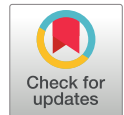


Experimed

Research Article

Open Access

Differential Myokine Responses to Swimming: Highlighting Meteorin-like (Metrnl) as a Unique Marker of Adaptation



Alten Oskay¹  , Ozgen Kilic Erkek² , Hande Senol³ , Mert Ozen¹ , Egem Burcu Unal⁴ ,
Melek Bor Kucukatay² 

¹ Department of Emergency Medicine, Faculty of Medicine, Pamukkale University, Denizli, Turkiye

² Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli, Turkiye

³ Department of Biostatistics and Medical Informatics, Faculty of Medicine, Pamukkale University, Denizli, Turkiye

⁴ Therapeutic Apheresis Unit, Adana City Training and Research Hospital, Adana, Turkiye

Abstract

Objective: We examined time-dependent changes in the levels of meteorin-like protein (Metrnl), interleukin (IL)-7, IL-8, and follistatin-like 1 (FSTL1) myokines in mouse gastrocnemius-soleus tissue and plasma samples following acute and long-term swimming exercises.

Materials and Methods: Seventy 8–12 weeks-old adult male BALB/c mice were classified as control (sedentary) and exercise groups. Either an acute or chronic exercise regime was applied to the exercise group. Samples were collected at 0 h, 3 h, and 48 h. The acute exercise group completed a 30-min single swimming session. The chronic exercise group completed the 30-min swimming exercise every day, 5 days a week, for 6 weeks. Metrnl, IL-7, IL-8, and FSTL1 levels in the m. gastrocnemius-soleus muscle and plasma samples were determined by enzyme-linked immunosorbent method (ELISA) kits.

Results: No changes were observed in the Metrnl, IL-7, IL-8, and FSTL1 levels in the gastrocnemius-soleus muscle complex following acute or 6 weeks of swimming exercise ($p>0.05$). IL-7, IL-8, and FSTL1 levels did not change in the plasma in response to the exercises ($p>0.05$). Plasma Metrnl levels were higher at 3 h after 6 weeks of chronic exercise compared with the control (sedentary) and the 3 h samples of the acute swimming (3.49 ± 1.75 ng/mL, 1.75 ± 0.48 ng/mL, and 1.66 ± 0.33 ng/mL; $p<0.001$, respectively) groups.

Conclusion: These findings suggest that unlike IL-7, FSTL1, and IL-8, only Metrnl may play a role in mediating adaptation to long-term exercise in mice.


Keywords

Follistatin-like 1 protein · Interleukin-7 · Interleukin-8 · Meteorin-like protein · Swimming



Citation: Oskay A, Kilic Erkek O, Senol H, Ozen M, Unal EB, & Bor Kucukatay M Differential Myokine Responses to Swimming: Highlighting Meteorin-like (Metrnl) as a Unique Marker of Adaptation. *Experimed* 2025; 15(3): 232–243. DOI: 10.26650/experimed.1690894

 This work is licensed under Creative Commons Attribution-NonCommercial 4.0 International License. 

 2025. Oskay, A., Kilic Erkek, O., Senol, H., Ozen, M., Unal, E. B. & Bor Kucukatay, M.

 Corresponding author: Alten Oskay oskayten@gmail.com



INTRODUCTION

The skeletal muscle is the largest organ, accounting for approximately 40% of the body weight (1). Exercise has various positive effects on distant tissues and organs, as well as on the muscle groups (2). Cytokines called myokines, released into the circulation by muscle cells, mediate some of these effects (2). Although it is known that exercise affects the whole organism depending on its characteristics, such as type, intensity, frequency, and duration, the most important beneficial effects are on the musculoskeletal, respiratory, cardiovascular, immune, and endocrine systems (2-5). These effects vary between episodic and long-term exercise (3, 4). With the cessation of exercise, these changes are known to gradually regress (5).

Recent studies have revealed many beneficial effects mediated by exercise-related myokines, describing their expression, mechanisms, and effects (6-8). However, the mechanisms of action of many myokines remain unclear. Of these, follistatin-like 1 (FSTL1) is proposed to be a multifunctional molecule with critical roles across various physiological systems. It has been shown to be essential for normal afferent synaptic sensory transmission (9), to exert cardioprotective effects via the Akt and ERK signaling pathways (10, 11), and to promote neovascularization in response to ischemia in skeletal muscle endothelial cells through the activation of the Akt-eNOS pathway (11). It is also associated with inflammation and influences cardiac remodeling through aerobic exercise (12). Meteorin-like (Metrnl) affects thermogenesis and the regulation of insulin sensitivity in metabolic syndrome and obesity (4). It also induces skeletal muscle hypertrophy through Peroxisome proliferator-activated receptor γ coactivator-1 α 4 (PGC-1 α 4) and exerts effects on the immune system (4, 13, 14). Interleukin-8 (IL-8) is considered an angiogenic chemo-myokine (15) and a proinflammatory factor exhibiting chemoattractant properties (16). It has been associated with osteoclastic activity (17) and is thought to play an important role in wound healing (18). Interleukin 7 (IL-7) is associated with inflammation and increases in the peripheral circulation in exercises such as high-intensity ultra-marathons (19). It primarily exerts its effects through the JAK/STAT signaling pathway (20) and plays a role in immune regulation and muscle hypertrophy (21).

Studies on the production and release of myokines into the peripheral circulation are mostly focused on resistance exercises and are conducted in the acute period (4, 21). In contrast, aerobic exercise studies usually involve weight-bearing activities such as walking, jogging, and cycling, which predominantly stress the muscles and joints of the lower extremities (22). The feature that distinguishes swimming

exercise from others is that the load on the muscle-joint system is lower; thus, such exercises are preferred in physiotherapy for certain disease conditions. Moreover, swimming is considered a natural form of physical activity in mice, making it a commonly used modality in animal exercise models (23).

The synthesis and release times of myokines change differentially during acute and chronic exercises, which may influence time-dependent adaptations to swimming exercise (24). Thus, we aimed to examine the time-dependent (at 0 h, 3 h, and 48 h) changes in IL-7, IL-8, FSTL1, and Metrnl myokines in the mouse gastrocnemius-soleus and plasma following acute and chronic swimming exercise. In this study, we evaluated the levels of these myokines following swimming exercise, which may be in response to minimizing the stress factors that may arise from exercise on mice that are natural swimmers, as well as minimizing the load on the joints thanks to swimming exercise, thereby preventing their potential impacts on our data. Our data could provide useful insights for developing new myokine-related agents and physiotherapy methods that may contribute to the monitoring and treatment of various diseases.

MATERIALS AND METHODS

Pamukkale University Animal Experiments Ethics Committee approved the study number PAUHADYEK-2019/29, dated 22.08.2019 and numbered 2019/06. We followed the national and institutional ethical guidelines throughout the experimental procedure.

Animals

We used 8–12 week-old adult male BALB/c mice. The animals were housed under standard conditions throughout the study (50 \pm 5% humidity, 22 \pm 2 $^{\circ}$ C, a 12-h light-dark cycle, etc.) and specially prepared cages and were kept under the control of a veterinarian (25-27). A total of 70 mice were divided into seven groups, with 10 mice in each group. The mice were checked daily by a veterinarian for general condition disorders and infections. The evaluation criteria included generally accepted animal welfare and behavior criteria such as the brightness and liveliness of the animals' fur, their general nutritional status, and normal movements. The mice were allowed free access to water and food and were all (exercising and non-exercising groups) subjected to daily handling. Animals that did not swim, were infected, or deteriorated during the study were planned to be excluded from the experiment. However, such problems were not observed during the study (25, 26).

