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The impact of training and biological sex on metabolic stress and antioxidant health

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Abstract—The present study was planned to evaluate the effect of experimental exercises on the histological and biochemical changes in the skeletal muscle, bone and general body functioning in males and females rabbit. There were control and exercise therapy groups in the study and both had male and female participants. It aims to look at changes in structure, metabolism, endocrine responses and balance of oxidative stress. Twenty adult rabbits (ten males and ten females) were randomly assigned to either a treadmill training group or a control group that did not exercise. The rabbits were between the ages of four and six months. The design allowed for the evaluation of treatment and sex effects simultaneously. Tissue slices of the control groups stained with Hematoxylin and Eosin showed normal tissue architecture.

The therapy groups nonetheless showed normal adaptation changes including an increase in trabecular bone density, an improvement in blood flow and hypertrophy of the muscle fibers. These data show that exercise increases the tissue's ability to modify shape and function. Biochemical testing showed that levels in treated animals were considerably higher of lactate and cortisol in treated animals compared to controls.

suggesting the metabolism is functioning harder and the stress reaction is being triggered. Levels of superoxide dismutase (SOD) were increased, indicating improvement in antioxidant defenses and levels of malondialdehyde (MDA) were reduced, indicating reduction in lipid peroxidation. Males showed high increases in muscle hypertrophy and metabolic indicators, whereas female had greater increases in antioxidants.

The two-way ANOVA test revealed significant effects of sex and therapy on all parameters studied ($P < 0.05$). Taken together, the data suggest that regulated exercise enhances bone health, muscle structure and oxidative balance, without any detrimental effects. Overall, our findings suggest that exercise training has great promise as a non-pharmacological intervention to improve tissue function and health in general, with obvious sex differences in the way they adaption.

Keywords — Histology, Training, Antioxidant, Metabolic stress, Rabbits.

INTRODUCTION

The study of sex differences in exercise efficiency is an important area of research with important implications for fitness and health across all populations (1). Sex-specific responses to exercise (2) provide information to guide sex-specific therapies that can improve health outcomes. The study was designed to investigate the effects of regular moderate exercise on bone density and muscle mass in rabbits and the findings may have implications for fitness and health in humans (3).

Rabbits are a good model for this study due to the physiological similarity with humans, especially in muscle and bone structure (4). The basic mechanisms underlying the exercise reactions (5) are the hormonal profiles, muscle fiber composition and metabolic capabilities being significantly different between genders. Previous studies have mentioned that if there is resistance, men tend to develop more muscles. training. Regular physical activity in females can produce higher gains in bone density (6). These differences highlight the importance of gender-specific recommendations for fitness (7).

Earlier researches suggests that regular exercise may improve bone density to a greater extent in women, while men are more likely to experience muscular hypertrophy (8). However, these variations and the underlying biological mechanisms still require thorough investigation. In this study, we filled in some of the gaps identified by these fascinating observations by studying both bone and muscle changes (bone density and muscle mass) over time in males and females' rabbits on a standardized exercise regimen (9).

MATERIALS AND MTHODS

Design and experimental Animals:

The experimental study was performed on 20 adult rabbits aged between 4 and 6 months (10 males and 10 females), which were randomly divided into two groups: control group without exercise and treadmill training. This design permitted assessment of both treatment and sex effects.

Animals were kept in controlled laboratory conditions with free access to food and water, at a temperature of 22–25 °C, relative humidity of 50 ± 5%, and under a cycle from light (12 h) to dark (12 h). In order to minimize stress, a two-week period was allowed for acclimatization.

Exercise Protocol:

The treadmill training program was conducted in a speed and time of 20 m/min, 20 min/d for 60 days on the treatment group. A moderate regimen was chosen to induce physiological adaptations without inflicting pathological stress (10).

Blood & Biochemical Analysis

At the conclusion of the experiment, animals were killed using chloroform inhalation. Blood was obtained from a heart puncture (≈5 ml) and centrifuged for 5 min at 3000 rpm, serum stored frozen at -20°C, and used to measure lactate (LA), cortisol, malondialdehyde (MDA) and superoxide dismutase (SOD) because these indicate the strength of oxidative state mechanisms of stress response and metabolic activity.

Myofibril quantification and histological analysis

Tissue preparation Skeletal muscle samples were fixed in 10% neutral buffered formalin, routinely processed, paraffin embedded and sectioned at 4–5 µm. Sections were stained with Hematoxylin and Eosin for assessment on cellular morphology and general tissue architecture. Histological examination primarily centered around the pattern of muscle fibers arrangement, nuclear morphology and presence of vascular or connective tissue abnormalities.

