Short Communication

Effect of aerobic exercise combined with anti-PD-L1 antibody injection on body weight and heart weight of breast cancer-bearing mice: Management in cancer cachexia

Amir Hossein Ahmadi Hekmatikar[1], Hamid Agha-Alinejad[1*], Aliakbar Yousefi-Ahmadipour[2,3,4,5], Mahdieh Molanouri Shamsi[1,6]

Abstract

Cancer is the leading cause of death worldwide, with breast cancer posing a high risk for women. Immunotherapy has shown efficacy, and exercise is recognized for its role in cancer management. Combining both may enhance therapeutic outcomes. This study examined 30 female BALB/c mice (average weight: 17.76g), divided into five groups (n=6 each). After treadmill acclimation, they underwent two 6-week training protocols and a 4-week protocol post-cancer induction. Data analysis was performed using one-way ANOVA. The findings revealed significant differences in body weight among the EIEA and EIA groups compared to the control group. Similarly, in the heart weight analysis, both EIEA and EIA groups showed significant differences compared to the control ($p<0.05$). Notably, the combination of exercise and anti-PD-L1 antibody treatment effectively prevented weight loss in both body mass and heart weight. This protective effect may be attributed to the mitigation of cachexia, a common complication in cancer that leads to severe weight loss and muscle wasting. These results suggest that integrating physical activity with immunotherapy could serve as a potential strategy to counteract cancer-induced weight deterioration.

Key Words: **Physical activity, Anti-PD-L1 antibody, Body weight, Heart weight, Breast cancer**

Introduction

Complex multicellular organisms consist of billions of specialized cells, each performing distinct functions. Throughout their lifespan, these cells are exposed to various factors that may induce genetic mutations (Vincze et al., 2022). While many mutations have no significant impact, some allow cells to bypass cell cycle regulation, leading to uncontrolled proliferation—a hallmark of cancer (Novikov et al., 2021). Cancer is a multifaceted biological process that varies based on cell type and tumor location, yet all cancers share fundamental mechanisms of initiation and progression (Hönigova et al., 2022). Preventing cancer development is essential, regardless of its underlying causes. One of the most critical aspects of cancer biology is tumor behavior, which is largely influenced by tumor morphology (Almagro et al., 2022). Malignant tumors pose severe health risks, making treatment highly challenging (Almagro et al., 2022). As cancer advances, tumor cells often metastasize by detaching from the primary tumor and infiltrating surrounding tissues (Steeg, 2006; Sundling & Lowe, 2019; Zetter, 1998). This occurs after tumor formation, where cancer cells manipulate both cellular and non-cellular components through intricate signaling pathways, leveraging non-malignant cells to their advantage (Hanahan & Coussens, 2012). Several factors contribute to tumor progression and metastasis, with lactate playing a central role (Liu et al., 2022).

Immunotherapy primarily targets immune checkpoint regulators, particularly PD-1 and PD-L1. Within the tumor microenvironment, these molecules play key roles in tumor survival by evading immune surveillance. PD-1 is expressed on various immune cells, including T cells, B cells, monocytes, dendritic cells, and tumor-infiltrating lymphocytes. In the cancer immunity cycle, PD-1 and its ligand PD-L1 collaborate to help tumors resist immune-mediated apoptosis, thereby driving tumor progression. Interestingly, research suggests that administering an anti-PD-L1 antibody can trigger apoptotic responses in tumors and extend survival in mice with breast cancer (Kitagawa et al., 2020; Lei et al., 2020).The field of exercise immunology has long suggested that physical activity

1. Department of Sport Science, Faculty of Humanities, Tarbiat Modares University, Tehran 14117-13116, Iran. 2. Immunology of Infectious Diseases Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. 3. Molecular Medicine Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. 4. Depatment of Laboratory Sciences, Faculty of Paramedicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. 5. Cancer and Stem Cell Research Laboratory, Faculty of Paramedicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. 6. Department of Physical Education & Sport Sciences, Yazd University, Yazd, Iran.