The mice were classified as the control (sedentary) and exercise groups. The exercise group was also divided into



two groups as acute and chronic swimming (25, 26). Each of the acute and chronic exercise swimming was divided into three groups at 0, 3, and 48 h in terms of the time passed from the end of the exercise to the end of the experiment. A total of 7 groups were obtained (Table 1). The animals were randomly assigned to the groups. In the chronic swimming group, exercise was started when the mice were 10 weeks old. It was applied for 6 weeks and in the 16th week, all animals were sacrificed on the same day immediately after the last swimming session, 3 h later and 48 h later. After the familiarization period, mice in the acute swimming group underwent a single 30-min exercise session at week 16, followed by cessation of all acute groups at 0, 3, and 48 h. Control (sedentary) mice were also sacrificed when they were 16 weeks old.

Table 1. Experimental groups

Experimental groups	Number of animals
Control	10
Acute swimming 0. Hour	10
Acute swimming 3. Hour	10
Acute swimming 48. Hour	10
Chronic swimming 0. Hour	10
Chronic swimming 3. Hour	10
Chronic swimming 48. Hour	10

Swimming Exercise Administration

Swimming training was performed in the Experimental Surgery Application and Research Center in plastic tanks filled with tap water (68 cm × 44 cm × 34 cm) similar to our previous work (25, 26). The temperature of water was kept at 32 ± 3 °C (25, 27). In order to familiarize the mice of the exercise groups with swimming, all animals of the exercise groups were subjected to swimming for 10 min on the first day, and the exercise was lengthened to 30 min on the third day (25, 26, 28). On the last day when 30 min of swimming was applied, the acute exercise groups were sacrificed at 0, 3, and 48 h following 30 min of swimming. The chronic groups swam for 6 weeks following the familiarization. Swimming exercise was applied 30 min/day, 5 days/week, 6 weeks to this group (25, 26) and the mice were sacrificed 0, 3, and 48 h following the last exercise session. The conditions under which the sedentary (non-exercised control) and exercise groups were the same except for the application of acute and chronic swimming procedures. The animals were matched for daily handling, water exposure, or other environmental factors to minimize confounding variables. The control (sedentary) group was subjected to swim 2 times for 10 min each. This procedure was applied to eliminate the stress caused by exposure to water (25, 26). After each swimming

exercise, the mice were dried thoroughly with a towel and then placed in their cages (25, 27). We ensured that all mice were age-matched (16 weeks old) at the time the tissue and blood were collected.

Euthanasia, Blood and Tissue Samples

While the mice were under intraperitoneal ketamine-HCl / Xylazin-HCl (75 mg/kg / 10 mg/kg) anesthesia, approximately 0.5–0.7 mL of blood was collected from their hearts with a sterile syringe, and the experiment was terminated. The collected blood was discharged into heparinized (15 U/mL) tubes, centrifuged with 1555 × g for 20 min, and the separated plasma samples were stored at –80°C for later analysis. For standardization, approximately 0.20 g of the right gastrocnemius-soleus tissue samples were first weighed, homogenized in phosphate-buffered saline (PBS), and centrifuged at 1555 × g for 20 min. The resulting supernatants were collected into 1.5 mL of eppendorf tubes and stored at –80 °C for later analysis (25, 26). During the collection and handling of the blood and tissue samples, the cold chain protocols were strictly maintained to preserve the sample integrity.

Enzyme-Linked Immunosorbent Assays (ELISA)

The double-antibody sandwich ELISA was used to measure IL-7, Metrnl, IL-8, and FSTL1 levels in mouse plasma and the gastrocnemius-soleus muscle complex. Metrnl, IL-7, IL-8, and FSTL1 in the gastrocnemius-soleus muscle complex were measured using ELISA (SinoGeneClon Biotech Co. Hangzhou, China, Cat. No. SG-31275, SG-30226, SG-30224 and SG-34986, respectively), following the manufacturer's recommendations. Plasma Metrnl, IL-7, IL-8, and FSTL1 levels were measured using ELISA kits (AFG Bioscience, Shanghai, China, Cat. No. EK731275, EK730226, EK732214 and EK730881, respectively) in accordance with the manufacturer's recommendations. Standards and samples (10 µL) were added to wells pre-coated with monoclonal antibodies specific for mouse Metrnl, IL-8, IL-7, and FSTL1. Biotin-labeled detection antibodies and streptavidin-HRP were then added to form immune complexes. After incubation under the manufacturer-recommended conditions, the wells were washed to remove unbound substances. Chromogenic substrates were added, producing a blue color, which turned yellow upon the addition of the stop solution. The absorbance was measured at 450 nm using a microplate reader (Shimadzu UV-1601; Shimadzu, Kyoto, Japan). Analyte concentrations were calculated based on standard curves.



Study Group Design

We observed a strong effect size in a reference study ($d=1$) (20). As we had planned for seven groups in this study and assuming that a lower effect size can be reached ($f=0.8$), we estimated that when at least 49 mice were included in the study (at least 7 mice for each group), 80% power could be obtained at the 95% confidence level.

Statistical Analyses

The data were analyzed using the SPSS 25.0 (IBM SPSS Statistics 25 software; Armonk, NY: IBM Corp.) package program. Continuous variables are given as the mean \pm standard deviation (SD). A normality test was performed initially. A one-way analysis of variance was performed for the comparison of independent group differences when parametric test assumptions were provided. When the parametric test assumptions were not met, the Kruskal–Wallis analysis of variance tests were used to compare independent group differences. In all analyses, $p<0.05$ was considered statistically significant.

RESULTS

Myokine Analyses in the Gastrocnemius-soleus Complex

Figures 1–4 show Metrnl, IL-8, IL-7, and FSTL1 levels in the gastrocnemius-soleus muscle complex immediately and 3 and 48 h after the acute and long-term exercise regimens, respectively. No statistically significant alteration was observed in the concentrations of these myokines in any of the tissue samples collected at 0, 3, and 48 h after short-term and chronic exercises ($p>0.05$).

Plasma Myokine Analysis

Figure 5 demonstrates that the plasma Metrnl concentration was significantly higher at 3 h after 6 weeks of chronic exercise compared to the control (sedentary) and the 3 h samples of the acute swimming (3.49 ± 1.75 ng/mL, 1.75 ± 0.48 ng/mL, and 1.66 ± 0.33 ng/mL; $p<0.001$, respectively) groups. However, under the same exercise protocols, no statistically significant change in the plasma levels of IL-7, FSTL1, and IL-8 between the study groups was observed ($p>0.05$; Figure 6–8).

