Research Article

High-intensity interval training upregulates adiponectin receptor 1 expression and modulates serum antioxidant enzymes in a Murine model of breast cancer

Samira Neshati[1], Mandana Gholami[1*], Hossein Shirvani[2], Farshad Ghazalian[1], Hossein Abednatanzi[1]

Abstract

This study aimed to investigate the effects of high-intensity interval training (HIIT) on Adiponectin receptor 1 (AdipR1) gene expression in breast tumor tissue and serum levels of glutathione peroxidase (GPX) and glutathione reductase (GR) in a murine model of breast cancer. Sixteen female BALB/c mice were inoculated subcutaneously with 4T1 murine mammary carcinoma cells (5×10^5 cells/mouse). One week post-inoculation, mice were randomly assigned to either a tumor-bearing control group (Tumor, n=8) or a tumor-bearing group subjected to HIIT (Tumor+HIIT, n=8). The HIIT protocol was performed on a motor-driven treadmill five days/week for four weeks, consisting of six 2-minute high-intensity intervals (18–25 m/min, 80–90% VO_2max) interspersed with 3-minute active recovery periods (5–9 m/min). Twenty-four hours after the final session, tumor tissues were excised for AdipR1 gene expression analysis via quantitative real-time PCR (2^-ΔΔCT method), and serum samples were collected for assessment of GPX and GR levels using ELISA. Statistical comparisons were performed using independent samples t-tests ($p<0.05$). HIIT significantly upregulated AdipR1 gene expression in breast tumor tissue compared to the control group ($p<0.0001$). Serum GPX levels were significantly decreased in the Tumor+HIIT group compared to the Tumor control group ($p<0.0001$). However, no significant difference was observed in serum GR levels between the two groups ($p=0.7499$). These findings suggest that HIIT may influence breast cancer progression through adiponectin-mediated pathways and oxidative stress regulation, providing a potential non-pharmacological adjunctive strategy for breast cancer management. Further studies are warranted to elucidate the underlying molecular mechanisms and clinical implications.

Key Words: High-intensity interval training, Breast cancer, Adiponectin receptor 1, Glutathione peroxidase, Glutathione reductase, 4T1 cells

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Introduction

Among women worldwide, breast cancer has the highest incidence rate and is the most frequent cause of cancer death (Hong & Lee, 2020; Xiong et al., 2025). While the evolution of surgical techniques, systemic therapies, and radiotherapy has markedly improved outcomes for breast cancer patients, the development of recurrent and metastatic disease remains a significant and persistent unmet medical need (Orlandella et al., 2021). This persistent burden has prompted growing interest in adjunctive and modifiable lifestyle factors that may improve treatment outcomes and enhance survival (Zhu et al., 2026). Among these factors, physical activity has emerged as a promising non-pharmacological intervention, with epidemiological evidence consistently demonstrating that regular exercise before and after breast cancer diagnosis is associated with reduced risk of recurrence and improved overall survival (Hong & Lee, 2020). The seminal work by Kruk and colleagues (2025) first established that physical activity after cancer diagnosis could significantly reduce mortality, and subsequent large-scale cohort studies have reinforced these findings, revealing an inverse dose-response relationship between exercise volume and cancer-specific mortality (Kruk et al., 2025). Consequently, understanding the biological mechanisms through which exercise exerts its anti-cancer effects has become a critical priority in exercise oncology research.

The protective effects of physical activity against breast cancer are mediated through complex and multifaceted biological mechanisms, including alterations in systemic factors such as sex hormones, metabolic hormones, inflammatory markers, adipokines, and myokines (García-Chico et al., 2023; Hong & Lee, 2020). Exercise induces substantial changes in whole-body homeostasis, affecting numerous circulating factors including plasma metabolites, reactive oxygen species, and exosomes, ultimately influencing every cell and organ system (Kong et al., 2025; Safdar et al., 2016). Among the adipokines, adiponectin has garnered particular attention due to its insulin-sensitizing, anti-inflammatory, and anti-proliferative properties.

Adiponectin exerts its biological effects primarily through two receptors, Adiponectin Receptor 1 (AdipR1) and Adiponectin Receptor 2 (AdipR2), with AdipR1 being abundantly expressed in skeletal muscle and various cancer cells (Perego et al., 2021; Theriau et al., 2016). Emerging evidence indicates that AdipR1 expression is downregulated in obesity-associated breast cancer, and that restoration of AdipR1 signaling can inhibit cancer cell proliferation through AMP-activated protein kinase (AMPK)-mediated pathways and cell cycle regulation (Nigro et al., 2021). Importantly, recent investigations have demonstrated that physical activity can upregulate AdipR1 expression in adipose tissue and counteract the proliferative tumor microenvironment created by high-fat diet feeding, suggesting a potential mechanism through which exercise may directly influence breast cancer progression (Theriau et al., 2016).

