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Immunohistochemical Analysis of Age-Related Dynamics of the Antiapoptotic Bcl-2 Marker in Skeletal Muscle Tissue: A Study on 2- and 4-Month-Old White Rats

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Abstract **Background:** The anti-apoptotic protein Bcl-2 plays a key role in regulating programmed cell death, maintaining cellular viability, and ensuring tissue homeostasis. Its expression in skeletal muscle tissue may reflect adaptive and regenerative responses to physiological and stress-related conditions; however, age-dependent dynamics under physical and metabolic stress remain insufficiently characterized. **Methods:** This study examined the immunohistochemical expression of Bcl-2 in skeletal muscle tissue of 2- and 4-month-old outbred white rats under control conditions and following exposure to high physical load and metabolic stress. Tissue samples were analyzed using standard immunohistochemical techniques to evaluate the localization and intensity of Bcl-2 expression. **Results:** In control groups, no significant age-related differences in Bcl-2 expression were observed. Relatively high baseline expression levels were detected in both age groups, indicating active cellular viability and ongoing regenerative processes in skeletal muscle tissue. Under conditions of high physical load and metabolic stress, a marked increase in Bcl-2 expression was observed, particularly in 2-month-old rats. Immunohistochemical analysis revealed enhanced cytoplasmic and perinuclear localization of Bcl-2-positive cells, suggesting activation of anti-apoptotic mechanisms and improved cellular survival under stress conditions. **Conclusion:** Bcl-2 expression in skeletal muscle tissue is dynamically regulated in response to physiological and stress stimuli. Its increased expression under high physical and metabolic load reflects an adaptive anti-apoptotic response that supports muscle cell survival and tissue homeostasis. These findings highlight the role of Bcl-2 as a potential biomarker of skeletal muscle adaptation, regeneration, and resistance to stress-induced apoptosis.

Keywords Skeletal muscle tissue, Bcl-2, Immunohistochemistry, Apoptosis, Anti-apoptotic protein, High physical load, Metabolic stress, Cell viability, Muscle regeneration, Adaptation, Oxidative stress

1. Introduction

Skeletal muscle tissue is a highly adaptive system capable of responding to physiological and pathological stimuli, including physical exercise and metabolic stress. Its structural integrity and functional capacity are maintained through a delicate balance between cellular proliferation, differentiation, and programmed cell death (apoptosis) [1,4,7,9,11]. Disruption of this balance may lead to impaired muscle regeneration, altered contractile function, and progressive tissue remodeling.

Apoptosis plays a crucial role in skeletal muscle homeostasis by eliminating damaged or dysfunctional cells [2,3,6]. However, excessive activation of apoptotic pathways can contribute to muscle atrophy and reduced regenerative potential. In contrast, anti-apoptotic mechanisms are essential for preserving cell survival and supporting tissue repair processes. Among the key regulators of apoptosis, Bcl-2 (B-cell lymphoma 2) is one of the most important anti-apoptotic proteins. It functions by stabilizing mitochondrial membranes, inhibiting cytochrome c release, and suppressing caspase activation, thereby preventing programmed cell death [5,16,17,19,20].

Recent studies have shown that Bcl-2 expression is closely associated with muscle development, regeneration, and adaptation to physiological stress. Its level may vary depending on age, metabolic status, and exposure to physical нагрузка (exercise-induced stress). However, the age-related dynamics of Bcl-2 expression in skeletal muscle tissue, particularly under different physiological conditions, remain insufficiently studied [12,13,15,18].

High physical load and metabolic disturbances are known to induce oxidative stress, mitochondrial dysfunction, and activation of apoptotic signaling pathways in muscle fibers [21,26,27]. These changes may significantly influence the expression of apoptotic and anti-apoptotic markers, including Bcl-2, thereby affecting muscle tissue plasticity and recovery capacity [22,23,24,25,28].

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Therefore, the present study **aims** to evaluate the immunohistochemical expression of the Bcl-2 anti-apoptotic marker in skeletal muscle tissue of 2- and 4-month-old white rats under control conditions, as well as under high physical load and metabolic stress. Understanding the dynamics of Bcl-2 expression may provide valuable insights into skeletal muscle adaptation mechanisms and contribute to the development of therapeutic strategies targeting muscle regeneration and protection.