DISCUSSION

There is a growing interest to uncover the myokines that mediate the effects of different types of exercise at different time points following the exercise protocol to provide useful insights for the development of new myokine-related agents to mimic the positive effects of exercise (7, 12, 19, 29–31). In this

study, the Metrnl concentrations in the plasma were increased at 3 h after 6 weeks of swimming exercise compared to both the control (sedentary) group and the 3 h plasma samples following acute exercise. However, no significant changes were observed in FSTL1, IL-7, or IL-8 concentrations in the gastrocnemius-soleus muscle complex or plasma of mice in response to swimming. These data suggest that FSTL1, IL-7, and IL-8 do not play a role in swimming exercise adherence over the periods we evaluated, and that among these myokines, only Metrnl may mediate adaptation to long-term swimming exercise. The benefits of exercise on metabolism have been widely investigated in the literature (6–8). Long-term aerobic exercise has been demonstrated to be associated with positive alterations, such as improvement in cardiac and vascular structures, cholesterol values, oxidant/antioxidant balance (7), and reduction in inflammation (8). However, these effects are time-dependent; they eventually return to baseline after the exercise is terminated (5, 7, 22, 32–35). Swimming differs from other types of exercises in that it imposes a minimal mechanical load on the musculoskeletal system. Therefore, this type of exercise is frequently used for individuals with musculoskeletal disorders or reduced mobility. Swimming is also a natural behavior of mice (23).

Some metabolic effects of exercise are thought to be mediated by myokines. Myokines are released into the circulation following muscle contraction and show autocrine, paracrine, or endocrine effects in various organs (2). For these reasons, the gastrocnemius-soleus muscle, which is frequently used by mice during swimming exercise, and plasma samples were used in our study. Myokines are secreted into the circulation in a time-dependent manner following exercise, and their plasma concentrations can vary significantly based on both the molecular function of the myokine and the timing of sample collection (7). In our study, sampling was performed at 0, 3, and 48 h post-exercise to capture acute, early-phase, and late-phase responses. The 0 h time point allows for the assessment of immediate exercise-induced changes. Our 3-h time point was selected to capture the early secretion of myokines, and the 48-h time point was chosen to assess sustained or delayed myokine responses. Furthermore, the changes observed at 48 h may reflect secondary processes such as muscle adaptation, tissue remodeling, or delayed immune responses. Thus, the selected time points were designed to encompass both the rapid dynamics and the prolonged effects of exercise on the circulating levels of IL-7, IL-8, FSTL1, and Metrnl. This approach allows for a comprehensive evaluation of both transient and more sustained responses, improving the interpretability and translational relevance of our findings.



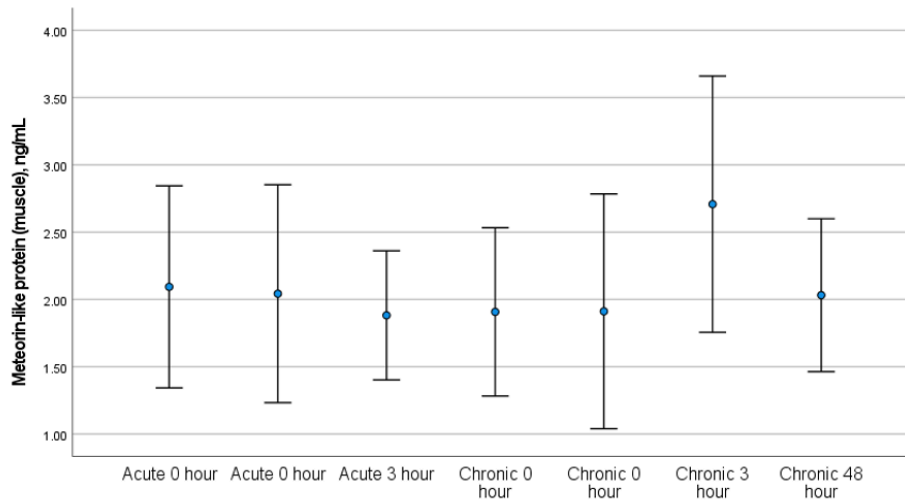


Figure 1. Meteorin-like protein concentrations (ng/mL) in the right gastrocnemius-soleus muscle complex of the experimental groups. No statistically significant alteration was observed in the concentrations of Metrnl in any of the samples collected at 0, 3, and 48 h after short-term and chronic exercises ($p > 0.05$; $F = 1.509$).

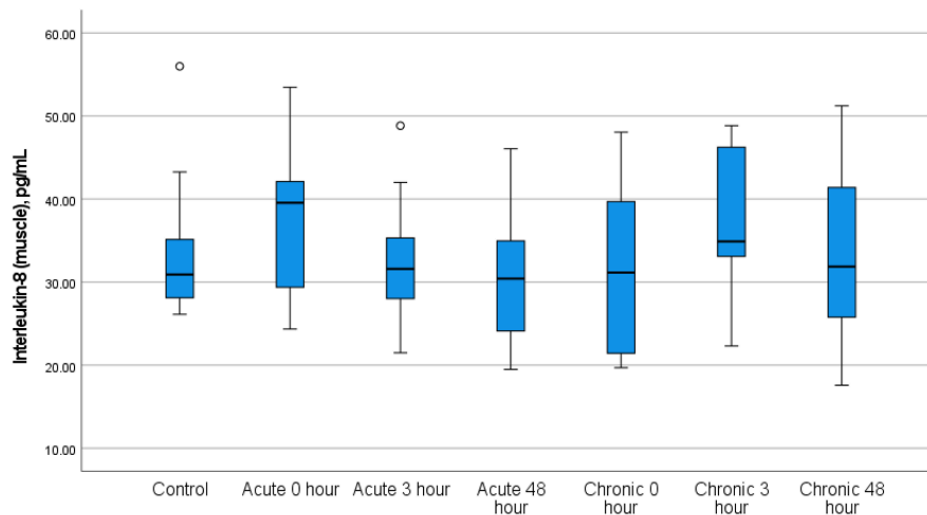


Figure 2. Interleukin-8 concentrations (pg/mL) in the right gastrocnemius-soleus muscle complex of the experimental groups. No statistically significant alteration was observed in the concentrations of IL-8 in any of the samples collected at 0, 3, and 48 h after short-term and chronic exercises ($p > 0.05$; $kw = 4.219$).

Metrnl is a myokine that is present in adipose, muscular, and neuronal tissues (4, 36, 37). It is expressed in the skeletal muscle and heart immediately after acute endurance exercise (4). In mice, Metrnl mRNA expression increased in *m. triceps brachii* at 240 min following acute downhill running exercise, but its expression in the *m. quadriceps femoris* remained unchanged (4). Human studies revealed that, in samples collected from the vastus lateralis muscle of healthy volunteers at rest, 1 h, and 4 h after performing acute resistance exercise plus 30 min of aerobic exercise, Metrnl mRNA expression was higher at 1 h, with a less but significant increase at 4 h (4). As an example of chronic exercise, 8–16 weeks of both aerobic and combined exercise have been shown to promote increased blood Metrnl levels

(38, 39), which decrease at 60 min following the session (39). In the current study, no alteration was observed in Metrnl concentrations in the acute period, but at 180 min after exercise, plasma Metrnl levels were found to be higher in the chronic exercise group than in the control (sedentary) and acute groups at the same time point. However, this significant value did not persist at 48 h.

