





Analysis of the effects of a continuous magnetic field on the heart and diaphragm of elderly Wistar rats

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ABSTRACT

Introduction and aim. The aim was to investigate the histomorphometric effects of applying a static magnetic field (MF) of different intensities to the diaphragm muscle and heart of aging rats.

Material and methods. Continuous MF of three different intensities were applied for five consecutive days (10 hours in total). Morphological and histological variables in the diaphragm and heart were assessed, including capillary density, cardiomyocyte diameters, histopathological index, and the presence of regenerative changes.

Results. In the diaphragm, there was a significant reduction in the number of capillaries in the treated groups ($p < 0.05$), with no changes in the other morphological variables ($p > 0.05$). In the heart, there were no differences in cardiac mass or in the heart weight/body weight ratio ($p > 0.05$), indicating no macroscopic hypertrophy. However, the intensity of 2500 G led to an increase in the area of the cardiomyocytes and their nuclei ($p < 0.05$), suggesting an adaptive response to the overload. There were no significant changes in the histopathological index or in muscle degeneration characteristics ($p > 0.05$).

Conclusion. Exposure to MF influenced the microcirculation of the diaphragm and promoted cellular changes in the heart, especially at higher intensities, without causing apparent damage to the tissue.

Keywords. diaphragm, heart, magnetic field therapy

Introduction

Living organisms are constantly exposed to the natural magnetic field (MF) present at the Earth's surface, which influences various natural events and animal behavior. With technological advances, artificial magnetic fields have been created through devices for communication, security, and medical equipment.¹ Consequently, human exposure to magnetic fields has increased, highlighting the importance of clarifying their effects on living organisms.²

Recently, the effects of MF on biological tissues have received increasing attention. The biomagnetic energy present in cellular activity, biochemical factors, and bodily fluids can, when exposed to MF, produce therapeutic effects in various tissues. These effects range from treating vascular disorders and musculoskeletal diseases to burn treatment, the prevention of type 2 diabetes mellitus, bone remodeling, and stem cell proliferation.^{3–10}

In magnetotherapy, magnets are applied directly to the site of an injury. This therapy exerts beneficial effects

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on the biological tissues it targets, such as during the wound healing process. The primary objectives of this application are to reduce pain, accelerate healing, and increase scar strength.^{11–13} However, current research seeks to understand not only the therapeutic benefits of magnetic fields but also their potential adverse effects on living organisms. Technological advances and the use of various magnetic sources in therapeutic procedures have led to increasingly complex human exposure to magnetic fields. Despite this, there are few reports on the effects of magnetic fields on the morphology of tissues such as those of the diaphragm and the heart.²

It is known that MFs interact with and affect living tissues. In the case of red blood cells, exposure to an MF causes them to migrate toward the region of highest field intensity. This occurs because blood is a biomagnetic fluid capable of magnetization. The hemoglobin within red blood cells, which contains iron, reacts to magnetic fields.^{14–16} This interaction can increase the attraction of red blood cells to the MF, a phenomenon that could potentially create overload on the heart and respiratory system.¹⁷ Regarding the respiratory system, the diaphragm functions continuously to maintain breathing. With aging, its morphology changes: it becomes flatter, less elastic, exhibits reduced movement amplitude, and produces less protein. These changes weaken the muscle and can lead to a decline in its function.¹⁸ Given these considerations, research aimed at understanding the mechanisms and effects of applying a continuous magnetic field to the diaphragm and cardiac muscle has become highly relevant.

Aim

The aim of this study was to analyze the histomorphometric effects of applying a continuous magnetic field at three different intensities on the diaphragmatic and cardiac muscle in an experimental model using elderly Wistar rats.

Material and methods

This was an experimental study using a quantitative approach. All procedures were approved by the Ethics Committee on Animal Use of the State University of Western Paraná (UNIOESTE), under protocol number 16-21, in compliance with the Arouca Law (Law No. 11,794/2008).

Animals and experimental conditions

Twenty-four Wistar rats, approximately 20 months old and weighing between 218 and 340 g, were obtained from the UNIOESTE Central Animal Facility. The animals were housed in polypropylene cages with *ad libitum* access to food and water, under controlled temperature ($21\pm 1^\circ\text{C}$) and a 12-hour light/dark photoperiod.

