR) male mice were purchased from Samtako Co. (Osan, Korea) and used for experiment. The mice were housed in a controlled environment with a temperature of 23℃ ± 2℃, humidity of 50% ± 10%, and illumination of 150-300 lux on a 12-hour light/dark cycle. The mice were acclimated for one week before the experiments were conducted. A protein diet was obtained from Samtako Co., specifically Altromin #1314 (Protein 27%, Fat 14%, Carbohydrates 59%) from Altromin Co. (Lage, Germany). The protein-free diet (Protein-Free Diet) was custom-made by Saeronbio Co. (Korea), with its composition detailed in Table 1. Both food and water were provided ad libitum. This study followed the Saintone Institutional Animal Care and Use Committee (IACUC) (2024-SAINTONE IACUC-001).

Table 1. Protein-free diet formula sheet

Formula	Protein-free Diet (g/Kg)
Casein	0
DL-Methionine	0
Sucrose	631.8
Corn Starch	200
Corn Oil	54.6
Cellulose	66.462
Vitamin mix	10
Ethoxyquin, antioxidant	0.01
Mineral Mix, Ca-P Deficient	13.37
Calcium Phosphate, dibasic	23.72
Calcium Carbonate	0.038

Experimental Design and Swimming Exercise

The experiment was designed with four groups, each consisting of eight mice. PEG was given a protein diet combined with swimming exercise, PNEG received a protein diet without swimming exercise, NPEG received a protein-free diet with swimming exercise, and NPNEG was fed a protein-free diet without swimming exercise. Swimming, an inherent capability of rodents, offers distinct advantages over treadmill exercise (Liu et al., 2018). The mice were trained according to a moderate-intensity swimming regimen described by Liu et al. This regimen included two phases: an adaptation phase and a training phase. During the first week of adaptation, the swimming duration started at 15 minutes on the first day and gradually increased to 60 minutes by the fifth day. The primary goal of the adaptation phase was to mitigate water-induced stress without triggering physiological changes associated with physical training (Contarteze et al., 2008). Following this, the mice swam for 60 minutes daily, 5 days a week, for 6 weeks. Swimming was conducted in groups of eight mice, as previous studies have shown that the intensity of swimming exercise significantly increases due to animal interactions (Iemitsu et al., 2004). Throughout the exercise sessions, supervision was maintained to ensure proper execution. After completing the exercise and protein-modified feeding protocols, all animals were anesthetized, and blood was collected via cardiac puncture. A portion of the collected blood was used for hematological analysis, while the remaining was reserved for ELISA assays. The heart, liver, spleen, and brain tissues were harvested. The tissues were washed with 1X PBS and weighed. Subsequently, brain tissues were immersed in RNAlater (Thermo, USA) and stored until further use in experiments.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from brain tissues preserved in RNAlater using QIAzol (QIAGEN, Valencia, CA). A 100 mg brain tissue sample was homogenized in 1 ml QIAzol reagent using a Centrifuge Tube Sample Pestle (SciLab, Korea). The homogenized samples were incubated at room temperature for 5 minutes and added 0.2 ml chloroform. The mixture was incubated at room temperature for an additional 3 minutes. After centrifugation at 12,000 × g for 15 minutes at 4°C, the aqueous phase was transferred to a new 1.5 ml centrifuge tube, avoiding the interphase. Subsequently, 0.5 ml of isopropanol was added, and the mixture was incubated at room temperature for 10 minutes. The samples were

centrifuged at 12,000 × g for 10 minutes at 4°C, and the supernatant was discarded. The pellet was washed with 1 ml of 75% ethanol and centrifuged at 7,500 × g for 5 minutes at 4°C. The supernatant was completely removed, and the RNA pellet was resuspended in RNase-free water. The concentration (ng/μl), purity (260/280 nm), and integrity (260/230 nm) of the extracted RNA were measured using a spectrophotometer. cDNA was synthesized using the Han cDNA Synthesis Kit (HanLAB, Korea). Approximately 1 μg of total RNA per 20 μl reaction and the synthesized cDNA was stored at -20°C.