Studies have also shown that the release of Metrnl varies with cold temperatures (4). In ice swimmers, serum Metrnl levels remained unchanged at 30 min following the swimming session (6). Our experiment was conducted in healthy mice under isothermal conditions. Unlike aerobic exercises, such as running and cycling, swimming causes minimal mechanical trauma to the muscles and joints of the lower extremities. For

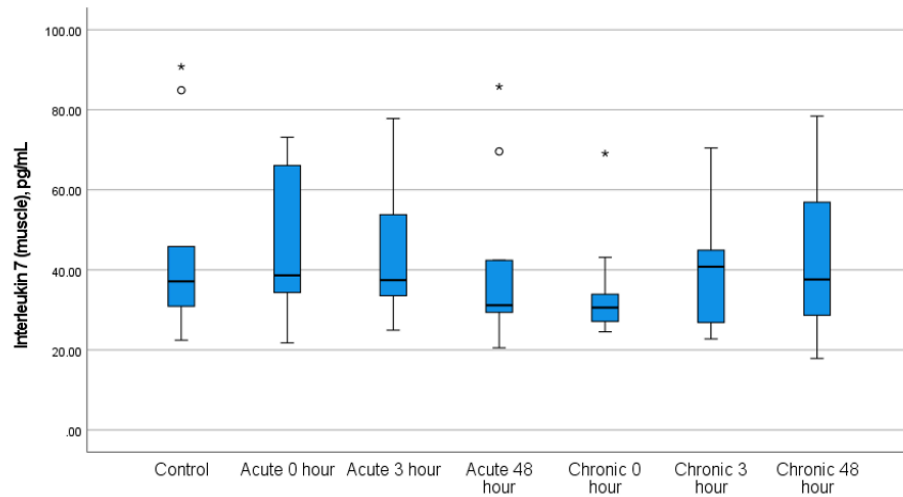


Figure 3. Interleukin-7 concentrations (pg/mL) in the right gastrocnemius-soleus muscle complex of the experimental groups. No statistically significant alteration was observed in the concentrations of IL-7 in any of the samples collected at 0, 3, and 48 h after short-term and chronic exercises ($p>0.05$; $kw= 4.307$).

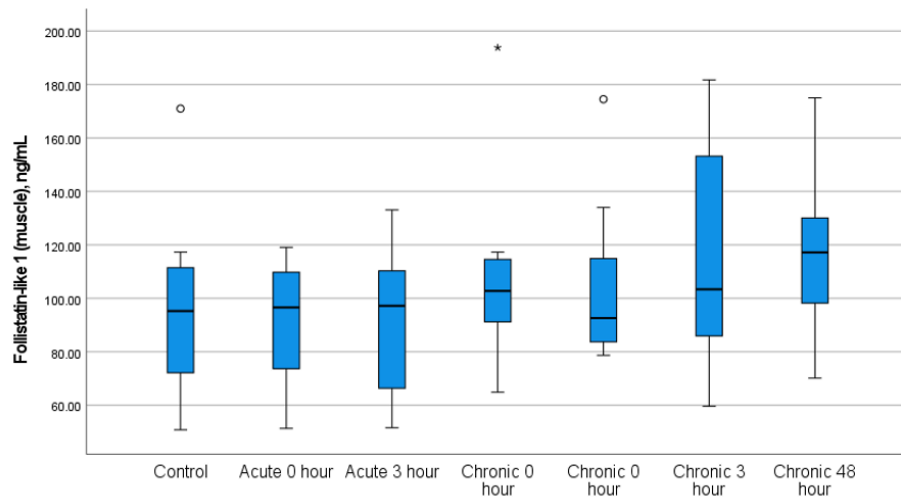


Figure 4. Follistatin-like 1 protein concentrations (ng/mL) in the right gastrocnemius-soleus muscle complex of the experimental groups. No statistically significant alteration was observed in the concentrations of FSTL1 in any of the samples collected at 0, 3, and 48 h after short-term and chronic exercises ($p>0.05$; $kw=5.79$).

these reasons, we may have missed detecting a change in the Metrnl concentrations in the gastrocnemius-soleus muscle tissue. However, the increase in plasma Metrnl concentrations during the adaptation process to exercise in the chronic period shows that the beneficial effects of this myokine may originate from other tissue groups during swimming and should be further investigated. Furthermore, this result may guide the prescription of swimming exercises for the treatment of certain disease conditions.

The delayed increase in plasma Metrnl levels after chronic swimming may reflect metabolic and immunological adaptations rather than acute mechanical stimuli. Metrnl is regulated by PGC-1 α and AMPK pathways, which are activated through long-term endurance exercise and promote

mitochondrial biogenesis and oxidative metabolism (4, 40). Additionally, adipose tissue and alternatively activated (M2) macrophages are known sources of Metrnl, and chronic exercise promotes tissue remodeling and anti-inflammatory signaling, particularly through the IL-4/IL-13 pathways (41). These processes may explain the transient rise at 180 min but not at 48 h, suggesting that Metrnl acts within a limited post-exercise recovery window. Its role appears to be more related to immune-metabolic homeostasis than to acute exercise-induced stress.

Studies examining the IL-8 response to exercise in the literature reveal contradictory results. IL-8 levels were found to vary depending on the intensity, duration, type of the exercise, and time elapsed after exercise until measurement

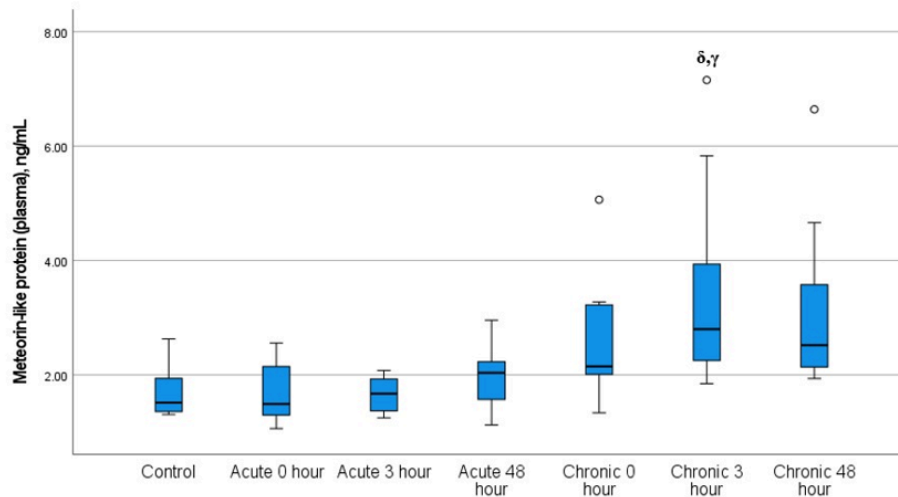


Figure 5. Plasma meteorin-like protein concentrations (ng/mL) of the experimental groups. δ : $p < 0.001$, compared with the control (sedentary) group; γ : $p < 0.001$, compared with the 3-h plasma sample of the acute exercise group. Plasma Meteorin concentration was significantly higher at 3 h after 6 weeks of chronic exercise compared to the control (sedentary) (δ) and the 3-h samples (γ) of the acute swimming (3.49 ± 1.75 ng/mL, 1.75 ± 0.48 ng/mL, and 1.66 ± 0.33 ng/mL; $p < 0.001$, respectively) groups (kw=26.129).