A light microscope and an image analysis system were used to measure the diameter of the muscle fibers. To assess for hypertrophic changes, diameters were measured from randomly selected muscle fibers from multiple fields. From morphometric studies mean values for each group were calculated to compare the treated animals with the control animals (11).

Statistical analyses

Data expressed in Mean ± Standard deviation [SD]. Statistical analysis was done by Tow-way ANOVA SPSS. All differences were considered statistically significant at $P \leq 0.05$.

REULTS AND DISCUSSION

More importantly, this study found that regardless of sex (male vs. female), repeated daily treadmill exercise produced a rather well coordinated series of biochemical, morphometric and histological adaptations in rabbit skeletal muscle indicating improved physiological performance without pathological injury. Blood parameters, including serum lactate level (ran in male groups selected as trained with high intensity), higher significant response of trained groups was recorded ($p \leq 0.05$) for this variable in comparison to control one by figure (1). This is consistent with the idea of the lactate shuttle, which holds that lactate functions as a signaling molecule and an energy substrate that controls metabolic adaptation (Brooks, 2020).

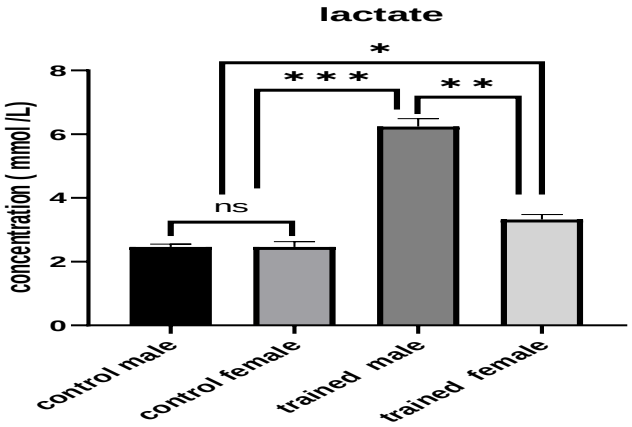

Figure 1. The effect of treadmill on Serum lactate concentration. Serum cortisol levels were significantly increased ($P \leq 0.05$) in trained animals, with higher values in males. Indicating activation of the hypothalamic-pituitary-adrenal axis and greater energy mobilization in response to physical stress (12).

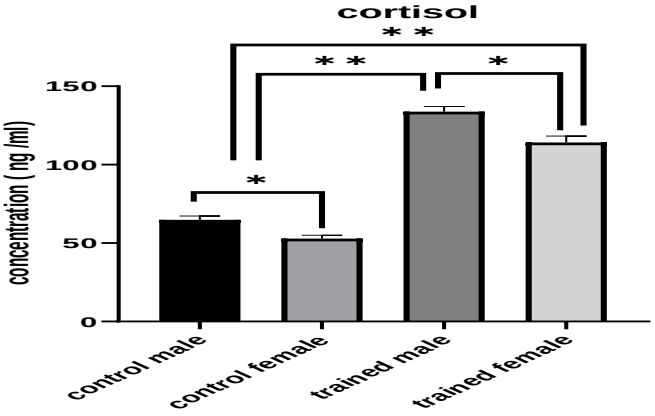

Figure 2. The effect of treadmill on Serum cortisol concentration. Malondialdehyde (MDA), an oxidative stress marker, dropped significantly in trained groups compared to controls. Indicating lower lipid peroxidation and enhanced cellular resistance to oxidative damage, consistent with exercise-induced hormesis (13). .

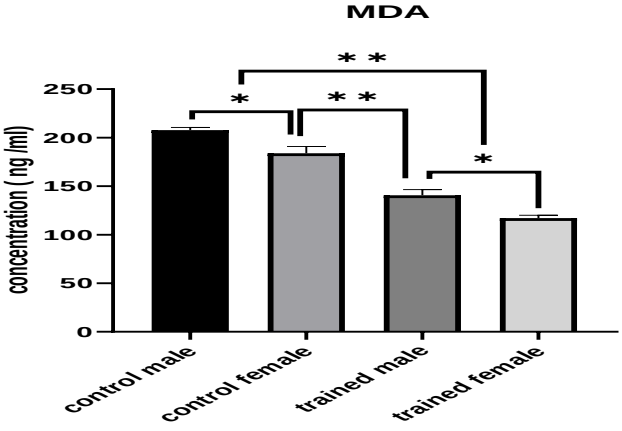

Figure 3. The effect of treadmill on Serum MDA concentration. shows a significantly increase ($P < 0.05$) in superoxide dismutase (SOD) levels in trained females, Indicating greater

antioxidant defense mechanisms. This sex-related variation is likely due to estrogen-mediated activation of antioxidant enzymes and higher mitochondrial efficiency (14).