Experimental groups

The rats were randomized into four groups (n=6 per group) using the website <https://www.graphpad.com/quickcalcs/randomize1/>, a sample size based on previous studies from our laboratory:¹⁹

- Placebo group (GP): received copper weights (without magnetism) simulating the placebo effect.
- Group 1200 G (1200 G): exposed to a Neodymium magnet with a magnetic induction of 1200 Gauss (0.12T).
- Group 1800 G (1800 G): exposed to 1800 Gauss (0.18T).
- Group 2500 G (2500 G): exposed to 2500 Gauss (0.25T).

Static magnetic field exposure protocol (MF)

Exposure to the MF was administered using magnets affixed to custom-made cotton vests worn on the rats' backs (Fig. 1). The magnets and placebo blocks had identical dimensions (20 mm×10 mm) and similar mass (3–4 g). Animals were exposed individually for 2 hours daily over 5 consecutive days while housed in cages

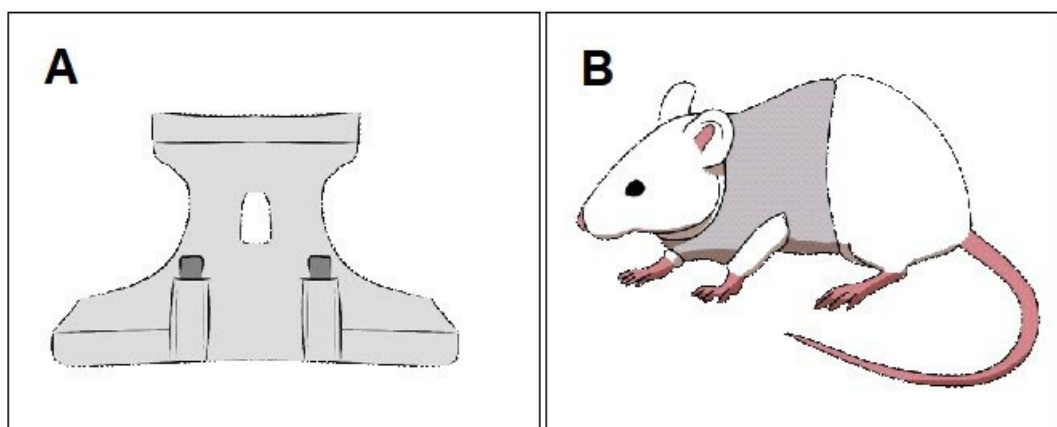


Fig. 1. Static magnetic field (SMF) exposure protocol, A: Experimental vest with view of the inner face, showing the position of the magnets (blue arrows), B: Animal with the experimental vest and magnets on the inner face

without bedding to prevent external interference. This exposure protocol was designed to simulate a therapeutic regimen applicable to humans.

Euthanasia and tissue collection

On the sixth day, the rats were euthanized via intraperitoneal injection of ketamine hydrochloride and xylazine hydrochloride. Following confirmation of the absence of reflexes, the heart and the right costal portion of the diaphragm were collected. The organs were fixed, respectively, in Methacarn (diaphragm) and 10% formalin (heart), then transferred to 70% alcohol and processed for paraffin embedding.

Histological processing and morphometric analysis

The hearts were sectioned (7 μm), stained with Hematoxylin-Eosin (HE) and analyzed in cross-sections. The following were evaluated:

- Sectional area of cardiomyocytes and their nuclei (μm^2);
- Larger and smaller diameters (μm).

For the diaphragm, sections (7 μm thick) were stained with hematoxylin and eosin (H&E) and analyzed across 10 microscopic fields at 40 \times magnification. In these fields, fibers, nuclei, and capillaries were quantified, along with their relative proportions. The assessment also included the presence of central nuclei and alterations such as vacuolated, hypertrophic, and hyper-eosinophilic fibers.

The slides were analyzed using an Olympus BX60 microscope coupled with an Olympus DP71 camera. Images were captured at 400 \times magnification and analyzed with Image-Pro Plus 6.0 software (Media Cybernetics®, USA). For the analysis of fiber and nuclear area, as well as the larger and smaller diameters of fibers, 100 fibers per tissue sample were measured. For all other analyses, 10 microscopic fields were assessed.

Distinct morphometric approaches were applied to cardiac and diaphragmatic tissues due to intrinsic anatomical and structural differences between these muscle types. Cardiac muscle was analyzed primarily through cardiomyocyte cross-sectional area and nuclear measurements in ventricular sections, whereas diaphragmatic analysis included fiber density, capillary quantification, and qualitative histopathological features. These methodological differences reflect established tissue-specific analytical standards rather than inconsistency in experimental design. However, this heterogeneity should be considered when interpreting and comparing tissue-specific responses.