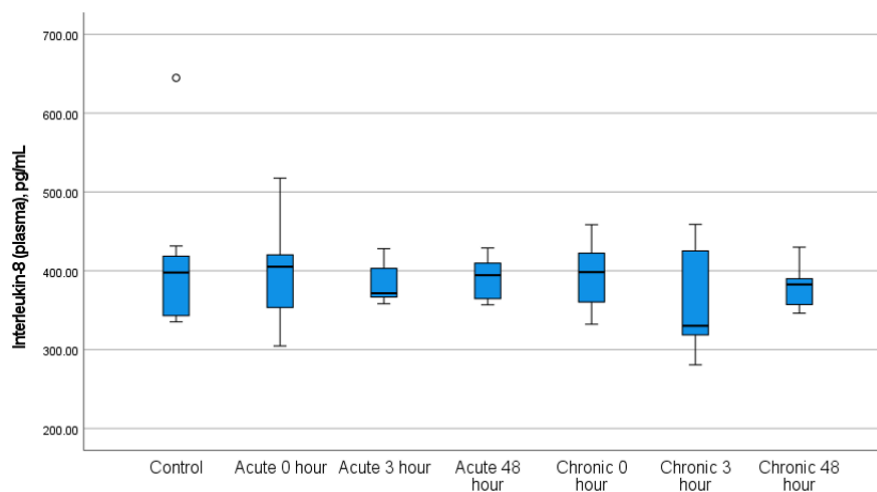


Figure 6. Plasma interleukin-8 concentrations (pg/mL) of the experimental groups. No statistically significant alteration was observed in the plasma concentrations of IL-8 in any of the samples collected at 0, 3, and 48 h after short-term and chronic exercises ($p > 0.05$; kw=5.647).

(22, 32-35, 42). Changes in IL-8 levels have been discussed, considering the proinflammatory and angiogenic properties of this myokine (43). Landers-Ramos et al. found that there was no difference between the plasma IL-8 levels at baseline in the trained and non-trained groups (44). Changes in IL-8 levels in resistance exercises, both in the acute and chronic periods, are evident (3, 42). Although there are studies showing that IL-8 levels increase 30 min after 30 min of acute treadmill exercise (44), Dorneles et al. demonstrated that IL-8 concentrations increased immediately after high-intensity interval exercise but returned to the basal value within 30 min. In contrast, IL-8 levels remained unchanged

during this timeframe following moderate-intensity interval training. The authors argued that IL-8 affected by the intensity of exercise due to its proinflammatory properties (45). Studies have shown that monitoring time-dependent changes in post-exercise myokine levels is important for revealing the role of related myokines in the process of adaptation to acute and chronic exercise. The 6-week chronic exercise protocol used in our study was shorter in duration than the above-mentioned studies. We observed that plasma and gastrocnemius-soleus muscle IL-8 levels did not change in the samples collected at 0, 3, and 48 h after either acute or long-term weight-free swimming exercises. Our data suggest that IL-8 does not play

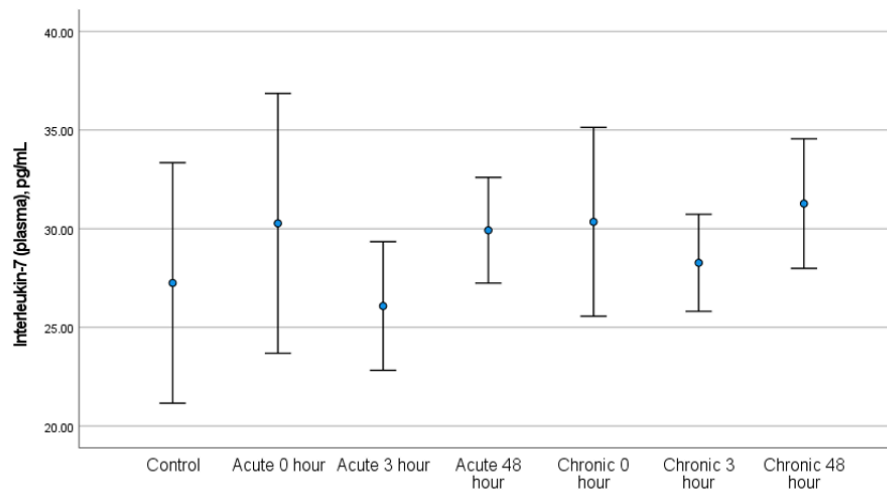


Figure 7. Plasma interleukin-7 concentrations (pg/mL) of the experimental groups. No statistically significant alteration was observed in the plasma concentrations of IL-7 in any of the samples collected at 0, 3, and 48 h after short-term and chronic exercises ($p > 0.05$; $F = 1.55$).

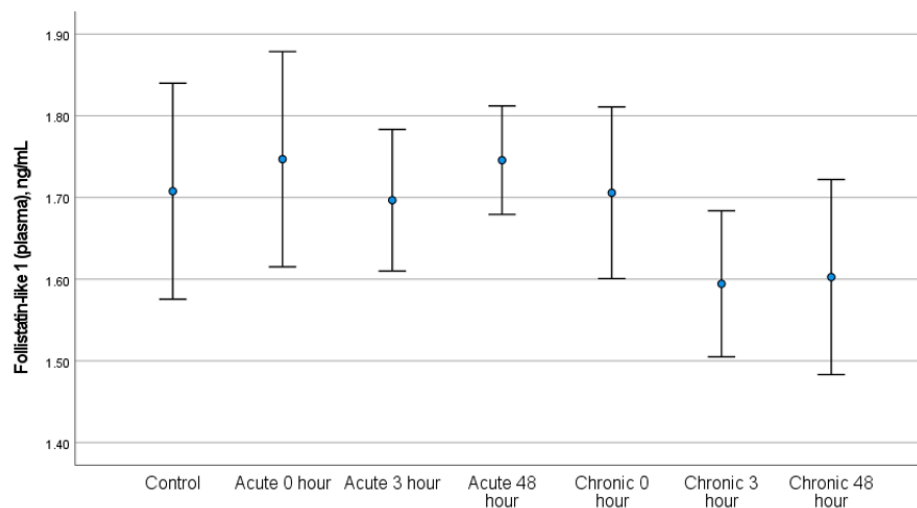


Figure 8. Plasma follistatin-like 1 protein concentrations (ng/mL) of the experimental groups. No statistically significant alteration was observed in the plasma concentrations of FSTL1 in any of the samples collected at 0, 3, and 48 h after short-term and chronic exercises ($p > 0.05$; $F = 3.369$).