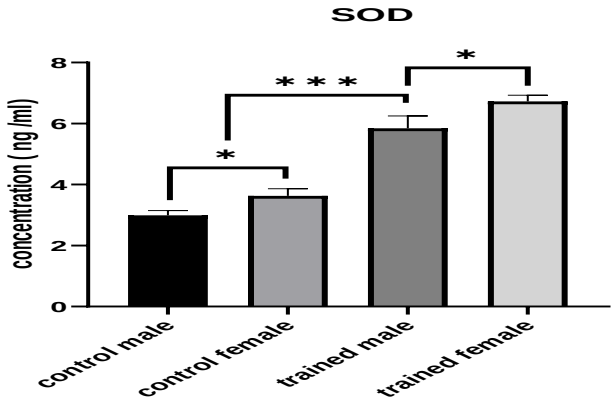

Figure 4. The effect of treadmill on Serum SOD concentration. Morphometric analysis showed a significantly increase ($P < 0.05$) in muscle fiber diameter in trained groups compared to controls in Table (1). Indicating exercise-induced hypertrophy. The larger increase found in males indicates stronger anabolic signaling, higher testosterone levels, and increased activation of satellite cells, which promote protein synthesis and muscle growth (15).

Table 1. The Mean of Muscle Fiber Diameter (µm) in Controls and Exercise Groups, the descriptive statistical analysis showed clear differences in muscle fiber diameter among the experimental groups. The male exercise group recorded the highest mean value (51.7 ± 4.1 µm), while the female control group showed the lowest value (36.7 ± 2.8 µm).

Groups	Mean ± SD (µm)	P-value
Male Control	42.2 ± 3.6	≤ 0.05
Female Control	36.7 ± 2.8	≤ 0.05
Male Exercise	51.7 ± 4.1	≤ 0.01
Female Exercise	45.3 ± 3.6	≤ 0.05

Both exercise groups demonstrated increased muscle fiber diameter compared with their respective control groups, indicating exercise-induced hypertrophy. Males exhibited higher values than females in both control and exercise groups, reflecting sex-related differences in muscle morphology. Statistical analysis revealed significant differences among groups, particularly in the male exercise group ($P \leq 0.01$).

In contrast, males showed a milder increase in fiber diameter, most likely because they rely more on oxidative metabolism rather than glycolytic hypertrophy. These data show that exercise induces structural remodeling of skeletal muscle through mechanical loading and metabolic adaptation, with clear sex variations in response magnitude. Male showed a subtler increase in fiber diameter, most likely due to their reliance on oxidative metabolism rather than glycolytic hypertrophy. These findings indicate that exercise causes structural remodeling of skeletal muscle through mechanical stress and metabolic adaptation, with clear sex differences in response magnitude.

Hematoxylin and Eosin staining confirmed these observations at 30 days (fig.5, fig.6) demonstrating normal muscle architecture with parallel fibers with peripheral nuclei and minimal interstitial space in control groups while trained accumulations show enlarged muscle fiber size, occasional central nuclei indicating regeneration, and increased vascularization in endomysial spaces in figure (7), (8).

The presence of these features is indicative of ongoing remodeling and will be associated with delivery of improved nutrition and oxygen supply. To note, there was no degeneration, necrosis or inflammatory infiltration suggesting the exercise protocol used.

Integrated biochemical, histological and morphometric analysis indicate that exercise is a potent regulator of oxidative status and improves metabolic activity and endocrine response to achieve a better muscle shape/function.

Furthermore, similar sex-related differences, with males exhibiting larger metabolic and hypertrophic responses and females demonstrating superior antioxidant capacity, underscore the role of hormonal and metabolic variables in defining adaptive responses to exercise. Collectively, our findings demonstrate that regular moderate-intensity exercise generates favorable systemic and cellular changes, improving both structural integrity and functional efficiency of skeletal muscle. (16,17).