All morphometric measurements were performed by trained evaluators blinded to group allocation using predefined standardized criteria. Each parameter was measured once per predefined field or fiber. No formal intra- or inter-observer reliability analysis was conducted.

Histopathological index

Lesions were classified as mild, moderate, or severe using the index described by Zazula et al.²⁰ which accounts for inflammatory disorders as well as regressive and progressive changes. The total injury score was calculated using the formula $X = a \times w$, where a represents the degree of injury and w its pathological impact. For both the index application and general histological evaluation, the assessors were blinded to the animal's treatment group.

Statistical analysis

For the adopted sample size ($n=6$ per group), assuming an effect size of 0.8 (Cohen's d), $\alpha=0.05$, and four groups, the estimated statistical power was 80%. The assumed effect size was based on previous histomorphometric investigations conducted by our research group using comparable experimental models involving skeletal and cardiac muscle. These prior studies demonstrated moderate to large morphometric differences following biological interventions. No formal pilot study was conducted specifically for the present experimental design. Although the calculation supports adequate power for detecting large effects, the study may not have been sufficiently powered to identify subtle histomorphometric changes, particularly considering the increased biological variability associated with aged animals. Therefore, small effect sizes cannot be excluded.

The data were tabulated in Excel® and analyzed using SPSS (Statistical Package for the Social Sciences, IBM, Armonk, NY, USA) software. Analysis was performed using generalized linear models (GLM), followed by a Least Significant Difference (LSD) post hoc test. A p -value of less than 0.05 ($p<0.05$) was considered statistically significant. Results are expressed as mean \pm standard deviation. The GLM framework was chosen for its robustness and flexibility in accommodating either linear or gamma distributions, depending on the characteristics of the data.

Results

Body mass of the animals

Animal body weights were recorded twice during the experiment: first as a baseline measurement (EV1) one day prior to the initiation of MF exposure, and again immediately before euthanasia (EV2). No significant differences in body weight were observed between the four groups at either time point (Table 1).

Table 1. Body weight data (b), heart weight (h) and b/h ratio

		GP	1200 G	1800 G	2500 G
Body weight (g)	EV1	291 \pm 26.6	275 \pm 18.0	292 \pm 34.3	290 \pm 24.5
	EV2	279 \pm 25.0	265 \pm 18.1	275 \pm 35.5	278 \pm 22.8
Heart weight (g)		1.256 \pm 0.051	1.194 \pm 0.045	1.315 \pm 0.050	1.258 \pm 0.047
Relationship b/h (%)		0.45 \pm 0.01	0.45 \pm 0.01	0.48 \pm 0.01	0.45 \pm 0.01