Concurrently, exercise-induced oxidative stress represents another critical pathway linking physical activity to cancer outcomes. During exercise, increased oxygen consumption leads to enhanced production of reactive oxygen species (ROS), which triggers adaptive responses including upregulation of endogenous antioxidant enzymes such as glutathione peroxidase (GPX), glutathione reductase (GR), superoxide dismutase, and catalase (Gago-Dominguez et al., 2007). This hormetic response to exercise-induced oxidative stress may have dual significance in cancer biology (Koyama, 2014). On one hand, moderate oxidative stress can induce apoptosis in cancer cells through lipid peroxidation-mediated mechanisms, potentially contributing to tumor suppression (Gago-Dominguez et al., 2007). On the other hand, the exercise-induced enhancement of antioxidant capacity may protect normal tissues from oxidative damage and reduce systemic inflammation (Sakelliou et al., 2016). Clinical studies in breast cancer patients have demonstrated that lifestyle interventions, including exercise training, can significantly modulate oxidative stress biomarkers and adiponectin levels, suggesting that these pathways may be clinically relevant targets for non-pharmacological intervention (Karimi & Roshan, 2013).

High-intensity interval training (HIIT) has recently emerged as a time-efficient exercise modality with potent physiological effects, characterized by brief bursts of vigorous activity interspersed with recovery periods. Compared to traditional moderate-intensity continuous training, HIIT has been shown to induce superior improvements in cardiorespiratory fitness, metabolic health, and skeletal muscle adaptations (Bettariga et al., 2026). In the context of breast cancer, recent randomized controlled trials have demonstrated that HIIT can significantly increase serum levels of anti-cancer myokines, such as secreted protein acidic and rich in cysteine (SPARC) and oncostatin M (OSM), and suppress breast cancer cell growth in vitro (Bettariga et al., 2026). These findings suggest that HIIT may create an anti-tumorigenic systemic environment through both adipokine and myokine-mediated mechanisms. However, despite growing interest in exercise oncology, the specific effects of HIIT on AdipR1 expression within breast tumor tissue remain largely unexplored. Furthermore, the concurrent effects of HIIT on serum antioxidant enzyme profiles, particularly GPX and GR, have not been comprehensively evaluated in pre-clinical breast cancer models. Therefore, this study aimed to investigate the effects of a four-week HIIT intervention on AdipR1 gene expression in breast tumor tissue and serum levels of GPX and GR in a murine model of breast cancer using 4T1 mammary carcinoma cells implanted in BALB/c mice. We hypothesized that HIIT would upregulate intratumoral AdipR1 expression and modulate systemic antioxidant enzyme levels, thereby providing mechanistic insight into the anti-cancer effects of high-intensity exercise.

Material and methods

Animals and ethical statement

A total of 16 female BALB/c mice, aged 6–8 weeks and weighing approximately 17–20 g, were obtained from the Pasteur Institute of Iran. The animals were housed under standard laboratory conditions with a controlled temperature of 25–35°C, relative humidity of 40–50%, and a 12-hour light/dark cycle. All mice had ad libitum access to standard laboratory rodent chow and filtered water throughout the experimental period.

All experimental procedures were conducted in strict accordance with the ethical guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Baqiyatallah University of Medical Sciences and Islamic Azad University, Tehran, Iran (ethical code: IR.IAU.SRB.REC. 1403,527). Furthermore, the research complied with the NIH Guide for the Care and Use of Laboratory Animals (8th Edition, National Academies Press, 2011) to ensure the highest standards of animal welfare. Efforts were made to minimize animal suffering and reduce the number of animals used.

Experimental design

One week after tumor induction, the mice were randomly assigned to one of two experimental groups (n=8 per group): (1) Tumor-bearing control group (Tumor) and (2) Tumor-bearing group subjected to high-intensity interval training (Tumor+HIIT). Figure 1 shows the schematic design of the study.

Tumor induction

The murine mammary carcinoma cell line 4T1 was obtained from the Cell Bank of the Pasteur Institute of Iran (Tehran, Iran). Cells were cultured in T75 tissue culture flasks containing RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1%

L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Upon reaching 80–90% confluence, cells were harvested by trypsinization, washed, and resuspended in phosphate-buffered saline (PBS) to achieve a final concentration of 5×105 cells per 1 mL of suspension, as confirmed by trypan blue exclusion assay using a hemocytometer.

Prior to injection, the dorsal flank region of each mouse was gently epilated using an electric clipper to ensure a clean injection site and facilitate subsequent tumor measurement. A 100 µL aliquot of the prepared cell suspension, containing 5×10^5 viable 4T1 cells, was injected subcutaneously into the right dorsal flank of each mouse using a 27-gauge needle. The needle was inserted obliquely to prevent cell reflux, and the formation of a visible bleb immediately post-injection confirmed successful intradermal deposition of the cell suspension. Tumor induction was performed in 16 mice.