Realtime Loop-Mediated Isothermal Amplification

Realtime Loop-Mediated Isothermal Amplification (RT-LAMP) is a rapid and sensitive molecular diagnostic technique designed to amplify specific DNA sequences at a constant temperature (60°C to 65°C), unlike traditional PCR, which requires cyclic temperature changes. This method allows for the efficient amplification of large amounts of DNA within a short timeframe, typically yielding results within 30 to 60 minutes, making it faster than conventional PCR. RT-LAMP employs 4 to 6 sets of primers that bind to six distinct regions of the target DNA, providing exceptionally high specificity. This high specificity minimizes nonspecific amplification, ensuring accurate analytical results. The amplification process can also monitored in real time by detecting the fluorescence or turbidity changes associated with DNA synthesis. This allows for continuous observation of the reaction as it progresses.

The relative quantification of BDNF, IGF-1, and VEGF expression in brain tissues from each group was analyzed using the RT-LAMP technique. The outer forward primer (F3), outer backward primer (B3), forward inner primer (FIP), backward inner primer (BIP), loop forward primer (LF) and loop backward primer (LB) for each target gene were designed based on sequences provided by the National Center for Biotechnology Information (NCBI) using the NEB® primer design tool. The primer information is provided in Table 2. The RT-LAMP reaction mixture consisted of 10 μl of RT-LAMP 2X Master mix (Elpisbio, Korea), 2 μl of 10X primer mixture (2 μM F3/B3, 16 μM FIP/BIP, and 4 μM LF/LB), 2 μl of cDNA, and RNase-free water to make a final volume of 20 μl. The 20 μl RT-LAMP reaction mixture was placed in 0.1 μl PCR strips and amplified using an isoQuarkM4 (RevoSketch, Korea) at 65°C for 30 minutes. After completion of all reactions, the relative quantification of gene expression for each group was performed using the Delta-Delta Ct method.

Table 2. Primer sequences used for RT-LAMP analysis

Gene Name	NCBI Gene ID	Primers	Sequence(5'-3')
BDNF	12064	M.BDNF_F3	CTCTGGAGAGCGTGAATGG
		M.BDNF_B3	AGTAGAGGAGGCTCCAAAGG
		M.BDNF_FIP	TCCTCATCCAGCAGCTCTTCGAAGAGGTCTGACGACGACAT
		M.BDNF_BIP	CCAGAAGGTTCGGCCCAACGCACTTGACTGCTGAGCATCA
		M.BDNF_LF	TGCTCAAAAGTGTCAGCCAGTG
		M.BDNF_LB	GAAAACCATAAGGACGCGGACT
IGF1	16000	M.IGF1_F3	TCTTCTACCTGGCGCTCTG
		M.IGF1_B3	AGCTCCGGAAGCAACACT
		M.IGF1_FIP	GAGCATCCACCAGCTCAGCCCACCTTCACCAGCTCCAC
		M.IGF1_BIP	GGACCGAGGGGCTTTTACTTCACATCCACAATGCCTGTCTGA
		M.IGF1_LF	GCAAAGGGTCTCTGGTCCAG
		M.IGF1_LB	CAAGCCCACAGGCTATGGC
VEGF	22339	M.VEGF_F3	GTTCCAACCAGAAGTTTGG
		M.VEGF_B3	TACCAGGCCTCTTCTTCC
		M.VEGF_FIP	CGATTCTTCCAGGCAGTGCCGCTTTGTGGATCCCCATG
		M.VEGF_BIP	CTCCACTTCTGAGGGGCCTACACCGTGTCTTCTCTTGC
		M.VEGF_LF	GCGGGGTGCTTTTGTAGACTAT
		M.VEGF_LB	AGGCCTCCCACAGGTGT
GAPDH	14433	M.GAPDH_F3	ATGGCCTTCCGTGTTCCT
		M.GAPDH_B3	GCATCGAAGGTGGAAGAGTG
		M.GAPDH_FIP	GCCTGCTTCACCACCTTCTTGACAATGTGTCCGTCGTGGATC
		M.GAPDH_BIP	TCTGAGGGCCCACTGAAGGGAGTTGCTGTTGAAGTCGCA
		M.GAPDH_LF	TCATCATACTTGGCAGGTTTCTCC
		M.GAPDH_LB	CATCTTGGGCTACACTGAGGAC