a role in the acute and long-term adherence to moderate-intensity swimming exercises (within the scope of our sample collection times). Although IL-8 is a known exercise-induced myokine, its expression is primarily activated via mechanical stress-sensitive pathways such as NF- κ B, MAPK, and Ca²⁺-dependent signaling (46, 47). However, weight-free swimming likely imposes a lower mechanical load compared with resistance or weight-bearing exercises, which may result in insufficient activation of these pathways. On the other hand, if more advanced techniques such as gene expression were used in our study, we might have found statistical differences. IL-7 plays a role in the regulation of immunity. It is also associated with myogenic differentiation and muscle

hypertrophy (21, 48). Following its identification in human myotubes, it has been suggested that it may also have myokine properties (21). We could not find any information in the literature investigating the alterations in IL-7 concentrations following acute and prolonged swimming exercises. Studies examining IL-7 concentrations after aerobic exercises have presented certain limitations, and they are generally limited to acute exercise training (33, 49). Changes in IL-7 levels following exercise have been associated with the role of IL-7 in preventing atrophy (50). In young women, the microarray method could not detect IL-7 levels in the serum samples collected just before and after a 1-h cycling exercise. In the same study, the researchers measured IL-8

levels using the ELISA method, but they could not obtain a significant result (49). In a study comparing cytokine changes in trail running and ultra-trail running, IL-7 concentrations increased only in the ultra-trail group following exercise. In this case, it can be argued that longer-term, long-distance exercises in high-altitude areas may be necessary to increase IL-7 levels (19). In the current study, following 6 weeks of swimming exercise limited to 30 min per day, no difference was observed in the gastrocnemius-soleus muscle tissue and plasma concentrations of IL-7 compared with the control subjects. Within the scope of our study, swimming exercises were performed at the temperature and altitude of natural life. The low mechanical load and non-weight-bearing nature of swimming may have been insufficient to stimulate IL-7 gene expression in myocytes and in healthy animals without inflammation or immunodeficiency, IL-7 levels may remain relatively stable despite exercise. It is also possible that IL-7 responses may not be detectable at the sampling times used.

FSTL1 is a myokine that has been intensively studied in recent years in terms of cardiac and vascular disease diagnosis and treatment (11, 12). Increased FSTL1 levels after acute myocardial infarction are associated with angiogenesis, protection of cardiomyocytes, and myocardial fibrosis (12). It has been released from the intact myocardial tissue (12). It is also an important myokine for lung development (51). FSTL1 stimulate the secretion of proinflammatory cytokines and inflammation (52). The fact that FSTL1 was not changed in our study can be attributed to the minimal triggering of inflammation markers in the applied exercise model. In contrast, in long-term swimming adherence, a decrease in the plasma FSTL1 concentration was observed (although it did not reach a statistically significant level), which may be consistent with a decrease in inflammation and oxidative stress between 3 and 48 h after the last training session. Although the level of FSTL1 was not statistically significantly different in muscle groups that are largely used during swimming, such as the gastrocnemius-soleus muscle, following prolonged exercise, decreased plasma concentration may indicate release from body parts other than this muscle group. If mRNA and protein expressions could be investigated, we might have found a statistical difference.

Studies conducted by applying aerobic exercise in the literature provide different results for FSTL1 levels based on the duration, intensity of the exercise and the post-exercise measurement time (53, 54). There are studies showing that FSTL1 increases in the peripheral circulation immediately after acute sprint interval exercise, remains elevated for 15 min, and does not differ from the basal level in the blood taken at 30 min (30, 31). In a manuscript involving rats, there was

a significant decrease in FSTL1 mRNA expression in skeletal muscle and cardiac tissue after acute treadmill exercise; no difference was observed in terms of mRNA expression in white adipose and liver tissue, while protein levels in skeletal muscle and heart tissue were not altered (30). In the same study, FSTL1 levels in the serum increased significantly immediately following exercise (30). These findings, consistent with our data, remove skeletal muscle tissue as the main source of increased circulating FSTL1 levels.

Despite the advantages of the swimming exercise used in our study, certain limitations of swimming as an exercise type should be acknowledged. First, swimming may induce different physiological stress patterns compared to land-based exercises due to hydrostatic pressure, exposure to water, and breathe control demands, potentially affecting hormonal and metabolic responses (55). Although swimming generally has a lower energy expenditure rate compared to certain land-based aerobic activities, in untrained individuals it may result in higher energy costs due to increased water resistance and thermoregulatory demands, potentially affecting fatigue development and exercise tolerance (56-58). Additionally, the practical implementation of swimming protocols—particularly in animal models—can be technically challenging and may introduce variability in exercise intensity and duration (59). These factors should be considered when interpreting the physiological effects of swimming and when comparing it with other forms of aerobic exercise in both clinical and experimental settings. Additionally, care was taken to use the commercial kits preferred in the literature, as much as the budget allowed. However, the possibility that different results could have been obtained if the measurements were made with more sensitive kits/methods should not be ruled out. Within the scope of the study, while the time-dependent changes in the relevant myokines were examined, only the changes in post-exercise 0, 3, and 48 h could be evaluated. Possible myokine changes outside these time periods have not yet been elucidated. In addition, only the plasma and gastrocnemius-soleus muscle complex concentrations of the relevant myokines could be examined, and mRNA and protein expressions could not be investigated. The study was only conducted based on a male gender. These are the study limitations.

CONCLUSION

After 6 weeks of swimming exercise, the plasma concentrations of Metrnl were higher in mice at 3 h than in the plasma samples of the control (sedentary) group or the 3 h samples of the acute exercise group. However, there was no significant change in FSTL1, IL-7, or IL-8 concentrations



in the gastrocnemius-soleus muscle complex or plasma samples of mice in response to swimming exercises. Our data suggest that FSTL1, IL-7, and IL-8 do not play a role in swimming exercise adherence over the periods we evaluated. Among the myokines assessed, only the Metrnl myokine may mediate adaptation to long-term exercise. To clearly reveal the myokines that mediate the positive effects of exercise on the body, it may be recommended to explore the effects of various myokines in different types of exercise and at different time periods following exercise. It would be beneficial to plan these studies in other tissues (e.g., muscle groups) and to include not only the concentration level of myokines and cytokines but also their genetic expressions. It should be noted that examining the physiological pathways of Metrnl myokine might provide important data in this context.



Ethics Approval	Pamukkale University Animal Experiments Ethics Committee approved the study number PAUHADYEK-2019/29, dated 22.08.2019 and numbered 2019/06.
Peer-review	Externally peer-reviewed.
Author Contributions	Conception/Design of Study – A.O., E.B.U., M.B.K.; Data Acquisition – A.O., O.K.E., M.O., E.B.U., M.B.K.; Data Analysis/Interpretation – A.O., H.S., M.O., M.B.K.; Drafting Manuscript – A.O., O.K.E., M.O., H.S., M.B.K.; Critical Revision of Manuscript – H.S., M.O., M.B.K.; Final Approval and Accountability – A.O., O.K.E., H.S., M.O., E.B.U., M.B.K.
Conflict of Interest	The authors declare no conflict of interest.
Financial Disclosure	The Pamukkale University Scientific Research Projects Coordination Unit supported the study (Project No: 2020SABE005).