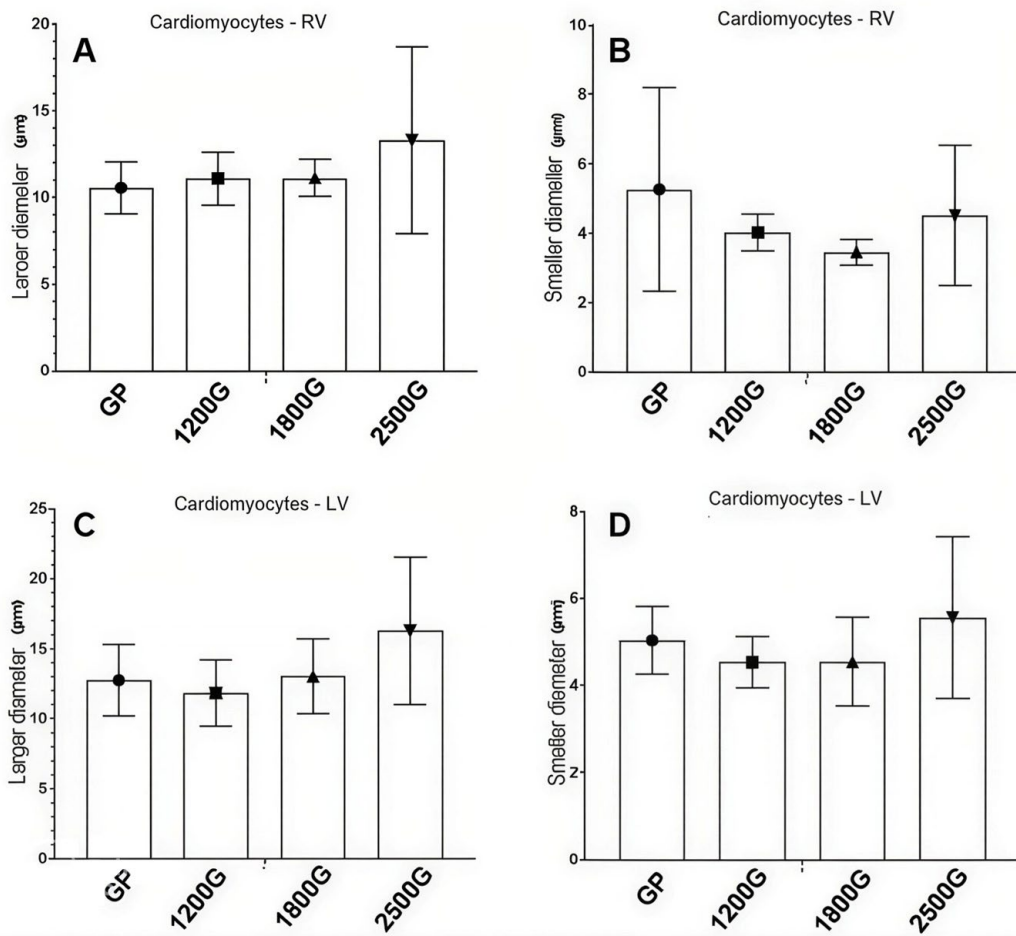


Fig 2. Histomorphometric analysis of Wistar rats exposed to different magnetic field intensities, A: major diameter of the right ventricle (RV) cardiomyocytes, B: minor diameter of the RV cardiomyocytes, C: major diameter of the left ventricle (LV), and D: minor diameter of the LV, placebo group (GP), exposure to 1200 Gauss magnet (1200 G), exposure to 1800 Gauss magnet (1800 G) and exposure to 2500 Gauss magnet (2500 G), values expressed as mean±standard deviation of the mean ($p>0.05$)

Cardiomyocyte diameter

No significant differences were observed between the groups regarding the maximum and minimum diameters of cardiomyocytes in the right ventricle (RV) (Fig. 2A, B) and the left ventricle (LV) (Fig. 2C, D) ($p>0.05$).

Cardiomyocyte area

A significant difference in right ventricular (RV) cardiomyocyte area was observed between the groups (GP, 1200 G, 1800 G, and 2500 G; $p=0.015$). Specifically, the 2500 G group exhibited a larger cardiomyocyte area compared to the GP, 1200 G, and 1800 G groups (Fig. 3A).

In the left ventricle (LV), a significant difference was also found between groups ($p=0.021$). Post-hoc analysis revealed that the 1800 G and 2500 G groups exhibited larger cardiomyocyte diameters compared to the GP and 1200 G groups ($p<0.05$; Fig. 3B).

Area of cardiomyocyte nuclei

The area of right ventricular (RV) cardiomyocyte nuclei differed significantly between groups ($p=0.016$). Post-

hoc comparisons indicated that the 2500 G group exhibited a larger nuclear area than both the GP group ($p=0.003$) and the 1800 G group ($p=0.030$) (Fig. 4A).

A significant difference was also observed in the area of left ventricular (LV) cardiomyocyte nuclei between groups ($p=0.001$). The 2500 G group exhibited a larger nuclear area compared to all other groups (Fig. 4B). In summary, exposure to the 2500 G magnetic field was associated with an increase in nuclear area for cardiomyocytes in both the right and left ventricles.

Diaphragm – histological analysis

In the histomorphometric analysis of the diaphragm muscle, the GP (placebo group) exhibited muscle fibers with preserved morphology. These fibers maintained a polygonal shape, were arranged in a fascicular pattern, and were multinucleated with peripherally located nuclei (Fig. 5A).

In the 1200 G, 1800 G, and 2500 G groups, various morphological alterations were observed in the diaphragm muscle. These included amorphous, hypertrophic, and hyper eosinophilic fibers; fibers with cyto-