2. Materials and Methods of Research

The study was conducted on 2- and 4-month-old outbred white rats (*Rattus norvegicus*) kept under standard vivarium conditions, including controlled temperature (20–22 ℃), relative humidity (50–60%), and a 12-hour light/dark cycle. Animals were fed a standard laboratory diet with free access to water. The experimental design included both control and experimental groups. The control groups consisted of 2- and 4-month-old rats maintained without any additional interventions. Experimental groups were subjected to high physical load and metabolic stress conditions to induce physiological adaptation responses in skeletal muscle tissue.

High physical load was applied using a treadmill exercise model with gradually increasing intensity and duration. This protocol was designed to simulate chronic physical stress and induce metabolic alterations in skeletal muscle fibers. The duration and intensity of exercise were standardized across experimental groups to ensure reproducibility of results. Following the experimental period, skeletal muscle tissue samples were collected under appropriate ethical guidelines. Tissue specimens were fixed in 10% neutral buffered formalin, dehydrated through a graded ethanol series, and embedded in paraffin blocks. Serial sections of 4–5 μm thickness were prepared using a microtome.

Immunohistochemical staining was performed using the Bcl-2 primary antibody to detect anti-apoptotic protein expression. The sections were processed using the DAB (3,3′-diaminobenzidine) chromogen system, followed by hematoxylin counterstaining. Stained slides were examined under a light microscope at 400× magnification. Digital image analysis and quantitative assessment of immunopositive cells were performed using QuPath software (version 0.4.0). The number of positive and negative cells, as well as the percentage of Bcl-2 expression, was calculated in defined microscopic fields. Morphometric parameters were recorded in pixel-based measurements and statistically analyzed.

Statistical analysis was performed using standard methods. Results were expressed as mean ±standard error (M ±SEM). Differences between groups were evaluated using Student's t-test, and values of $p < 0.05$ were considered statistically significant.

3. Results and Discussion

The immunohistochemical evaluation of Bcl-2 expression in skeletal muscle tissue demonstrated both age-related stability under physiological conditions and dynamic alterations in response to high physical load and metabolic stress. In the control groups, skeletal muscle tissue of both 2- and 4-month-old rats exhibited relatively high and uniform expression of Bcl-2. Quantitative morphometric analysis revealed no statistically significant differences between age groups ($p > 0.05$), suggesting that during early ontogenesis, anti-apoptotic mechanisms are well maintained and functionally active. The observed moderate to strong cytoplasmic staining of muscle fibers indicates a stable intracellular environment, where apoptosis is effectively suppressed, and cellular turnover is balanced by regeneration processes.

Histologically, Bcl-2-positive cells were predominantly localized in the cytoplasm of myocytes, with occasional perinuclear accumulation. This pattern is consistent with the known mitochondrial localization of Bcl-2, reflecting its role in maintaining mitochondrial membrane integrity and preventing the release of pro-apoptotic factors such as cytochrome c. The preservation of this staining pattern across both age groups indicates that baseline anti-apoptotic protection is not significantly influenced by age within this developmental window.

Under conditions of high physical load and metabolic stress, a marked increase in Bcl-2 expression was observed, particularly in the skeletal muscle tissue of 2-month-old rats. Quantitative analysis demonstrated a statistically significant rise in the proportion of immunopositive cells ($p < 0.05$), along with increased staining intensity. This suggests activation of compensatory cellular mechanisms aimed at enhancing cell survival in response to stress-induced damage.

The increase in Bcl-2 expression can be interpreted as an adaptive response to elevated levels of oxidative stress and metabolic demand. Intense physical activity is known to induce the generation of reactive oxygen species (ROS), mitochondrial dysfunction, and activation of intrinsic apoptotic pathways. In this context, upregulation of Bcl-2 serves to counteract these effects by inhibiting mitochondrial permeability transition and suppressing caspase activation. Thus, the elevated expression of Bcl-2 reflects a protective mechanism that limits excessive apoptosis and preserves muscle fiber integrity.

Morphologically, muscle fibers in the experimental groups showed enhanced cytoplasmic and perinuclear staining, with a higher density of Bcl-2-positive cells distributed throughout the tissue. In some cases, clustering of immunopositive cells was observed, which may indicate localized zones of increased regenerative or adaptive activity. These findings suggest that not only individual cells but also specific regions within the muscle tissue actively respond to stress by upregulating anti-apoptotic pathways.