Enzyme-Linked Immunosorbent Assay

Blood was centrifuged at 1,000 × g for 10 minutes at 4°C to separate the plasma layer. The separated plasma was then diluted 6, 8, 12, or 24 times with sample diluent, depending on the remaining volume. The concentrations of Corticotropin-Releasing Factor-Binding Protein (CRHBP), Epinephrine (EPI), and Cortisol (Cor) in the diluted plasma were measured using enzyme-linked immunosorbent assay (ELISA) kits provided by ABclonal Biotechnology (USA)

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical significance between groups was determined using one-way ANOVA followed by Tukey's post-hoc test. A p-value of <0.05 was considered statistically significant.

Results

Effects on Organ Development

After the completion of the experiment, the experimental animals were euthanized, and blood was maximally

removed through cardiac puncture. The heart, liver, spleen, and brain were then carefully harvested. The collected organs were washed 2-3 times with Phosphate Buffered Saline (PBS), and their weights were measured using a precision scale.

As shown in Fig. 1, statistically significant differences were found in the heart, liver, and spleen between PNEG vs NPNEG, PNEG vs NPEG, NPNEG vs PEG, and PEG vs NPEG. However, no significant differences were observed between PNEG and PEG, or between NPNEG and NPEG.

In the brain, significant differences were observed between NPNEG and PEG, as well as between PEG and NPEG. These results suggest that the experimental conditions had varying effects on organ development across the different groups.