*Author for correspondence: halinejad@modares.ac.ir

AH AH: 0009-0001-3331-8272; H AA: 0000-0003-3608-822X; A YA: 0000-0001-7007-2518; M MS: 0000-0003-4665-5363

Received 4 September 2024; Accepted 24 December 2024

(PE) can enhance immune function safely and effectively (Nieman, 2024). Notably, PE has been linked to improvements in adaptive immunity, particularly in T cell function (Nieman, 2024; Simpson et al., 2024).

Several studies indicate that exercise-induced immunological changes may promote apoptotic processes within tumors (Fortner et al., 2023; Friedenreich et al., 2021). Furthermore, research suggests that physical activity can prolong the survival of mice with breast cancer (Cannioto et al., 2021; Friedenreich et al., 2021; Wang & Zhou, 2021). While numerous studies have explored the independent effects of anti-PD-L1 antibody administration and physical activity on tumor dynamics and overall health, no research has yet examined their combined influence. To address this gap, the present study investigates the simultaneous effects of anti-PD-L1 antibody treatment and physical activity on tumor weight and survival outcomes in mice with breast cancer.

Materials and Methods

The Ethics Committee in Biomedical Research of Tarbiat Modares University of Tehran (IR.MODARES.AEC.1403.023) approved this study. All animal procedures were conducted in compliance with the guidelines for the care and use of laboratory animals and ethical principles in animal research as endorsed by the Iranian Council for the Control of Animal Experiments. This study adheres to the ARRIVE guidelines.

Animals

Thirty female mice, weighing between 18-20 grams and approximately at 4 weeks of age, were purchased from the mouse-breeding center of Rafsanjan University of Medical Sciences. They were housed in special Plexiglas cages under controlled environmental conditions, with an average temperature of 22 ± 1.4 °C, humidity of 50 ± 4%, and a 12-hour light-dark cycle. The mice had free access to the laboratory animal food and water. At the beginning of the training program, the mice were familiarized with the treadmill for adaptation. Five cancer-afflicted mice and five healthy mice warmed up for 10 minutes and began training at a speed of 6 m/min. The mice were habituated to the treadmill for four consecutive days before treadmill performance testing. The adaptation period involved 5 minutes of running at a speed of 6 m/min, followed by 5 minutes at speeds ranging from 6 to 12 m/min. For the exercise test, the mice warmed up for 3 minutes at a speed of 6 m/min. Then, they trained at a speed of 3 m/min for 3 minutes with no incline, and every 3 minutes, the treadmill speed increased by 3 m/min until the mice reached fatigue (Conner et al., 2014; Ferreira et al., 2007; Shamsi et al., 2017; Tobias et al., 2023). Finally, the exercise-training program was formulated based on the maximum running speed achieved by the mice.

Cell line

The 4T1 mouse breast cancer cell line was cultured in RPMI1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin (PenStrep) under conditions of 80% humidity, 5% CO2, and a temperature of 37°C, all maintained under sterile conditions. After incubation, the culture medium was renewed every 48 hours, continuing this process three times until approximately 70% of the cell culture plate was populated with cancer cells. Then, using 5% trypsin enzyme and rapid pipetting, the cells were detached. The harvested cells were washed with RPMI1640 medium, creating a suspension with a density of one million cells per milliliter. After trypsinization and washing with PBS buffer, the cells were counted, and dilutions of 510^5 to 110^6 cells were prepared. Finally, to induce cancer, an injection was administered near the breast area.

Protocol 1 (exercise before cancer induction)

All groups, except for the control group, underwent training for 60 minutes per session, 5 sessions per week, during the first 6 weeks without the induction of cancer or administration of antibodies. The mice were trained on a 5-lane treadmill.