Author Details

Alten Oskay

¹ Department of Emergency Medicine, Faculty of Medicine, Pamukkale University, Denizli, Turkiye

0000-0003-4373-6280 oskayten@gmail.com

Ozgen Kilic Erkek

² Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli, Turkiye

0000-0001-8037-099X

Hande Senol

³ Department of Biostatistics and Medical Informatics, Faculty of Medicine, Pamukkale University, Denizli, Turkiye

0000-0001-6395-7924

Mert Ozen

¹ Department of Emergency Medicine, Faculty of Medicine, Pamukkale University, Denizli, Turkiye

0000-0001-6653-3756

Egem Burcu Unal

⁴ Therapeutic Apherisis Unit, Adana City Training and Research Hospital, Adana, Turkiye

0000-0002-9499-2316

Melek Bor Kucukatay

² Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli, Turkiye

0000-0002-9366-0205

REFERENCES

- Hiruma E. Ch 1: Muscles and bones. In: Dugue G, Bruce RT, editors. Osteosarcopenia India: Elsevier; 2022.p.1-18.
- Severinsen MCK, Pedersen BK. Muscle-organ crosstalk: The emerging roles of myokines. *Endocr Rev* 2020; 41 (4): 594–609. Erratum in: *Endocr Rev* 2021; 42(1): 97-9.
- Kraemer WJ, Hatfield DL, Comstock BA, Fragala MS, Davitt PM, Cortis C, et al. Influence of HMB supplementation and resistance training on cytokine responses to resistance exercise. *J Am Coll Nutr* 2014; 33(4): 247-55.
- Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, et al. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *Cell* 2014; 157(6): 1279-91.
- CDC-Centers for Disease Control and Prevention: Chapter 3 - Physiologic responses and long-term adaptations to exercise. *Physical activity and health: A report of the surgeon general executive summary (online)*. (cited 2022 July 03). Available from: URL: <https://www.cdc.gov/nccdphp/sgr/chap3.htm>.
- Mu S, Huang C, Chen B, Zhao P, Xu H, Zhang W, et al. Sclerostin decreases in regular swimmers after ice swimming and is associated with meteorin-like protein serum levels. *Front Physiol* 2023; 14: 1210457.
- Algaidi SA, Eldomiaty MA, Elbastwisy YM, Almasry SM, Desouky MK, Elnaggar AM. Effect of voluntary running on expression of myokines in brains of rats with depression. *Int J Immunopathol Pharmacol* 2019; 33: 2058738419833533.
- Acikel Elmas M, Cakici SE, Dur IR, Kozluca I, Arinc M, Binbuga B, et al. Protective effects of exercise on heart and aorta in high-fat diet-induced obese rats. *Tissue Cell* 2019; 57: 57-65.
- Li KC, Zhang FX, Li CL, Wang F, Yu MY, Zhong YQ, et al. Follistatin-like 1 suppresses sensory afferent transmission by activating Na⁺, K⁺-ATPase. *Neuron* 2011; 69(5): 974-87.



10. Oshima Y, Ouchi N, Sato K, Izumiya Y, Pimentel DR, Walsh K. Follistatin-like 1 is an Akt-regulated cardioprotective factor that is secreted by the heart. *Circulation* 2008; 117(24): 3099-108.
11. Ouchi N, Oshima Y, Ohashi K, Higuchi A, Ikegami C, Izumiya Y, et al. Follistatin-like 1, a secreted muscle protein, promotes endothelial cell function and revascularization in ischemic tissue through a nitric-oxide synthase-dependent mechanism. *J Biol Chem* 2008; 283(47): 32802-11.
12. Xi Y, Gong DW, Tian Z. FSTL1 as a potential mediator of exercise-induced cardioprotection in post-myocardial infarction rats. *Sci Rep* 2016; 6: 32424.
13. Schnyder S, Handschin C. Skeletal muscle as an endocrine organ: PGC-1 α , myokines and exercise. *Bone* 2015; 80: 115-25.
14. Ushach I, Burkhardt AM, Martinez C, Hevezi PA, Gerber PA, Buhren BA, et al. Meteorin-like is a cytokine associated with barrier tissues and alternatively activated macrophages. *Clin Immunol* 2015; 156(2): 119-27.
15. Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehler JE, Burdick MD, et al. CXCL chemokines in angiogenesis. *J Leukoc Biol* 2000; 68(1): 1-8.
16. Lira SA, Zalamea P, Heinrich JN, Fuentes ME, Carrasco D, Lewin AC, et al. Expression of the chemokine N51/KC in the thymus and epidermis of transgenic mice results in marked infiltration of a single class of inflammatory cells. *J Exp Med* 1994; 180(6): 2039-48.
17. Bendre MS, Montague DC, Peery T, Akel NS, Gaddy D, Suva LJ. Interleukin-8 stimulation of osteoclastogenesis and bone resorption is a mechanism for the increased osteolysis of metastatic bone disease. *Bone* 2003; 33(1): 28-37.
18. Rennekampff HO, Hansbrough JF, Kiessig V, Doré C, Sticherling M, Schröder JM. Bioactive interleukin-8 is expressed in wounds and enhances wound healing. *J Surg Res* 2000; 93(1): 41-54.
19. Skinner S, Nader E, Stauffer E, Robert M, Boisson C, Cibiel A, et al. Differential impacts of trail and ultra-trail running on cytokine profiles: An observational study. *Clin Hemorheol Microcirc* 2021; 78(3): 301-10.
20. Wang XS, Li BZ, Hu LF, Wen PF, Zhang M, Pan HF, et al. Perspectives of the relationship between IL-7 and autoimmune diseases. *Clin Rheumatol* 2013; 32(12): 1703-9.
21. Haugen F, Norheim F, Lian H, Wensaas AJ, Dueland S, Berg O, et al. IL-7 is expressed and secreted by human skeletal muscle cells. *Am J Physiol Cell Physiol* 2010; 298(4): C807-16.
22. Larsen AI, Aukrust P, Aarmland T, Dickstein K. Effect of aerobic exercise training on plasma levels of tumor necrosis factor alpha in patients with heart failure. *Am J Cardiol* 2001; 88(7): 805-8.
23. Arshadi S, Bakhtiyari S, Haghani K, Valizadeh A. Effects of fenugreek seed extract and swimming endurance training on plasma glucose and cardiac antioxidant enzymes activity in streptozotocin-induced diabetic rats. *Osong Public Health Res Perspect* 2015; 6(2): 87-93.
24. Ravi Kiran T, Subramanyam MV, Asha Devi S. Swim exercise training and adaptations in the antioxidant defense system of myocardium of old rats: relationship to swim intensity and duration. *Comp Biochem Physiol B Biochem Mol Biol* 2004; 137(2): 187-96.
25. Kilic-Erkek O, Caner V, Abban-Mete G, Baris IC, Bor-Kucukatay M. Determination of the pathways of potential muscle damage and regeneration in response to acute and long-term swimming exercise in mice. *Life Sci* 2021; 272: 119265.
26. Ünal EB, Kılıç Erkek Ö, Bor Küçükatay M. An experimental study on investigation of myokine responses to acute and chronic swimming exercise in mice. *Turkiye Klinikleri J Med Sci* 2024; 44(2): 60-8.
27. Scomparin DX, Grassioli S, Gomes RM, Torrezan R, de Oliveira JC, Gravena C, et al. Low-Intensity swimming training after weaning improves glucose and lipid homeostasis in MSG hypothalamic obese mice. *Endocr Res* 2011; 36(2): 83-90.
28. Su X, Yu X, Chen R, Bian W. Swimming improves platelet dysfunction in mice fed with a high-fat diet. *Arch Physiol Biochem* 2023; 129(1): 198-203.
29. Frydelund-Larsen L, Penkowa M, Akerstrom T, Zankari A, Nielsen S, Pedersen BK. Exercise induces interleukin-8 receptor (CXCR2) expression in human skeletal muscle. *Exp Physiol* 2007; 92(1): 233-40.
30. Kon M, Tanimura Y, Yoshizato H. Effects of acute endurance exercise on follistatin-like 1 and apelin in the circulation and metabolic organs in rats. *Arch Physiol Biochem* 2022; 128(5): 1254-8.
31. Kon M, Ebi Y, Nakagaki K. Effects of acute sprint interval exercise on follistatin-like 1 and apelin secretions. *Arch Physiol Biochem* 2021; 127(3): 223-7.
32. Schild M, Eichner G, Beiter T, Zügel M, Krumholz-Wagner I, Hudemann J, et al. Effects of acute endurance exercise on plasma protein profiles of endurance-trained and untrained individuals over time. *Mediators Inflamm* 2016; 2016: 4851935.
33. Andersson H, Bøhn SK, Raastad T, Paulsen G, Blomhoff R, Kadi F. Differences in the inflammatory plasma cytokine response following two elite female soccer games separated by a 72-h recovery. *Scand J Med Sci Sports* 2010; 20(5): 740-7.
34. Bote ME, García JJ, Hinchado MD, Ortega E. An exploratory study of the effect of regular aquatic exercise on the function of neutrophils from women with fibromyalgia: role of IL-8 and noradrenaline. *Brain Behav Immun* 2014; 39: 107-12.
35. Tan J, Guo L. Swimming intervention alleviates insulin resistance and chronic inflammation in metabolic syndrome. *Exp Ther Med* 2019; 17(1): 57-62.
36. Li ZY, Zheng SL, Wang P, Xu TY, Guan YF, Zhang YJ, et al. Subfatin is a novel adipokine and unlike Meteorin in adipose and brain expression. *CNS Neurosci Ther* 2014; 20(4): 344-54.
37. Zheng SL, Li ZY, Song J, Liu JM, Miao CY. Metrnl: a secreted protein with new emerging functions. *Acta Pharmacol Sin* 2016; 37(5): 571-9.
38. Bae JY, Woo J, Kang S, Shin KO. Effects of detraining and retraining on muscle energy-sensing network and meteorin-like levels in obese mice. *Lipids Health Dis* 2018; 17(1): 97.
39. Bonfante ILP, Duft RG, Mateus KCDS, Trombeta JCDS, Finardi EAR, Ramkraps APB, et al. Acute/chronic responses of combined training on serum prothermogenic/anti-inflammatory inducers and its relation with fed and fasting state in overweight type 2 diabetic individuals. *Front Physiol* 2022; 12: 736244.
40. Jung TW, Lee SH, Kim HC, Bang JS, Abd El-Aty AM, Hacimüftüoğlu A, et al. METRNL attenuates lipid-induced inflammation and insulin resistance via AMPK or PPAR δ -dependent pathways in skeletal muscle of mice. *Exp Mol Med* 2018; 50(9): 1-11.
41. Li Z, Gao Z, Sun T, Zhang S, Yang S, Zheng M, Shen H. Meteorin-like/Metrnl, a novel secreted protein implicated in inflammation, immunology, and metabolism: A comprehensive review of preclinical and clinical studies. *Front Immunol* 2023; 14: 1098570.
42. Kironenko TA, Milovanova KG, Zakharaova AN, Sidorenko SV, Klimanova EA, Dyakova EY, et al. Effect of dynamic and static load on the concentration of myokines in the blood plasma and content of sodium and potassium in mouse skeletal muscles. *Biochemistry (Mosc)* 2021; 86(3): 370-81.
43. Pedersen BK, Akerström TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. *J Appl Physiol* (1985) 2007; 103(3): 1093-8.
44. Landers-Ramos RQ, Jenkins NT, Spangenburg EE, Hagberg JM, Prior SJ. Circulating angiogenic and inflammatory cytokine responses to acute aerobic exercise in trained and sedentary young men. *Eur J Appl Physiol* 2014; 114(7): 1377-84.
45. Dorneles GP, Haddad DO, Fagundes VO, Vargas BK, Kloecker A, Romão PR, et al. High intensity interval exercise decreases IL-8 and enhances the immunomodulatory cytokine interleukin-10 in lean and overweight-obese individuals. *Cytokine* 2016; 77: 1-9.
46. Kim MS, Lim WK, Park RK, Shin T, Yoo YH, Hong SH, et al. Involvement of mitogen-activated protein kinase and NF- κ B activation in Ca $^{2+}$ -induced IL-8 production in human mast cells. *Cytokine* 2005; 32(5): 226-33.
47. Chan MH, Carey AL, Watt MJ, Febbraio MA. Cytokine gene expression in human skeletal muscle during concentric contraction: evidence that IL-8, like IL-6, is influenced by glycogen availability. *Am J Physiol Regul Integr Comp Physiol* 2004; 287(2): R322-7.
48. Sawa Y, Arima Y, Ogura H, Kitabayashi C, Jiang JJ, Fukushima T, et al. Hepatic interleukin-7 expression regulates T cell responses. *Immunity* 2009; 30(3): 447-57.
49. García JJ, Bote E, Hinchado MD, Ortega E. A single session of intense exercise improves the inflammatory response in healthy sedentary women. *J Physiol Biochem* 2011; 67(1): 87-94.