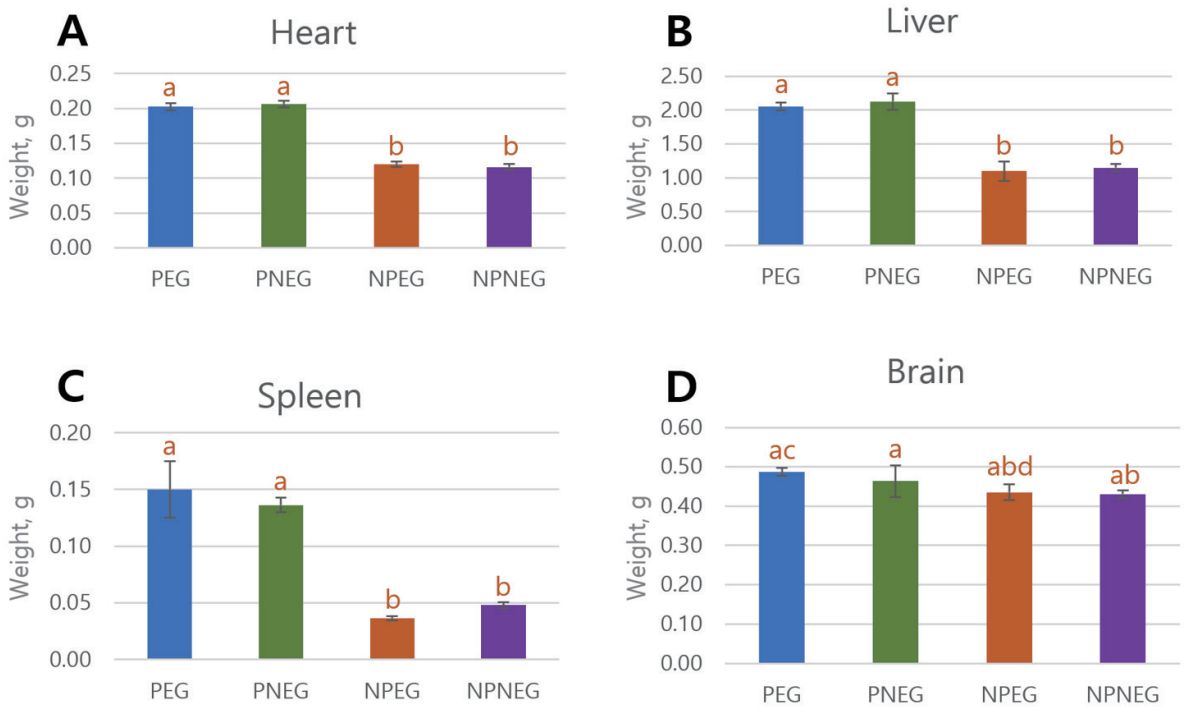

Fig. 1. Organ Weights According to Experimental Treatment. Heart (A), Liver (B), Spleen (C), Brain (D). Mice (N=8/group) were treated based on the respective group's protein intake and swimming exercise. Data are presented as mean ± SD. *Statistical significance between groups ($p<.05$).

Analysis of Cholesterol and Albumin Levels

After the experiment was completed, the animals were euthanized, and blood was collected via cardiac puncture. The blood was then analyzed using the Spotchem™ EZ VET from Arkray, Japan, according to the provided manual (Fig. 2). The total cholesterol analysis revealed a statistically significant difference between PNEG and NPNEG. However, no significant differences were observed in comparisons between PNEG and PEG, PNEG and NPEG, NPNEG and PEG, NPNEG and NPEG, or PEG and NPEG.

In the albumin analysis, statistically significant differences were observed between PNEG and NPNEG, PNEG and NPEG, and NPNEG and PEG. On the other hand, there were no significant differences between PNEG and PEG, or between NPNEG and NPEG.

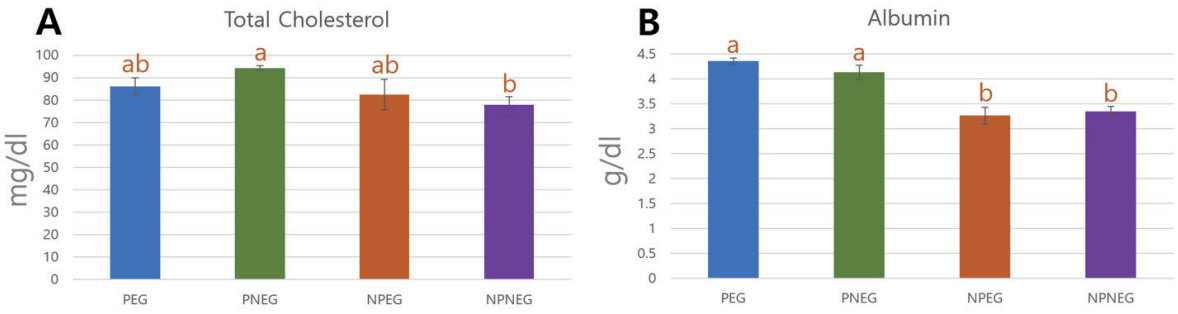

Fig. 2. Differences in Total Cholesterol and Albumin Levels Confirmed by Blood Analysis. Total Cholesterol (A) and Albumin (B). Mice (N=8/group) were treated according to their respective group's protein intake and swimming exercise. Data are presented as mean ± SD. *Statistical significance is indicated for group comparisons ($p<.05$).

Analysis of Hemoglobin level, Hematocrit percentage, Mean Corpuscular Hemoglobin and Platelet count

Blood analysis was conducted using the Scil Vet abc Plus hematology analyzer from Scilvet, Germany, following cardiac puncture (Fig. 3). Hemoglobin analysis showed a statistically significant difference between PNEG and NPNEG, and significant differences were also found between PNEG and NPEG, NPNEG and PEG, and PEG and NPEG. However, no significant differences were observed between PNEG and PEG, or between NPNEG and NPEG.

In the hematocrit analysis, statistically significant differences were found between PNEG and NPNEG, PNEG and NPEG, NPNEG and PEG, and PEG and NPEG. The difference between NPNEG and PEG was substantial, with an average difference of -12.01%. However, no significant differences were observed between PNEG and PEG, or between NPNEG and NPEG.