Protocol 2 (exercise after cancer induction) + Anti-PDL-1 injection

After 6 weeks, the mice were injected subcutaneously with the 4T1 cell line in the upper right thigh area. Two weeks after the cell line injection, the tumor was palpable at the injection site. Upon tumor appearance in the first week, the mice were randomly assigned into the following groups: PCG (N=6), EIC (N=6), EIE (N=6), EIA (N=6), and EIE+A (N=6). The mice in the exercise groups trained 3 times a week, performing 3 sets of 10 minutes each, with 2 minutes and 30 seconds of active rest between sets (Jafari et al., 2021; Shamsi et al., 2017; Tobias et al., 2023) The exercise intensity of the rats was 60% of the maximum intensity they achieved (Jafari et al., 2021; Shamsi et al., 2017; Tobias et al., 2023). In the entire length of the research, the incline of the treadmill was zero. The patient control group did not engage in any physical activity nor receive antibodies after cancer induction. Anti-PDL-1 antibody was obtained in EIA and EIE+A groups according to Table 1. Antibody injection was performed in the tail.

Measurement of body weight and heart weight of mice

To accurately monitor the mice's body weight, daily measurements were taken and systematically recorded throughout the study. Additionally, following the surgical procedure, the heart weight of each mouse was carefully measured and documented to ensure precise data collection.

Table 1. Training protocol and injection dose of anti-PDL-1 antibody

Protocol	First week	Second week	Third week	fourth week		The first two weeks	Second first two weeks
Treadmill speed	14	16	18	18		14	16
Duration	60 minutes	60 minutes	60 minutes	60 minutes	Cancer induction	3 sets of 10 minutes	3 sets of 10 minutes
Number of sessions per week	5	5	5	5		3	3
The amount of antibody injection						10 micrograms	10 micrograms

To analyze the data, descriptive statistics were used to calculate descriptive indices, including mean and standard deviation. All statistical procedures were performed using IBM SPSS Statistics version 24 software at a significant level of P<0.05. The Kolmogorov-Smirnov test was used to check the normality of data distribution. Additionally, a one-way analysis of variance (ANOVA) was used to measure the research variables. Bonferroni post hoc test was also used for post-hoc measurements.

Results

The results of the present study in the section of changes in the weight of mice showed that there was a significant difference in the EIEA and EIA groups compared to the control group. Therefore, the results of the present study showed that the weight of the mice in these two groups decreased less than in the other groups (P<0.05) (Figure 1).

The results of the present study in the section on changes in the heart weight of mice showed that a significant difference was found in the EIEA and EIA groups compared to the control group. Therefore, the results of the present study showed that the heart weight of mice in these two groups decreased less than in the other groups. (P<0.05) (Figure 2).

Discussion

The primary aim of the present study was to investigate the effects of exercise and anti-PD-L1 antibody injection on body weight and heart weight in mice. The findings demonstrated a significant difference in body weight and heart weight in the EIEA and EIA groups compared to the control group. Specifically, the combination of exercise and anti-PD-L1 antibody administration effectively prevented weight loss in both body and heart mass. This suggests that these interventions may play a protective role against the decline in physiological parameters associated with

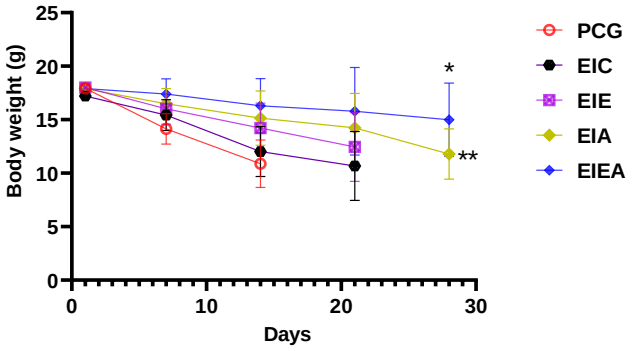

Figure 1. Weight changes of mice in all groups. Data are presented as mean ± standard deviation (SD).