50. Ahn N, Kim K. Effects of aerobic and resistance exercise on myokines in high fat diet-induced middle-aged obese rats. *Int J Environ Res Public Health* 2020; 17(8): 2685.
51. Sylvia M, Li VS, Buffing AA, van Es JH, van den Born M, van der Velden S, et al. The BMP antagonist follistatin-like 1 is required for skeletal and lung organogenesis. *PLoS One* 2011; 6(8): e22616.
52. Miyamae T, Marinov AD, Sowders D, Wilson DC, Devlin J, Boudreau R, et al. Follistatin-like protein-1 is a novel proinflammatory molecule. *J Immunol* 2006; 177(7): 4758-62.
53. Görgens SW, Raschke S, Holven KB, Jensen J, Eckardt K, Eckel J. Regulation of follistatin-like protein 1 expression and secretion in primary human skeletal muscle cells. *Arch Physiol Biochem* 2013; 119(2): 75-80.
54. Xu X, Zhang T, Mokou M, Li L, Li P, Song J, et al. Follistatin-like 1 as a novel adipomyokine related to insulin resistance and physical activity. *J Clin Endocrinol Metab* 2020; 105(12): dgaa629.
55. Poole GW, Crepin BJ, Sevigny M. Physiological characteristics of elite synchronized swimmers. *Can J Appl Sport Sci* 1980; 5(3): 156-60.
56. Barrero A, Erola P, Bescós R. Energy balance of triathletes during an ultra-endurance event. *Nutrients* 2014; 7 (1): 209-22.
57. Barbosa TM, Bragada JA, Reis VM, Marinho DA, Carvalho C, Silva AJ. Energetics and biomechanics as determining factors of swimming performance: updating the state of the art. *J Sci Med Sport* 2010; 13(2): 262-9.
58. Lambert CP, Flynn MG, Braun WA, Boardley DJ. The effects of swimming and running on energy intake during 2 hours of recovery. *J Sports Med Phys Fitness*. 1999; 39(4): 348-54.
59. Börzsei D, Szabó R, Hoffmann A, Harmath A, Sebestyén J, Osman J, et al. Multiple applications of different exercise modalities with rodents. *Oxid Med Cell Longev* 2021; 2021: 3898710.