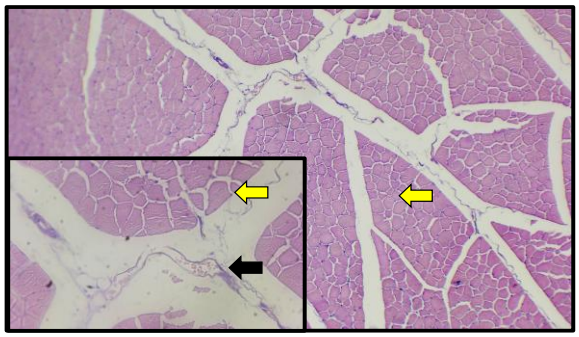

Figure 5. histological section in the muscle of female rabbit of control group shows cross section of muscle fiber with periphery placed nuclei (yellow arrow) with capillaries& loose connective tissue surrounds each muscle fiber (black arrow). H&E stain 10,40 X.

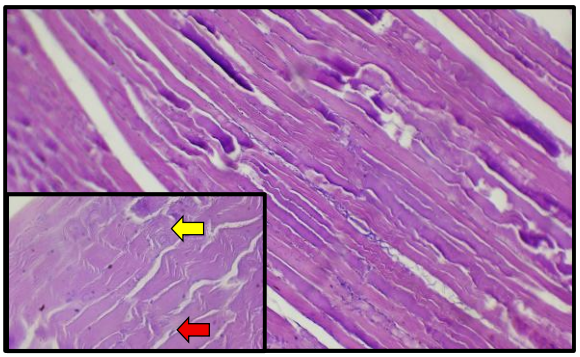

Figure 6. histological section in the muscle of male rabbit of control group shows cross section of muscle fiber with periphery placed nuclei (yellow arrow) with capillaries& loose

connective tissue surrounds each muscle fiber (black arrow). H&E stain 10,40 X).

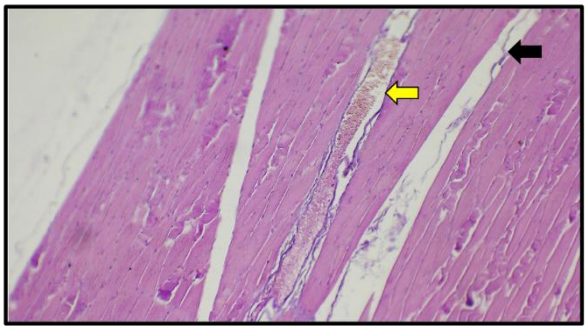

Figure 7. histological section in the muscle of female rabbit of exercise group shows hypertrophy of muscle fibers which appeared parallel to each other (yellow arrow) that muscle fibers were well organized with distinct striations with peripheral vesicular nuclei with typically shaped nuclear chain (red arrows) H&E stain 10,40 X).

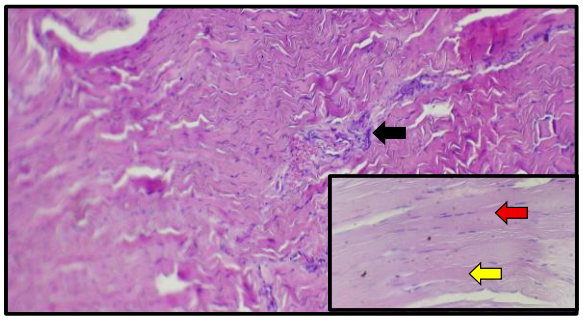

Figure 8. histological section in the muscle of male rabbit of exercise group shows dilated congested blood vessels (yellow arrow) loose connective tissue surrounds each muscle fiber (black arrow). H&E stain 10,40 X.

CONCLUSION

The present study demonstrates that treadmill exercise habitually induces major physiological adaptation in both skeletal muscle and metabolic variables in females and male's rabbits. Increased metabolic activity and activation of the stress response during exercise are confirmed with elevated serum lactate and cortisol levels, respectively. Conversely, oxidative stress decreased as indicated by a decrease in malondialdehyde (MDA) and an increase in superoxide dismutase (SOD).

The morphometric analysis showed a significant increase in muscle fiber cross-sectional area in the training groups, confirming exercise-induced hypertrophy. Preliminary observations of enhanced muscle structure, greater vascularization, and the absence of pathological changes. Notably, sex-related variations were found, with larger metabolic rate and with a lower body fat in male. Hypertrophic responses and females exhibiting higher antioxidant capacity.