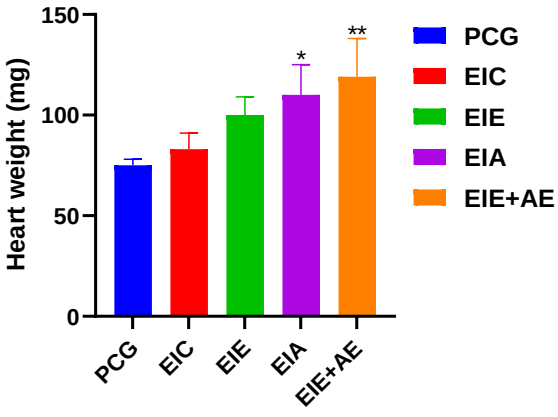

Figure 2. Changes in heart weight of mice in all groups. Data are presented as mean ± standard deviation (SD).

Table 2. Post-hoc test in the Body weight change group.

	groups	P	Effect size
PCG	EICs	P=0.991	1.118
	EIE	P=0.148	2.132
	EIA	P=0.001	3.738
	EIE+AE	P=0.001	6.231

Table 3. Post-hoc test in the Body weight change group

	groups	P	Effect size
PCG	EIC	P=0.169	3.491
	EIE	P=0.098	4.982
	EIA	P=0.001	5.221
	EIE+AE	P=0.001	6.162

disease progression. However, in the other experimental groups, significant weight reduction was observed, which appears to be linked to cachexia, a common pathological condition in cancer progression (Berriel Diaz et al., 2024; Ilonze et al., 2024; Liu et al., 2024).

Cachexia is a multifactorial syndrome characterized by severe weight loss, muscle wasting, and metabolic disturbances, often observed in chronic diseases such as cancer (Berriel Diaz et al., 2024; Ilonze et al., 2024; Liu et al., 2024). One of the key features of cachexia is the loss of both skeletal muscle and cardiac muscle mass, which can severely impact overall health and survival (Berriel Diaz et al., 2024; Ilonze et al., 2024; Liu et al., 2024). The present study supports this notion, as untreated mice exhibited substantial reductions in body and heart weight, indicative of cachexia-induced wasting (Berriel Diaz et al., 2024; Ilonze et al., 2024; Liu et al., 2024). However, the results also revealed that exercise combined with anti-PD-L1 antibody treatment successfully mitigated these effects (Ahmadi Hekmatikar et al., 2023; Rami et al., 2023; Tayebi et al., 2020). This suggests that these interventions may help counteract the metabolic imbalances that contribute to cachexia, potentially improving the overall health status of affected individuals.

There were also limitations in the current study. These limitations included the lack of measurement of variables related to muscle cachexia and other molecular analyses. Therefore, it is recommended that in future studies, researchers investigate information on mechanisms (e.g., muscle atrophy markers, inflammatory cytokines, and immune cell infiltration) and other molecular analyses (e.g., IL-6, TNF-α, myostatin).

Conclusion

In conclusion, the findings of this study highlight the beneficial effects of exercise and anti-PD-L1 antibody administration in preventing body and heart weight loss in cancer-afflicted mice. The observed protective impact against cachexia-induced weight reduction suggests that these interventions may have therapeutic potential in preserving physiological function during disease progression. Future research should further explore the underlying mechanisms responsible for these effects and evaluate the long-term benefits of such combined treatments in clinical settings.

What is already known on this subject?

Cancer is the leading cause of death worldwide, with breast cancer posing a high risk for women.

What this study adds?

The observed protective impact against cachexia-induced weight reduction suggests that these interventions may have therapeutic potential in preserving physiological function during disease progression.

Organ Cross-Talk Tips:

- The administration of anti-PD-L1 antibodies in cancer treatment involves organ crosstalk by modulating the tumor microenvironment, affecting immune responses and potentially influencing distant tumor sites through systemic communication mechanisms.

Acknowledgements

The support of the Shahid Sattari (NICICO) Collection for this study is gratefully acknowledged.

Funding

None.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Biomedical Research of Tarbiat Modares University of Tehran (IR.MODARES.AEC.1403.023).

Informed consent Animal study

Author contributions

Conceptualization: AH.AH.; Methodology: H.AA.; Software: H.AA.; Validation: A.YA.; Formal analysis: A.YA.; Investigation: M.MS.; Resources: M.MS.; Data curation: M.MS.; Writing - original draft: AH.AH.; Writing – review & editing: AH.AH.; Visualization: M.MS.; Supervision: H.AA. Project administration: M.MS.; Funding acquisition: H.AA.