The mean corpuscular hemoglobin (MCH) analysis revealed statistically significant differences between PNEG and NPNEG, PNEG and NPEG, NPNEG and PEG, NPNEG and NPEG, and PEG and NPEG. Conversely, no significant difference was observed between PNEG and PEG.

Significant differences were observed in the platelet count analysis between PNEG and NPNEG, PNEG and PEG, NPNEG and PEG, NPNEG and NPEG, and PEG and NPEG. The difference between PEG and NPEG was particularly notable, with a difference of 349.57 (10^3/mm³). However, no significant difference was found between PNEG and NPEG.

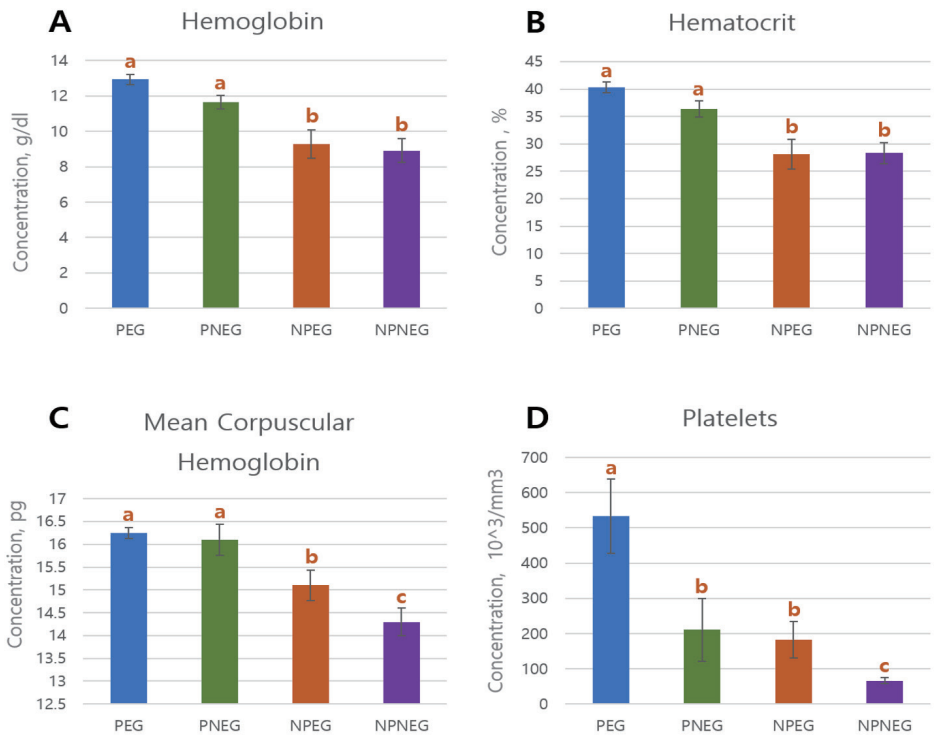

Fig. 3. Hemoglobin level, Hematocrit percentage, Mean Corpuscular Hemoglobin, and Platelet count results from blood analysis. Hemoglobin (A), Hematocrit (B), Mean Corpuscular Hemoglobin (C), Platelet (D). Mice (N=8/group) were treated based on the respective group's protein intake and swimming exercise. Data are presented as mean ± SD. *Statistical significance between groups ($p<.05$).

Validation of BDNF, IGF-1, and VEGF Expression Using Isothermal PCR

mRNA was extracted from brain tissue and cDNA was synthesized to assess BDNF, IGF-1, and VEGF gene expression patterns using the isothermal PCR method. All three genes BDNF, IGF-1, and VEGF expression levels were highest in the group that received a protein diet and participated in swimming exercises (Fig. 3.).

For BDNF expression, a statistically significant difference was observed between PNEG and NPNEG, with a mean difference of 0.47. Similarly, an important difference was found between PNEG and PEG, with a mean difference of 0.77. However, no significant differences were observed between PNEG and NPEG, NPNEG and PEG, or NPNEG and NPEG. In contrast, a statistically significant difference was noted between PEG and NPEG.