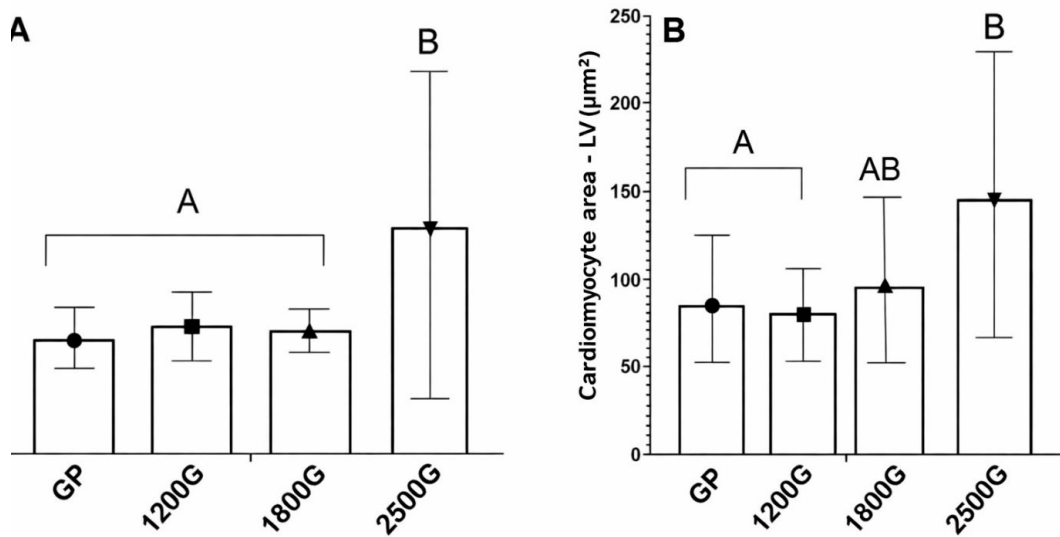


Fig. 3. Histomorphometric analysis of rat cardiomyocytes exposed to different magnetic field intensities, A: Right ventricle (RV) cardiomyocyte area, and B: left ventricle (LV) cardiomyocyte area, results expressed as mean standard deviation, placebo group (PG), exposure to 1200 Gauss magnet (1200 G), exposure to 1800 Gauss magnet (1800 G) and exposure to 2500 Gauss magnet (2500 G) ($p < 0.05$), different capital letters indicate significant differences between the groups exposed to the static magnetic field ($p < 0.05$)

plasmic vacuolization; fibers with altered sarcoplasmic staining; rounded fibers with differential staining; and fibers containing central nuclei (Fig. 5B–D).

Table 2. Histomorphometric measurements of the diaphragm muscle*

Parameters	GP	1200 G	1800 G	2500 G
Fiber density	546.50±88.71 ^A	515.00±40.53 ^A	509.17±99.57 ^A	524.67±55.31 ^A
Number of peripherals nuclei	1302.33±229.86 ^A	1324.67±166.08 ^A	1306.17±276.34 ^A	1433.67±181.52 ^A
Nuclei/fiber ratio	2.40±0.38 ^A	2.57±0.24 ^A	2.57±0.33 ^A	2.74±0.32 ^A
Total of capillaries	174.08±32.51 ^A	132.42±34.71 ^B	127.17±26.08 ^B	137.42±29.86 ^B
Relationship capillary/fiber	0.32±0.06 ^A	0.26±0.07 ^A	0.26±0.06 ^A	0.26±0.06 ^A
Hypertrophic and hyper eosinophilic muscle fiber	7.17±5.42 ^A	5.50±2.74 ^A	5.17±2.32 ^A	3.50±2.88 ^A
Muscle fiber with vacuolized cytoplasm	4.00±2.37 ^A	5.67±4.72 ^A	7.00±5.97 ^A	8.50±4.42 ^A
Muscle fiber with sarcoplasm with dye difference	27.17±25.81 ^A	22.33±9.85 ^A	13.17±5.42 ^A	13.67±6.59 ^A
Muscle fiber with central core	8.17±4.36 ^A	6.67±3.83 ^A	8.67±3.27 ^A	5.67±2.42 ^A
Histomorphometric index	11.33±1.12 ^A	9.83±0.98 ^A	9.17±0.91 ^A	9.33±0.93 ^A

* identical letters indicate statistical similarities

Histological assessment further revealed vascular congestion across all experimental groups. Additionally, significant disorganization and thickening of the perimysial connective tissue were observed in all groups examined (Fig. 5A–D).

Histomorphometric analysis of diaphragm muscle fibers revealed no significant differences between the four groups in muscle fiber density, number of peripheral nuclei, the nucleus-to-fiber ratio, or the capillary-to-fiber ratio (Table 2). However, regarding the total capillary count, significant reductions were observed in the exposed groups compared to the GP (placebo) group: 24% for 1200 G ($p < 0.011$), 27% for 1800 G ($p < 0.004$), and 22% for 2500 G ($p < 0.025$). In the analysis of the histopathological index, despite variations in individual scores, there was no statistically significant difference between the groups, a finding consistent with the histomorphometric data.

