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Spirulina-Induced Changes in Skeletal Muscle Morphology and Morphometry in Young and Mature Rats Under Physical and Metabolic Stress

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Abstract **Background:** High physical load and metabolic stress induce significant structural and functional alterations in skeletal muscle tissue. Nutritional and bioactive interventions, such as Spirulina supplementation, are considered potential modulators of muscle adaptation and recovery under stress conditions. **Methods:** This study investigated the effects of high physical load and metabolic stress on the morphological and morphometric characteristics of skeletal muscle tissue in 2- and 4-month-old outbred white rats. In addition, the therapeutic effects of Spirulina supplementation were evaluated. Histological and morphometric analyses were performed to assess muscle fiber structure, cellular organization, and microvascular parameters. **Results:** High physical load induced pronounced structural and metabolic disturbances in skeletal muscle tissue, including dystrophic and atrophic changes, disorganization of myofibrils, reduced mitochondrial content, and a shift of nuclei from peripheral to central localization. These alterations were associated with oxidative stress, hypoxic conditions, and increased energy demand. Spirulina supplementation significantly improved skeletal muscle morphology, evidenced by restoration of muscle fiber architecture, normalization of sarcoplasmic organization, increased mitochondrial density, and reestablishment of peripheral nuclear positioning. Morphometric evaluation demonstrated an increase in muscle fiber diameter, enhanced capillary density, and normalization of the nucleus-to-cytoplasm ratio, indicating improved microcirculation and metabolic activity. **Conclusion:** Spirulina exhibits pronounced antioxidant, antihypoxic, and anabolic effects, promoting skeletal muscle regeneration and enhancing adaptive capacity under conditions of physical and metabolic stress. These findings support its potential application as a functional supplement in sports medicine and rehabilitation to improve muscle recovery and performance.

Keywords Skeletal muscle, Spirulina, Morphometry, Morphology, Oxidative stress, Physical load, Metabolic stress, Muscle regeneration, Mitochondria, Capillary density, Fibrosis, Antioxidant activity, Adaptation, Rats

1. Introduction

Skeletal muscle tissue represents one of the most dynamic and adaptable systems in the organism, capable of responding to a wide range of physiological and pathological stimuli. Its structural and functional integrity is maintained through a complex interplay between anabolic and catabolic processes, including protein synthesis, energy metabolism, and programmed cell death (apoptosis) [1,4,7,9,11]. Under normal conditions, these processes are tightly regulated, ensuring tissue homeostasis and efficient regeneration.

However, exposure to high physical load and metabolic stress can disrupt this balance, leading to significant morphological and functional alterations in skeletal muscle [3,5,17,19,21]. Intense physical activity is associated with increased energy expenditure, hypoxia, and excessive production of reactive oxygen species (ROS), which contribute to oxidative stress and mitochondrial dysfunction. These changes may result in damage to muscle fibers, including disorganization of myofibrils, reduction in mitochondrial density, alterations in nuclear positioning, and activation of degenerative processes such as atrophy and fibrosis [2,6,8,10,12].

Age is an important factor influencing the adaptive capacity of skeletal muscle tissue. In younger organisms, higher metabolic activity and regenerative potential allow for more rapid recovery and effective adaptation to stress conditions [13,14,15,16,18]. In contrast, relatively older individuals may exhibit reduced plasticity, slower regeneration, and increased susceptibility to structural damage under similar conditions. Therefore, comparative analysis of different age groups is essential for understanding the mechanisms of muscle adaptation and recovery [20,24,27,29,30].

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In recent years, increasing attention has been given to the use of natural biologically active substances with antioxidant and cytoprotective properties to mitigate the negative effects of physical and metabolic stress. One such promising agent is Spirulina, a microalga rich in proteins, essential amino acids, vitamins, minerals, and bioactive compounds such as phycocyanin and carotenoids. Spirulina has been shown to possess strong antioxidant, anti-inflammatory, and antihypoxic properties, making it a potential candidate for enhancing muscle recovery and adaptation [22,23,25,26,28].

The **aim** of this study was to investigate the effects of high physical load and metabolic stress on the structural and morphometric characteristics of skeletal muscle tissue in experimental rats, and to evaluate the therapeutic potential of Spirulina supplementation in improving muscle morphology, enhancing regeneration, and promoting adaptive responses under physical and metabolic conditions.

2. Materials and Methods

The study was performed on 2- and 4-month-old outbred white rats (*Rattus norvegicus*). All animals were obtained from a certified laboratory breeding facility and kept under standardized vivarium conditions, including a temperature of 20–22 ℃, relative humidity of 50–60%, and a 12-hour light/dark cycle. Rats were provided with a standard laboratory diet and water ad libitum. Prior to the experiment, animals were acclimatized for 7 days.