In the analysis of IGF-1 expression, statistically significant differences were observed between PEG and Groups 1, 2, and 4. No significant differences were found among PNEG, NPNEG, and NPEG.

A significant difference was found between PNEG and NPNEG in VEGF expression. Similarly, PNEG and PEG also showed a statistically significant difference. However, no significant differences were observed between PNEG and NPEG, NPNEG and PEG, NPNEG and NPEG, or PEG and NPEG.

These results indicate that protein intake and swimming exercise profoundly impact BDNF, IGF-1, and VEGF expression levels in brain tissue.

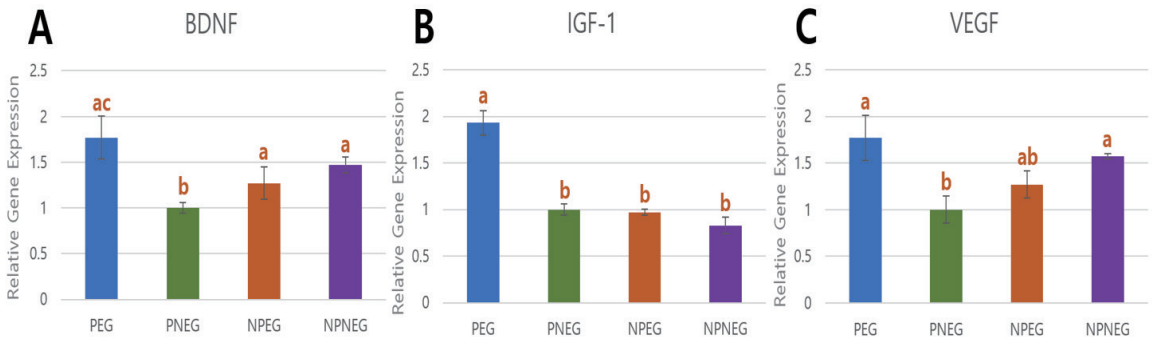

Fig. 4. Expression of mRNA in brain tissue. BDNF (A), IGF-1 (B), VEGF (C). Data are presented as the mean ± SD of values obtained using the ΔΔCt method. *Statistical significance between groups (p<.05).

Expression of Stress-Related Hormones and Proteins

Fig. 5 shows the expression patterns of CRHBP, cortisol, and EPI as determined using the ELISA method. Analysis of CRHBP expression revealed statistically significant differences between PNEG and Groups 2 and 4. However, there was no significant difference between PNEG and PEG. A significant difference was also observed between NPNEG and Groups 3 and 4, as well as between PEG and NPEG. In the Cortisol expression analysis, significant differences were observed between PNEG and Groups 2 and 4, but no significant difference was found between PNEG and PEG. Significant differences were also detected between NPNEG and Groups 3 and 4, as well as between PEG and NPEG. For Epinephrine (EPI) expression, significant differences were found between PNEG and Groups 2 and 4, while no significant difference was observed between PNEG and PEG. Significant differences were noted between NPNEG, PEG and NPEG, and PEG and NPEG.

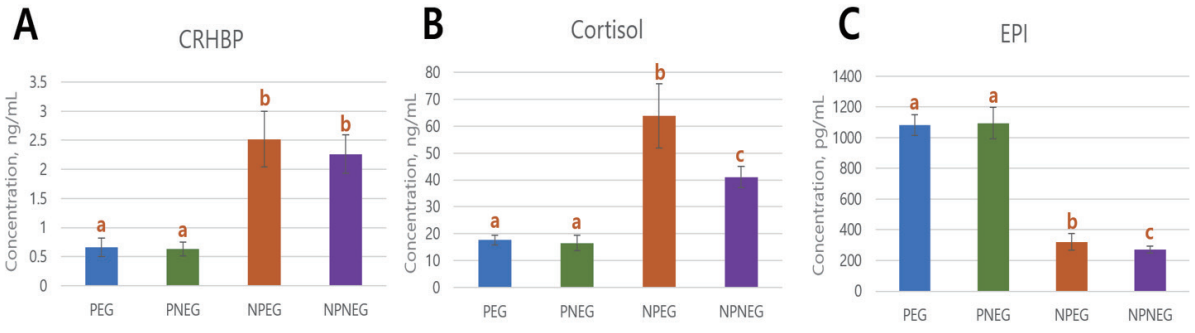

Fig. 5. Analysis of Stress-Related Hormone and Protein Expression. CRHBP (A), Cortisol (B), EPI (C). Data are presented as the mean ± SD. *Statistical significance between groups (p<.05).