Discussion

This study evaluated the histomorphometric effects of different intensities of a static MF on the diaphragm and heart in female rats. The main finding was a significant reduction in the total number of diaphragmatic capillaries in all treated groups, with no changes observed in the other measured variables. In cardiac tissue, both the absolute heart mass and the heart-to-body weight ratio remained unchanged, suggesting that exposure for five days (10 hours total) did not induce macroscopic cardiac hypertrophy.²¹

The literature indicates that MF can modulate microvascular tone and skin blood flow,^{3,22} enhancing circulation without necessarily triggering angiogenesis. This effect may explain the absence of increased capillary density or size in the diaphragm observed in this study. Furthermore, the interaction between the iron in

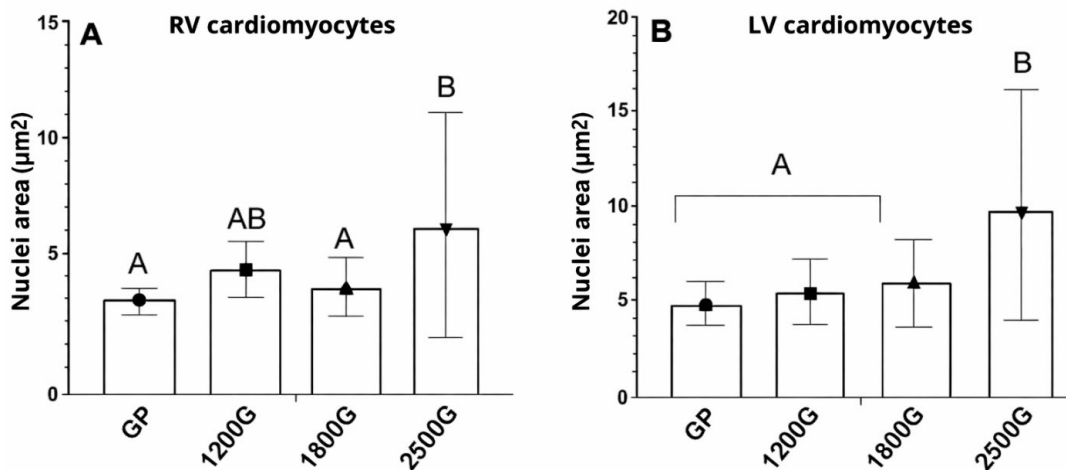


Fig. 4. Histomorphometric analysis of the cardiomyocytes of female rats exposed to different magnetic field intensities, A: Area of the nuclei of the right ventricle (RV), and B: area of the nuclei of the left ventricle (LV), placebo (GP), exposure to 1200 Gauss magnet, exposure to 1800 Gauss magnet and exposure to 2500 Gauss magnet, different capital letters indicate significant differences between the groups exposed to the static magnetic field ($p < 0.05$)