Experimental Groups and Study Design: The animals were divided according to age into two main cohorts (2-month-old and 4-month-old rats), each further subdivided into three experimental groups:

1. Intact control group
2. High physical load and metabolic stress group
3. High physical load and metabolic stress + Spirulina-treated group

The experimental design was intended to evaluate age-dependent differences in skeletal muscle response to stress conditions and the corrective effect of Spirulina supplementation. Induction of Physical Load and Metabolic Stress: High physical load was induced using a treadmill-based exercise model. Animals were subjected to repeated running sessions with gradually increasing intensity and duration to simulate chronic physical stress. This protocol was designed to induce metabolic overload, enhanced oxygen consumption, and oxidative stress in skeletal muscle tissue. The combination of prolonged exercise and energy demand was considered a model of metabolic disturbance. Spirulina Treatment Protocol: Animals in the treatment groups received Spirulina orally at a calculated dose based on body weight (mg/kg). Administration was performed daily throughout the experimental period. The dosage was selected to ensure systemic antioxidant and metabolic effects without causing toxicity. Tissue Sampling and Histological Preparation: At the end of the experimental period, animals were anesthetized and euthanized according to ethical standards. Samples of skeletal muscle tissue were collected from the hind limb region. Tissue specimens were immediately fixed in 10% neutral buffered formalin to preserve morphological structure. After fixation, samples were processed using standard histological techniques, including dehydration through graded ethanol solutions, clearing in xylene, and embedding in paraffin blocks. Serial sections of 4–5 μm thickness were obtained using a microtome.

Histological and Histochemical Methods: Histological evaluation was performed using Hematoxylin–Eosin (H&E) staining to assess general tissue architecture. Van Gieson staining was additionally applied to evaluate connective tissue components and collagen fiber distribution. Microscopic analysis was conducted using a light microscope under magnifications of 100× and 400×. Morphological parameters such as muscle fiber integrity, sarcoplasmic density, nuclear position, and signs of degeneration or regeneration were carefully examined.

Morphometric Assessment: Quantitative morphometric analysis was performed using QuPath (version 0.4.0). Digital images were analyzed to determine:

- Muscle fiber diameter (μm)
- Number of nuclei per muscle fiber
- Nucleus-to-cytoplasm ratio
- Capillary density per mm^2

Measurements were taken from multiple randomly selected microscopic fields to ensure statistical reliability.

Statistical Processing: All obtained data were expressed as mean ± standard error (M ± SEM). Statistical analysis was performed using Student's t-test to compare differences between groups. A probability level of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

The present study demonstrated significant morphological and morphometric alterations in skeletal muscle tissue of 2- and 4-month-old outbred white rats exposed to high physical load and metabolic stress, as well as a pronounced modulatory effect of Spirulina supplementation on tissue structure and adaptive responses. Effects of High Physical Load and Metabolic Stress: In rats subjected to prolonged physical нагрузка without treatment, marked degenerative changes were observed in skeletal muscle fibers. Histological analysis revealed disruption of myofibrillar organization, fragmentation of muscle fibers, and areas of sarcoplasmic vacuolization. A noticeable reduction in mitochondrial density was also detected, indicating impaired cellular energy metabolism.

In addition, nuclear displacement from the peripheral region toward the central part of muscle fibers was frequently observed, which is considered a characteristic feature of muscle fiber stress and degeneration. These changes were more pronounced in 4-month-old rats compared to 2-month-old animals, suggesting age-dependent differences in adaptive capacity and regenerative potential. Morphometric analysis confirmed these findings, showing a reduction in muscle fiber diameter, decreased capillary density, and an imbalance in the nucleus-to-cytoplasm ratio. These alterations reflect impaired microcirculation, reduced oxygen supply, and enhanced oxidative stress within skeletal muscle tissue.

Overall, these results indicate that high physical load combined with metabolic stress leads to structural disorganization of skeletal muscle, primarily driven by oxidative damage, mitochondrial dysfunction, and energy deficiency.

Effects of Spirulina Treatment: Administration of Spirulina significantly improved the morphological and functional state of skeletal muscle tissue in both age groups. Histological examination revealed restoration of muscle fiber integrity, improved alignment of myofibrils, and normalization of sarcoplasmic structure. Muscle fibers appeared more uniform, with clearly defined boundaries and reduced signs of degeneration. A notable increase in mitochondrial content was observed in treated groups, indicating enhanced cellular energy metabolism and improved oxidative capacity. Nuclear positioning also showed normalization, with most nuclei returning to their typical peripheral localization, suggesting recovery of muscle fiber structural organization.

Morphometric data further supported these observations. Spirulina-treated rats demonstrated an increase in muscle fiber diameter, reflecting improved protein synthesis and anabolic activity. Capillary density was significantly higher compared to untreated stressed animals, indicating improved microcirculation and oxygen delivery to muscle tissue. The nucleus-to-cytoplasm ratio also shifted toward physiological norms, suggesting restoration of cellular homeostasis.