Discussions

This study demonstrates the synergistic effects of swimming exercise and protein intake on the expression of major growth factors (BDNF, IGF-1, VEGF) and the regulation of stress-related hormones (CRHBP, cortisol, epinephrine) in mice. Regular physical activity and adequate protein nutrition are essential for optimal physiological development and stress response regulation in mammals (Kolieb et al., 2022; Serra et al., 2022). Additionally, swimming has been reported to enhance brain function and inhibit the accumulation of oxidatively damaged proteins (Huang et al., 2017; Radák et al., 2001).

The organ weights of the experimental groups, as confirmed in this study, indicate that protein intake is crucial for the development of the heart, liver, and spleen, emphasizing the importance of protein during the growth phase. However, in the brain, the PEG group showed significantly higher values than the NPNEG and NPEG groups, demonstrating the synergistic effect of protein and proper exercise.

Furthermore, the blood analysis conducted in this study provides important clues for understanding the physiological impact of swimming exercise and protein intake on various hematological indicators in mice. The PEG group showed higher hemoglobin levels than the NPNEG group, suggesting that sufficient protein intake combined with regular physical activity, such as swimming, can improve the oxygen-carrying capacity of the blood, thereby enhancing overall endurance and stress resistance in animals.

Cholesterol, as a component of the cell membrane, plays an essential role in maintaining cell structure and hormone synthesis. In this study, significant differences were observed between the PNEG and NPNEG groups, while no significant differences were found among the other groups. Albumin, synthesized in the liver, performs functions such as maintaining osmotic pressure, transporting nutrients, and detoxification. Significant differences in blood albumin levels between the protein-fed and protein-deprived groups indicate that protein intake plays a crucial role in albumin synthesis.

Hematocrit (HCT), which represents the percentage of red blood cells in the blood, is an important indicator of blood health. The PEG group showed significantly higher hematocrit levels than the NPNEG group, indicating that combining a protein diet with swimming exercise can increase the volume of red blood cells, highlighting the importance of protein. Mean corpuscular hemoglobin (MCH) measures the average amount of hemoglobin in a red blood cell, which reflects the oxygen-carrying capacity of red blood cells. MCH also underscores the importance of protein, with protein-deprived groups that underwent swimming showing significantly higher values, indicating an improvement in oxygen-carrying capacity due to swimming.

Platelets play a critical role in blood clotting and wound healing, and their count is an important indicator of the body's ability to respond to injury. In the protein-fed groups, those who underwent exercise showed significantly higher platelet counts, and even among the protein-deprived groups, those who underwent swimming showed significantly higher counts, indicating enhanced immunity to external stimuli. The PEG group showed significantly higher values than the other three groups, demonstrating the synergistic effect of combining protein intake with exercise.

The increase in BDNF and IGF-1 expression in the group that received both protein intake and swimming exercise (PEG group) reflects the positive effects of exercise and proper protein supply. BDNF is well-known for promoting neuroplasticity and cognitive function, particularly in response to physical activity, and has been reported to improve

spatial learning and memory performance (Lee et al., 2012; Liu et al., 2009; Merritt et al., 2015; Venezia et al., 2016; O'Leary et al., 2019). IGF-1 promotes growth and development in various tissues, including muscle and nervous tissue, and stimulates satellite cell activation and proliferation (Kraemer et al., 2020). The significant increase in VEGF expression, due to swimming exercise and appropriate protein supply, suggests active angiogenesis induced by exercise, which is essential for meeting the metabolic demands of tissues. Aerobic exercise has been reported to improve spatial learning and memory performance (Uysal et al., 2015), increase neural density in the hippocampus and frontal lobe, and elevate VEGF and BDNF expression levels (Drumond et al., 2012), demonstrating the neuroprotective and developmental effects of aerobic exercise (Alomari et al., 2013).