References

Ahmadi Hekmatikar, A., Nelson, A., & Petersen, A. (2023). Highlighting the idea of exerkines in the management of cancer patients with cachexia: novel insights and a critical review. BMC Cancer. 2023 Sep 20;23(1):889. doi: https://doi.org/10.1186/s12885-023-11391-3.

Almagro, J., Messal, H. A., Elosegui-Artola, A., van Rheenen, J., & Behrens, A. (2022). Tissue architecture in tumor initiation and progression. Trends Cancer. 2022 Jun;8(6):494-505. doi: https://doi.org/10.1016/j.trecan.2022.02.007

Berriel Diaz, M., Rohm, M., & Herzig, S. (2024). Nat Metab. 2024 Dec;6(12):2222-2245. doi: https://doi.org/10.1038/s42255-024-01167-9.

Cannioto, R. A., Hutson, A., Dighe, S., McCann, W., McCann, S. E., Zirpoli, G. R., . . . Hershman, D. L. (2021). Physical activity before, during, and after chemotherapy for high-risk breast cancer: relationships with survival. JNCI: J Natl Cancer Inst. 2021 Jan 4;113(1):54-63. doi: https://doi.org/10.1093/jnci/djaa046.

Conner, J. D., Wolden-Hanson, T., & Quinn, L. S. (2014). Assessment of murine exercise endurance without the use of a shock grid: an alternative to forced exercise. J Vis Exp(90), e51846. https://doi.org/10.3791/51846

Ferreira, J. C., Rolim, N. P., Bartholomeu, J. B., Gobatto, C. A., Kokubun, E., & Brum, P. C. (2007). Maximal lactate steady state in running mice: effect of exercise training. Clin Exp Pharmacol Physiol. 2007 Aug;34(8):760-5. doi: https://doi.org/10.1111/j.1440-1681.2007.04635.x.

Fortner, R. T., Brantley, K. D., Tworoger, S. S., Tamimi, R. M., Rosner, B., Farvid, M. S., . . . Eliassen, A. H. (2023). Physical activity and breast cancer survival: results from the Nurses' Health Studies. Breast Cancer Res. 2018 Oct 19;20(1):124. doi: https://doi.org/10.1186/s13058-018-1051-6.

Friedenreich, C. M., Ryder‐Burbidge, C., & McNeil, J. (2021). Physical activity, obesity and sedentary behavior in cancer etiology: epidemiologic evidence and biologic mechanisms. Breast Cancer Res. 2018 Oct 19;20(1):124. doi: 10.1186/s13058-018-1051-6. PMID: 30340503; PMCID: PMC6194749. Hanahan, D., & Coussens, L. M. (2012). Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer cell, 21(3), 309-322. doi: https://doi.org/10.1186/s13058-018-1051-6.

Hönigova, K., Navratil, J., Peltanova, B., Polanska, H. H., Raudenska, M., & Masarik, M. (2022). Metabolic tricks of cancer cells. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 188705.

Ilonze, O. J., Parsly Read-Button, L., Cogswell, R., Hackman, A., Breathett, K., Saltzman, E., & Vest, A. R. (2024). Controversies and conundrums in cardiac cachexia: key questions about wasting in patients with HFrEF. Heart Failure, 12(10), 1645-1660. doi: https://doi.org/10.1186/s13058-018-1051-6.

Jafari, A., Sheikholeslami-Vatani, D., Khosrobakhsh, F., & Khaledi, N. (2021). Synergistic effects of exercise training and vitamin D supplementation on mitochondrial function of cardiac tissue, antioxidant capacity, and tumor growth in breast cancer in bearing-4T1 mice. Frontiers in Physiology, 12, 640237. doi: https://doi.org/10.1186/s13058-018-1051-6.