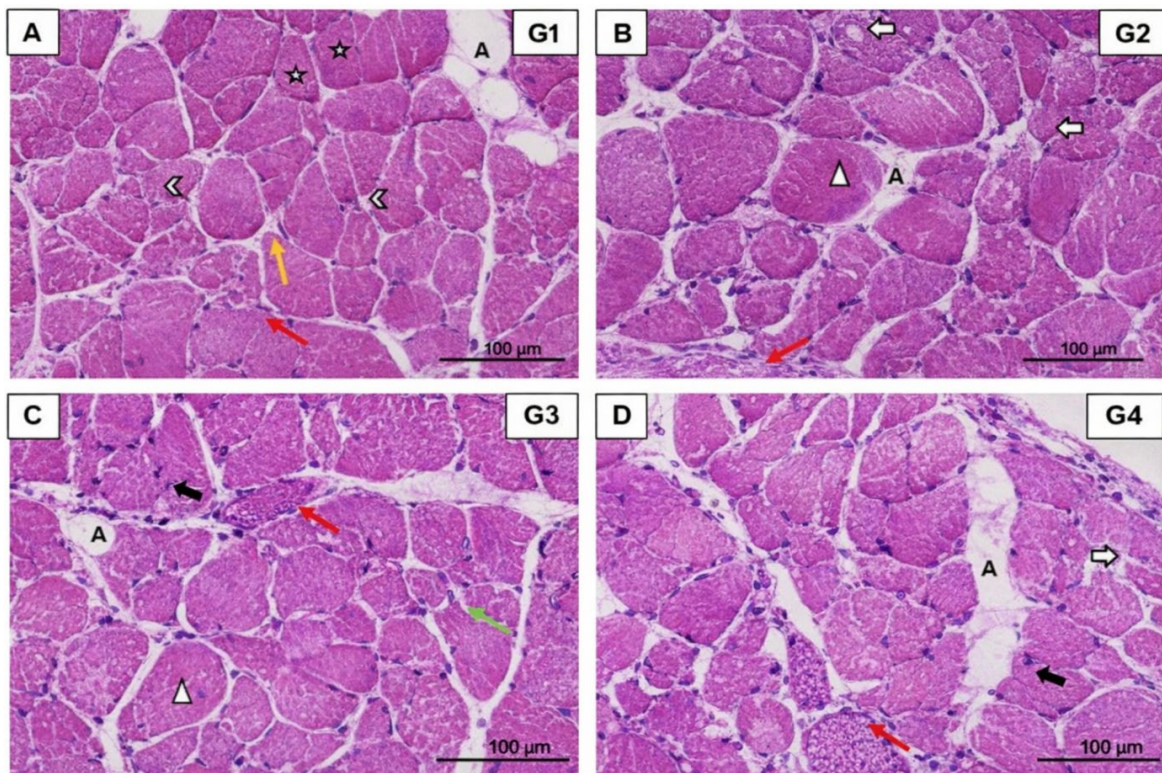


Fig. 5. Cross-sectional photomicrographs of the diaphragm muscle of Wistar rats stained with HE, A: control group (GP), with polygonal-shaped fibres (☆), peripheral nucleus (yellow arrow), disorganization and thickening of the connective tissue of the perimysium (letter A), blood capillaries (♣), congested blood vessels (red arrow), B: the lower intensity magnet exposure group (1200 G), with hypertrophic and hypereosinophilic muscle fibres (△), muscle fibers with vacuolized cytoplasm (white arrow), disorganized and enlarged connective tissue in the perimysium (letter A), congested blood vessels (red arrow), C: the medium-intensity magnet exposure group (1800 G), with hypertrophic and hypereosinophilic muscle fibers (△), disorganized and enlarged connective tissue in the perimysium (letter A), congested blood vessels (red arrow), muscle fibers with a central nucleus (black arrow), muscle fibers with a nucleus with a basophilic halo (green arrow), D: the higher intensity magnet exposure group (2500 G), with muscle fibers with vacuolized cytoplasm (white arrow), disorganized and enlarged connective tissue in the perimysium (letter A), congested blood vessels (red arrow), muscle fibers with a central nucleus (black arrow) and rounded muscle fibers with a dye difference (*)

hemoglobin and the applied MF may reduce blood viscosity.²³ This reduction could promote a more laminar flow, potentially contributing to the vascular congestion noted in the treated groups.

Morris and Skalak³ demonstrated that even short-term exposure to a MF can reduce edema in rats, supporting the hypothesis that brief applications can modulate microcirculation. This finding underscores the need for further research to determine the optimal exposure duration required to maximize the vascular response.

Although no significant differences were observed in global histomorphometric parameters of the diaphragm muscle, animals exposed to magnetic fields exhibited discrete morphological alterations, including hypertrophic and hyper eosinophilic fibers, cytoplasmic vacuolization, sarcoplasmic staining heterogeneity and centrally located nuclei, suggesting mild cellular stress and early myofiber remodeling without structural impairment. The diaphragm's high metabolic activity and continuous contractile function may favor compensatory and regenerative mechanisms that preserve overall muscle architecture despite focal alterations. Additionally, the significant reduction in the total number of capillaries in exposed groups, in the absence of changes in the capillary-to-fiber ratio, suggests microvascular remodeling rather than functional impairment. The presence of congested vessels and perimysial connective tissue thickening in all groups, including controls, indicates that these findings may reflect intrinsic characteristics of the diaphragm or technical factors related to tissue processing. Overall, the data indicate that magnetic field exposure induces subtle, non-progressive morphological and vascular changes consistent with an adaptive response rather than overt muscle pathology. The reduction in total capillary number without significant changes in the capillary-to-fiber ratio represents an apparent discrepancy. This finding may reflect sampling variability, microscopic field selection bias, or subtle microvascular rearrangement rather than true capillary rarefaction. Because endothelial markers, angiogenic signaling pathways, and functional perfusion measurements were not assessed, it is not possible to determine whether this represents structural remodeling, transient vascular redistribution, or methodological variability. Consequently, this interpretation must be considered speculative.