Age-Related Differences in Response: Comparative analysis between age groups revealed that 2-month-old rats exhibited a more rapid and pronounced response to Spirulina treatment. In this group, structural restoration of muscle fibers and normalization of morphometric parameters occurred more efficiently, reflecting higher regenerative capacity and metabolic plasticity in younger animals. In contrast, 4-month-old rats showed slower but more stable recovery patterns. Although improvement in muscle structure was evident, the extent of regeneration was relatively lower compared to younger animals. This suggests that age-related decline in adaptive capacity may influence the efficiency of muscle recovery under stress conditions.

Mechanisms of Spirulina Action: The beneficial effects observed in Spirulina-treated groups can be attributed to its rich composition of bioactive compounds, including proteins, essential amino acids, vitamins, minerals, and antioxidant pigments such as phycocyanin. These components are known to exert strong antioxidant, anti-inflammatory, and antihypoxic effects. Spirulina likely reduces oxidative stress by neutralizing reactive oxygen species, thereby protecting mitochondrial structures and preserving cellular energy metabolism. In addition, its protein-rich composition may stimulate muscle protein synthesis, contributing to the restoration of muscle fiber integrity and diameter.

Furthermore, improved capillary density observed in treated animals suggests that Spirulina may enhance angiogenic processes, thereby improving oxygen and nutrient delivery to skeletal muscle tissue. Collectively, these effects support enhanced tissue regeneration and adaptive capacity under conditions of physical and metabolic stress. Overall Interpretation. The findings of this study clearly demonstrate that high physical load and metabolic stress induce significant degenerative changes in skeletal muscle tissue, while Spirulina supplementation effectively mitigates these alterations. The observed improvements in muscle morphology and morphometry indicate that Spirulina plays a protective and restorative role in skeletal muscle adaptation. These results highlight the potential of Spirulina as a functional biologically active supplement for improving muscle recovery, enhancing resistance to oxidative stress, and supporting adaptive responses in conditions of increased physical demand.

4. Conclusions

The results of this study demonstrate that exposure to high physical load and metabolic stress induces pronounced morphological and morphometric alterations in skeletal muscle tissue of 2- and 4-month-old outbred white rats. These changes are characterized by disruption of myofibrillar organization, reduction in mitochondrial content, nuclear mislocalization, decreased capillary density, and overall impairment of muscle fiber integrity. The observed alterations were more severe in 4-month-old rats, indicating an age-dependent decline in adaptive and regenerative capacity.

Administration of Spirulina significantly attenuated stress-induced structural damage in skeletal muscle tissue. Spirulina supplementation promoted restoration of muscle fiber architecture, increased mitochondrial density, improved microcirculation, and normalized key morphometric parameters, including muscle fiber diameter and nucleus-to-cytoplasm ratio. These effects indicate a strong protective and restorative influence on skeletal muscle under conditions of physical and metabolic stress. The study further revealed that younger animals (2-month-old rats) exhibited a more rapid and pronounced response to Spirulina treatment, whereas older animals (4-month-old rats) showed slower but more stable recovery. This suggests that age plays an important role in determining the efficiency of muscle adaptation and regeneration.

Overall, the findings confirm that Spirulina possesses significant antioxidant, antihypoxic, and anabolic properties that contribute to the preservation and restoration of skeletal muscle structure under stress conditions. These results support the potential application of Spirulina as a biologically active supplement in sports medicine, rehabilitation, and conditions associated with muscle fatigue and metabolic imbalance.

5. Future Perspectives

Although the present study demonstrates the beneficial effects of Spirulina on skeletal muscle morphology and morphometry under conditions of high physical load and metabolic stress, several aspects remain to be further investigated in order to fully elucidate its mechanisms of action and potential clinical applications. Future research should focus on the molecular pathways underlying Spirulina -induced muscle protection, particularly the regulation of oxidative stress markers, mitochondrial biogenesis, and apoptosis-related signaling pathways. In this context, detailed analysis of pro- and anti-apoptotic proteins (such as Bcl-2 family members), as well as key regulators of energy metabolism, would provide deeper insight into its cytoprotective effects.

In addition, it is important to investigate the dose-dependent effects of Spirulina to determine the optimal therapeutic concentration for maximal efficacy and safety. Long-term experimental studies are also required to assess whether prolonged administration leads to sustained improvements in muscle structure and function or potential adaptive limitations. Further studies involving different age groups, sex differences, and various models of physical training would help to clarify the variability of response and improve the translational relevance of the findings. Expanding the research to include functional assessments of muscle performance (such as strength, endurance, and fatigue resistance) would also strengthen the physiological significance of morphological data.

Moreover, combining Spirulina with other antioxidant or metabolic modulators may reveal potential synergistic effects in enhancing skeletal muscle adaptation and recovery. Such combination strategies could be particularly relevant in sports medicine, rehabilitation programs, and the prevention of muscle degeneration associated with aging or chronic metabolic disorders.

In conclusion, Spirulina represents a promising natural bioactive compound; however, further multidisciplinary studies integrating morphology, biochemistry, and functional physiology are necessary to fully establish its role in skeletal muscle protection and regeneration.

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