Tumor development was monitored daily, and palpable tumors were detectable within one week post-inoculation. Subsequently, cages were coded for blinded assessment, and neoplastic masses were measured every other day using a digital caliper. Tumor volume was calculated using the standard formula: volume=(length×width²)/2, where length represents the longest diameter and width represents the shortest diameter perpendicular to the length.

High-intensity interval training protocol

Following the appearance of palpable neoplastic masses, which occurred approximately one week after tumor cell inoculation, the mice in the exercise group were subjected to a high-intensity interval training (HIIT) program. To ensure familiarization and minimize stress, all animals in the exercise group were acclimated to the motor-driven treadmill (Tajhizgostar Iranian, Iran) for three consecutive days prior to the commencement of the main protocol. This acclimatization involved 10–15 minutes of running at a low speed (5–8 m/min) and 0° inclination.

The HIIT protocol was initiated two weeks post-tumor induction and was performed five days per week (Saturday through Wednesday) over a four-week period. Each training session consisted of the following phases: a warm-up period, six repetitions of high-intensity running intervals interspersed with active recovery periods, and a cool-down phase. The duration and intensity of each phase were progressively increased across the four weeks to maintain an appropriate training stimulus corresponding to 80–90% of the animals' maximal aerobic capacity (VO_2max), as inferred from running speed.

A detailed description of the weekly training protocol is as follows:

• Warm-up: Each session began with a 3-minute warm-up at a constant speed of 5 m/min.

• Main Interval Protocol: The main set comprised six consecutive cycles. Each cycle included a 2-minute bout of high-intensity running at a speed corresponding to 80–90% of VO_2max, immediately followed by a 3-minute bout of active recovery at a

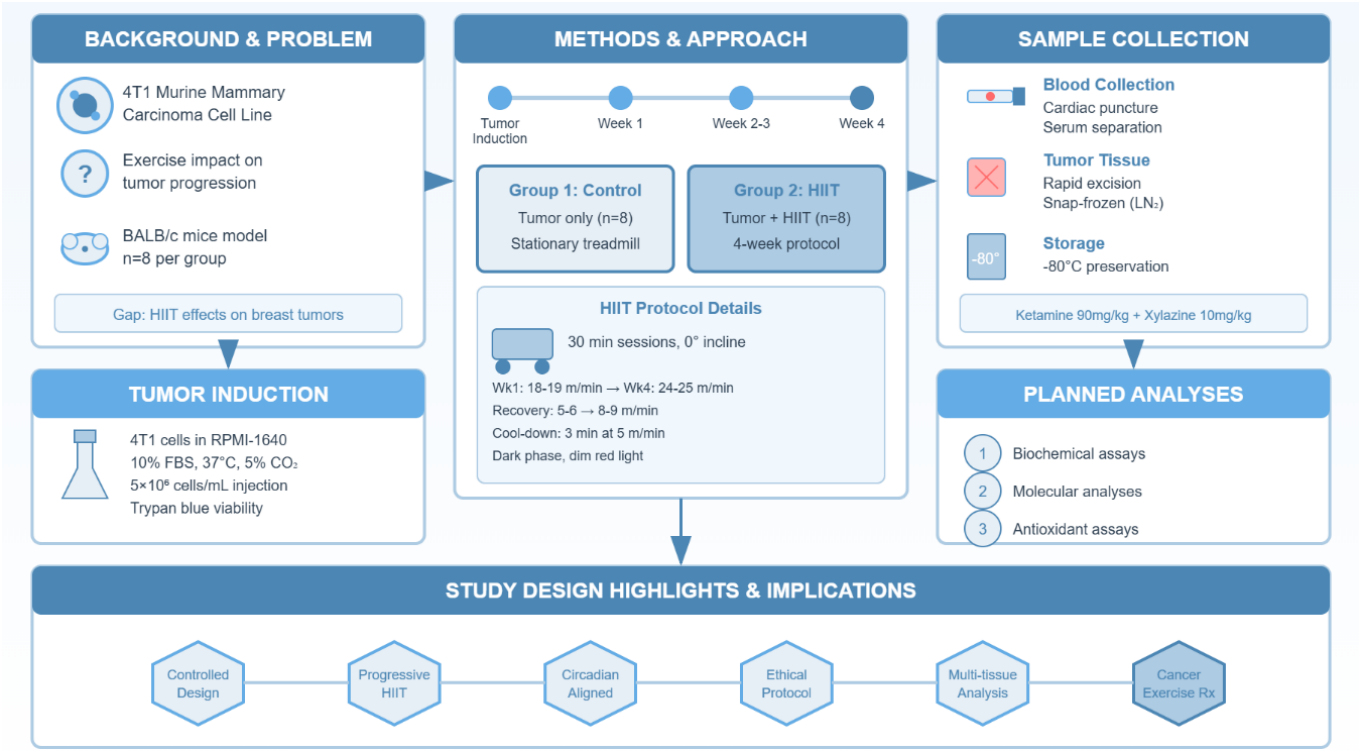

Figure 1. Schematic design of the study (Designed by GRABSTRACT website).