Kitagawa, S., Hakozaki, T., Kitadai, R., & Hosomi, Y. (2020). Switching administration of anti‐PD‐1 and anti‐PD‐L1 antibodies as immune checkpoint inhibitor rechallenge in individuals with advanced non‐small cell lung cancer: case series and literature review. Thoracic Cancer, 11(7), 1927-1933. doi: https://doi.org/10.1111/1759-7714.13483.

Lei, Q., Wang, D., Sun, K., Wang, L., & Zhang, Y. (2020). Resistance mechanisms of anti-PD1/PDL1 therapy in solid tumors. Frontiers in cell and developmental biology, 8, 672.

Liu, M., Ren, Y., Zhou, Z., Yang, J., Shi, X., Cai, Y., . . . Xu, C. (2024). The crosstalk between macrophages and cancer cells potentiates pancreatic cancer cachexia. Cancer cell, 42(5), 885-903. e884.

Liu, S., Zhao, H., Hu, Y., Yan, C., Mi, Y., Li, X., . . . Qin, J. (2022). Lactate promotes metastasis of normoxic colorectal cancer stem cells through PGC-1α-mediated oxidative phosphorylation. Cell death & disease, 13(7), 651. doi: https://doi.org/10.1111/1759-7714.13483.

Nieman, D. C. (2024). Exercise immunology: Novel insights. Journal of Sport and Health Science, 13(3), 277.

Novikov, N. M., Zolotaryova, S. Y., Gautreau, A. M., & Denisov, E. V. (2021). Mutational drivers of cancer cell migration and invasion. British journal of cancer, 124(1), 102-114.

Rami, M., Rahdar, S., Ahmadi Hekmatikar, A., & Awang Daud, D. M. (2023). Highlighting the novel effects of high-intensity interval training on some histopathological and molecular indices in the heart of type 2 diabetic rats. Frontiers in Endocrinology, 14, 1175585.

Shamsi, M. M., Chekachak, S., Soudi, S., Quinn, L., Ranjbar, K., Chenari, J., . . . Mahdavi, M. (2017). Combined effect of aerobic interval training and selenium nanoparticles on expression of IL-15 and IL-10/TNF-α ratio in skeletal muscle of 4T1 breast cancer mice with cachexia. Cytokine, 90, 100-108. doi: https://doi.org/10.1111/1759-7714.13483.

Simpson, R. J., Radom-Aizik, S., Baker, F. L., Spielmann, G., Teixeira, A. M., Hanson, E. D., & Pence, B. D. (2024). Current Advances in Exercise Immunology.

Steeg, P. S. (2006). Tumor metastasis: mechanistic insights and clinical challenges. Nature medicine, 12(8), 895-904.

Sundling, K. E., & Lowe, A. C. (2019). Circulating tumor cells: overview and opportunities in cytology. Advances in anatomic pathology, 26(1), 56-63.

Tayebi, S. M., Hekmatikar, A. A., Ghanbari-Niaki, A., & Fathi, R. (2020). Ghrelin behavior in exercise and training. J Med. Sci, 27, 85-111.

Tobias, G. C., Gomes, J. L. P., Fernandes, L. G., Voltarelli, V. A., de Almeida, N. R., Jannig, P. R., . . . Brum, P. C. (2023). Aerobic exercise training mitigates tumor growth and cancer-induced splenomegaly through modulation of non-platelet platelet factor 4 expression. Scientific Reports, 13(1), 21970. https://doi.org/10.1038/s41598-023-47217-2

Vincze, O., Colchero, F., Lemaître, J.-F., Conde, D. A., Pavard, S., Bieuville, M., . . . Maley, C. C. (2022). Cancer risk across mammals. Nature, 601(7892), 263-267.

Wang, Q., & Zhou, W. (2021). Roles and molecular mechanisms of physical exercise in cancer prevention and treatment. Journal of Sport and Health Science, 10(2), 201-210. https://doi.org/10.1038/s41598-023-47217-2

Zetter, B. R. (1998). Angiogenesis and tumor metastasis. Annual review of medicine, 1998;49:407-24. doi: https://doi.org/10.1146/annurev.med.49.1.407.