Exposure to the 2500 G magnetic field resulted in an increase in cardiomyocyte and nuclear area. This finding may indicate a structural alteration compatible with cellular remodeling; however, in the absence of functional (e.g., echocardiographic or hemodynamic) or molecular assessments, this interpretation remains speculative. Therefore, the present data should be regarded as descriptive morphometric observations rather than ev-

idence of adaptive or pathological remodeling.²⁴ This aligns with the work of Tasić et al.,²⁵ who reported arterial and cardiac hypertrophy following 30 days of exposure to a 16 mT field. Variations in results across studies can be attributed to differences in exposure protocols, field characteristics, and experimental models.

The reported effects of MF in the literature can vary. For instance, Goraca et al.²⁶ observed the induction of oxidative stress following exposure to dynamic magnetic fields, whereas Kimsa-Dudek et al.²⁷ identified a reduction in oxidative stress within fibroblasts. Such contrasting results underscore that the biological effects of MFs are highly dependent on the cell type studied and the specific experimental context, including the field's characteristics.

The use of aging female rats in this study represents a sample characteristic that may have influenced the observed results. The literature identifies aging, particularly the climacteric period, as a significant risk factor for cardiac dysfunction.^{28,29} Furthermore, documented morphological differences between the hearts of elderly males and females^{25,30,31} offer a plausible explanation for the divergence between the findings of this study and those from research conducted on young male subjects. And diets rich in protein and carbohydrates can cause vascular wall disorders and thus increase blood pressure in elderly rats.³²

From a clinical perspective, the observed increase in cardiomyocyte area may reflect adaptive responses to stress. This finding could be particularly relevant for populations subject to chronic MF exposure, such as industrial workers and frequent users of electronic devices.^{33,34} But, this interpretation is speculative, due to the lack of functional measures such as electrocardiography. The present findings are restricted to an experimental model involving short-term static magnetic field exposure in aged female rats. Extrapolation to human exposure scenarios should be avoided, as differences in exposure duration, magnetic field characteristics, and biological context may substantially influence outcomes.

Study limitations

This study presents several limitations. First, the relatively small sample size may have limited the ability to detect subtle histomorphometric differences, particularly in aged animals characterized by increased biological variability. Second, the short exposure duration (five consecutive days) restricts interpretation regarding chronic magnetic field effects. Third, the absence of functional cardiac or respiratory assessments prevents correlation between structural findings and physiological performance. Fourth, no molecular or biochemical analyses were performed, limiting mechanistic interpretation. Fifth, intra- and inter-observer reproducibility

analyses were not conducted, which may affect measurement robustness. Finally, the exclusive use of aged female animals restricts generalizability to other age groups and to males. These limitations require cautious interpretation of the findings strictly within the boundaries of the experimental model.

Conclusion

Exposure to a static magnetic field induced discrete histomorphometric alterations in diaphragmatic microvasculature and cardiomyocyte structure in aged female rats. However, given the absence of functional and molecular assessments, these findings should be interpreted as descriptive structural observations rather than evidence of adaptive or pathological remodeling. Further studies incorporating larger samples, longer exposure periods, and multimodal assessment are necessary to clarify the biological significance of these changes.

Declarations

Funding

There was no external financing.

Author contributions

Conceptualization, G.C.K., V.G.S., L.T.L., M.M.T., L.F.C.R. and G.R.F.B.; Methodology, M.M.T., L.F.C.R. and G.R.F.B.; Formal Analysis, M.M.T., L.F.C.R. and G.R.F.B.; Investigation, G.C.K., V.G.S. and L.T.L.; Resources, M.M.T., L.F.C.R. and G.R.F.B.; Data Curation, M.M.T., L.F.C.R. and G.R.F.B.; Writing – Original Draft Preparation, G.C.K. and V.G.S.; Writing – Review & Editing, L.T.L., M.M.T., L.F.C.R. and G.R.F.B.; Visualization, M.M.T.; Supervision, L.F.C.R.; Project Administration, G.R.F.B.; Funding Acquisition, L.T.L., M.M.T., L.F.C.R. and G.R.F.B.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The research project was approved by the Animal Use Ethics Committee of the State University of Western Paraná. (protocol 16-21).

Use of AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used DeepL and DeepSeek in order to assist in translating the text into English.

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