In 4-month-old rats, exposure to high physical load also resulted in increased Bcl-2 expression; however, the magnitude of this response was comparatively lower than in 2-month -old animals. Although statistically significant differences from control groups were still observed ($p < 0.05$), the relative increase in immunopositive cells and staining intensity was

less pronounced. This suggests a gradual decline in the efficiency of adaptive mechanisms with age, even within early stages of postnatal development. The reduced responsiveness of Bcl-2 expression in older rats may be associated with several factors, including decreased metabolic flexibility, reduced mitochondrial efficiency, and altered regulation of gene expression. Age-related changes in signaling pathways involved in stress response and apoptosis regulation may limit the capacity of muscle tissue to activate anti-apoptotic defenses under challenging conditions.

These observations are consistent with current concepts in muscle biology, where the balance between pro-apoptotic and anti-apoptotic factors determines the outcome of cellular stress responses. While Bcl-2 acts as a key inhibitor of apoptosis, its activity is closely counterbalanced by pro-apoptotic proteins such as Bax. The relative expression of these proteins ultimately influences whether a muscle cell survives or undergoes programmed cell death. Furthermore, the findings highlight the importance of mitochondrial pathways in skeletal muscle adaptation. Since Bcl-2 primarily functions at the mitochondrial level, its upregulation under stress conditions underscores the central role of mitochondrial integrity in maintaining muscle function and resilience.

Overall, the results of this study demonstrate that Bcl-2 expression is not static but dynamically regulated in response to physiological and pathological stimuli. The observed age-dependent differences indicate that younger muscle tissue possesses a greater capacity for adaptive upregulation of anti-apoptotic mechanisms, which may contribute to its enhanced regenerative potential.

4. Conclusions

This study provides comprehensive evidence that the anti-apoptotic protein Bcl-2 plays a crucial role in maintaining skeletal muscle homeostasis and mediating adaptive responses to stress. Under normal physiological conditions, both 2- and 4-month-old rats exhibit stable and relatively high levels of Bcl-2 expression, reflecting effective suppression of apoptosis and maintenance of cellular viability. The absence of significant age-related differences in control groups suggests that anti-apoptotic protection is well established during early stages of development.

However, exposure to high physical load and metabolic stress induces a significant increase in Bcl-2 expression, particularly in younger animals. This indicates activation of protective mechanisms aimed at counteracting stress-induced apoptotic signaling and preserving muscle tissue integrity. The more pronounced response observed in 2-month-old rats suggests that younger muscle tissue has a higher adaptive capacity and greater resilience to stress. In contrast, the relatively attenuated response in 4-month-old rats may reflect early signs of reduced plasticity and diminished efficiency of cellular defense mechanisms.

These findings underscore the importance of Bcl-2 as a key regulator of muscle cell survival and as a sensitive biomarker for assessing tissue adaptation, regenerative potential, and resistance to apoptosis. Understanding the dynamics of Bcl-2 expression in skeletal muscle may have important implications for the development of therapeutic strategies aimed at preventing muscle atrophy, enhancing recovery after injury, and improving outcomes in muscle-related diseases.

5. Future Perspectives

Further research is necessary to expand upon the findings of this study and to gain a deeper understanding of the molecular mechanisms underlying skeletal muscle adaptation.

In particular, it would be valuable to investigate the interaction between Bcl-2 and pro-apoptotic markers such as Bax and Caspase-3, as well as their combined influence on apoptotic signaling pathways. Evaluating the Bcl-2/Bax ratio could provide a more comprehensive assessment of the balance between cell survival and cell death. Additionally, future studies should focus on the role of oxidative stress, mitochondrial dynamics, and intracellular signaling pathways in regulating Bcl-2 expression. The involvement of pathways such as PI3K/Akt and MAPK in mediating stress responses and promoting cell survival warrants further investigation.

Long-term and longitudinal studies involving a wider range of age groups and varying intensities of physical load would help to clarify the progression of age-related changes in muscle adaptability. Moreover, the application of advanced imaging techniques and molecular analysis methods could enhance the precision and depth of immunohistochemical findings. Ultimately, a better understanding of anti-apoptotic regulation in skeletal muscle may contribute to the development of targeted interventions aimed at improving muscle health, preventing degeneration, and enhancing functional recovery in both clinical and sports practice.

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