The differential expression of stress-related hormones among experimental groups indicates that protein intake and exercise profoundly influence the body's stress response. Notably, the increase in cortisol and epinephrine levels in the NPEG group, which underwent swimming exercise under protein-deficient conditions, suggests that insufficient protein intake can exacerbate physiological stress related to high-intensity physical activity. These results align with previous research, emphasizing adequate nutrition's necessity in mitigating exercise-induced stress responses. The study suggests that the stress observed in the protein-deprived groups resulted from a nutritional deficiency, where aerobic exercise under protein-deficient conditions significantly increased stress and cortisol levels. This highlights the importance of sufficient protein intake during proper exercise. Cortisol is most highly expressed during peak stress (Kraemer et al., 1993; Kraemer et al., 1995) and plays a vital role in regulating energy homeostasis and metabolism (Munck et al., 1984). During exercise, cortisol increases the availability of metabolic substrates, protects against immune cell activity, and helps maintain vascular integrity (Duclos et al., 2003).

Epinephrine, which facilitates the "fight-or-flight" response, an automatic physiological reaction that occurs when the body faces acute stress, prepares the body for immediate physical action (Deeney et al., 2022). In the PNEG and PEG groups, EPI concentrations remained relatively stable and high. This suggests that protein intake is crucial and that, when combined with swimming exercise, the body's stress response can be effectively regulated. In contrast, in the protein-deprived NPEG and NPNEG groups, EPI concentrations were significantly lower, indicating that the body's ability to cope with stress may be impaired, as suggested by the low EPI levels. These findings indicate that the physiological response can change significantly when protein deficiency persists (Hanson et al., 2018). CRHBP, a protein that plays a critical role in the stress response, binds to the stress hormone Corticotropin-Releasing Hormone (CRH) and regulates its activation (Timofeeva et al., 2003). CRHBP expression was significantly elevated in the protein-deprived groups (NPNEG, NPEG), indicating that CRHBP expression increased to regulate stress by binding to CRH, thereby enhancing CRH metabolism and modulating the stress response (Chrousos et al., 2017).

Conclusions and Implications

The purpose of this study was to analyze the effect of protein intake and swimming exercise on the growth factors and stress-related hormones. The results of this study are as follows. First, BDNF and IGF-1 levels were highest in PEG, highlighting the beneficial effects of combining protein intake with swimming exercise. VEGF expression also showed significant differences, particularly between PNEG and NPNEG, and PNEG and PEG. Second, CRHBP, cortisol, and

epinephrine levels varied significantly across groups, especially between those subjected to different dietary and exercise regimens. This suggests a strong interaction between protein intake and exercise in modulating stress responses.

In conclusion, the study verifies that protein intake and swimming exercise synergistically effect the growth factor expression and stress hormone in mice. These findings provide valuable insights into the physiological adaptations to diet and exercise in mammals, which could inform future research on optimizing dietary and exercise interventions for health and stress management.

The interaction between protein intake and exercise in regulating the expression of growth factors and stress hormones emphasizes the importance of a holistic approach to mammalian health and development. The synergistic effects observed in this study highlight the importance of protein and exercise in maintaining health and managing stress, providing valuable insights into optimizing dietary and exercise interventions for enhanced well-being.

However, it should be noted that this study used ICR mice, which may limit the generalizability of the results to other species, particularly humans. Therefore, future studies should aim to replicate these findings across different animal models and human populations to better understand the broader applicability of these results. Additionally, since this study focused on a relatively short intervention period, long-term studies are needed to evaluate the long-term effects of protein intake and exercise on growth factor expression and stress regulation.

Conflicts of Interest

The authors declared no conflicts of interest.

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