All these data support the idea that exercise at a moderate intensity improves metabolic function, oxidative balance and tissue structure. as it increases Therefore, it has been an excellent strategy physiological function while minimizing issue damage. muscle architecture, vascularization and pathological alterations in the histological investigation using Hematoxylin and Eosin were confirmed. Crucially, sexual dimorphic responses were evaluated with improvements in both metabolic and hypertrophic.

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Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1) Devries, M. C., & Jakobi, J. M. (2021). Importance of considering sex and gender in exercise and nutrition research. In Applied Physiology, Nutrition, and Metabolism (Vol. 46, Issue 6, pp. iii–vii). NRC Research Press 1840 Woodward Drive, Suite 1, Ottawa, ON K2C 0P7.

2) Kim H, Kim SE, Sung MK. Sex and gender differences in obesity: biological, sociocultural, and clinical perspectives. The World Journal of Men's Health. 2025 Jul 14;43(4):758.

3) Rincon-Castanedo, C., Martín-Ruiz, A., Zazo, S., Luis Huertas, A. L., Valenzuela, P. L., Morán, M., Fleck, S. J., Santos-Lozano, A., Ramirez, M., & Rojo, F. (2023). Combined exercise intervention in a mouse model of high-risk neuroblastoma: effects on physical, immune, tumor and clinical outcomes. Exercise Immunology Review, 29.

4) Mosaddad, S. A., Hussain, A., & Tebyaniyan, H. (2024). Exploring the use of animal models in craniofacial regenerative medicine: a narrative review. Tissue Engineering Part B: Reviews, 30(1), 29–59.

5) Landen, S., Hiam, D., Voisin, S., Jacques, M., Lamon, S., & Eynon, N. (2023). Physiological and molecular sex differences in human skeletal muscle in response to exercise training. The Journal of Physiology, 601(3), 419–434.

6) Hong AR, Kim SW. Effects of Resistance Exercise on Bone Health. Endocrinol Metab (Seoul). 2018 Dec;33(4):435-444. doi: 10.3803/EnM.2018.33.4.435. PMID: 30513557; PMCID: PMC6279907.

7) Rubulotta, F., Rello, J., Moretti, A., & Mascia, L. (2025). Implementing a gender-specific perspective in critical care: An expert report from the 2024 iWIN International Meeting. Intensive and Critical Care Nursing, 87, 103946.

8) Dong G, Moparthy C, Thome T, Kim K, Yue F, Ryan TE. IGF-1 therapy improves muscle size and function in experimental peripheral arterial disease. Basic to Translational Science. 2023 Jun 1;8(6):702-19.

9) Nuzzo, J. L. (2023). Narrative review of sex differences in muscle strength, endurance, activation, size, fiber type, and strength training participation rates, preferences, motivations, injuries, and neuromuscular adaptations. The Journal of Strength & Conditioning Research, 37(2), 494–536.

10) Cui RM, Zheng M, Hong JB, Wang ZX, Cun YF, Gao SJ, Zhu YL, Yang ZB, Liu MW. Research progress on the

effects of macrophage derived exosomes on muscle factors IGF 1 and FGF 2 mediating musculoskeletal crosstalk molecular signaling pathway on bone metabolism (Review). Int J Mol Med. 2026 Mar;57(3):67. doi: 10.3892/ijmm.2026.5738. Epub 2026 Jan 23. PMID: 41574692; PMCID: PMC12851855.

11) Hawley, J.A., Hargreaves, M., Joyner, M.J. & Zierath, J.R. (2021). Integrative biology of exercise. Cell, 184(1), pp.63–77.

12) Damas, F., Libardi, C.A. & Ugrinowitsch, C. (2020). The development of skeletal muscle hypertrophy through resistance training. Sports Medicine, 50(2), pp.199–211.

13) Hackney, A.C. (2020). Stress and the neuroendocrine system: the role of exercise. Endocrine Reviews, 41(3), pp.1–27.

14) Kander, M.C. & Cui, Y. (2020). Sex differences in oxidative stress. Antioxidants, 9(10), 1000.

15) Schoenfeld, B.J. (2020). The mechanisms of muscle hypertrophy. Strength and Conditioning Journal, 42(1), pp.1–10.

16) Chen, X., Wang, Z. & Liu, Y. (2022). Mechanotransduction in bone remodeling. Bone Research, 10(1), pp.1–15.

17) Sabeh H. Comparative investigation of histological distribution of juxtaglomerular apparatus in renal parenchyma between wild (rattus norvegicus) and lab rats (rattus norvegicus). Kerbala Journal of Veterinary Medical Sciences. 2025 Jul 3;1(Supplement I):64-9.