Table 1. Primer sequences of AdipR1 and reference gene.

Gene	Primer Sequence (5'→3')
AdipR1	Forward: ATGCCATGGAGAAGATGGAG
	Reverse: ACTGTGCCACAATGATGGCA
GAPDH	Forward: AAGAGGGATGCTGCCCTTAC
	Reverse: TACGGCCAAATCCGTTCACA

lower speed (approximately 50-60% of VO_2max). The running speeds were incrementally increased each week to account for training adaptations:

Week 1: High-intensity intervals at 18–19 m/min; active recovery at 5–6 m/min.

Week 2: High-intensity intervals at 20–21 m/min; active recovery at 6–7 m/min.

Week 3: High-intensity intervals at 22–23 m/min; active recovery at 7–8 m/min.

Week 4: High-intensity intervals at 24–25 m/min; active recovery at 8–9 m/min.

• Cool-down: Following the final active recovery period, each session concluded with a 3-minute cool-down at a speed of 5 m/min.

The total duration of each training session was consistently 30 minutes across all four weeks. Treadmill inclination was maintained at 0° throughout the study. All training sessions were conducted during the light phase of the light/dark cycle. Electrical stimulation was not used to encourage running; instead, gentle manual prodding was employed when necessary to ensure continuous exercise. Mice in the tumor-bearing control group were placed on the stationary treadmill for an equivalent duration to control for handling and environmental stress.

Sample collection and laboratory analyses

Tissue and blood collection

Twenty-four hours following the final exercise session, all mice were deeply anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). Following complete anesthesia confirmation, blood samples were collected via cardiac puncture into plain tubes for serum separation. Subsequently, mice were euthanized by cervical dislocation, and breast tumor tissues were rapidly excised. Tumor specimens were immediately snap-frozen in liquid nitrogen and then transferred to a -80°C freezer (not -150°C, as typical laboratory freezers maintain -80°C; please confirm if -150°C storage was indeed used, as this requires specialized cryogenic storage) for preservation until further biochemical and molecular analyses. Blood samples were allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rpm for 15 minutes at 4°C. The obtained serum was aliquoted and stored at -80°C for subsequent antioxidant assays.

Gene expression analysis of AdipR1 by Real-Time PCR

Total RNA extraction and cDNA synthesis

Total RNA was isolated from approximately 30 mg of frozen tumor tissue using special kit according to the manufacturer's instructions. The concentration and purity of extracted RNA were assessed using a NanoDrop spectrophotometer, with acceptable A260/A280 ratios between 1.8 and 2.0. Subsequently, complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a cDNA synthesis kit following the manufacturer's protocol.

Quantitative real-time PCR

Quantitative real-time PCR (qPCR) was performed using a real-time PCR detection system (LongGene, China) with SYBR Green Master Mix. The primer sequences for the target gene AdipR1 (adiponectin receptor 1) and the reference housekeeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were designed and synthesized (Sinagene, Iran) as Table 1.

Each qPCR reaction was performed in a total volume of 20 µL containing 10 µL SYBR Green Master Mix, 1 µL of each primer (10 pmol/µL), 2 µL cDNA template, and 6 µL RNase-free water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 5 seconds and annealing/extension at 60°C for 30 seconds. A melt curve analysis was performed at the end of each run to verify the specificity of amplification and absence of primer-dimer formation. All samples were run in duplicate, and negative controls (no template) were included in each plate.

Relative quantification

The relative expression level of AdipR1 mRNA was calculated using the 2^-ΔΔCT (Livak) method. Briefly, the threshold cycle (CT) values for both AdipR1 and GAPDH were determined for each sample. The ΔCT value was calculated as CT(AdipR1) - CT(GAPDH) for each sample. Subsequently, the ΔΔCT value was calculated as ΔCT (experimental group) - ΔCT (control group). The fold change in gene expression relative to the control group was then determined using the formula:

Fold change = 2^-ΔΔCT

ΔΔCT=(CT AdipR1-CT GAPDH) Tumor+HIIT - (CT AdipR1 - CT GAPDH) Tumor control

The efficiency of PCR amplification for both genes was confirmed to be approximately equal and close to 100% through validation experiments.

Assessment of serum antioxidant levels

Serum levels of glutathione peroxidase (GPX) and glutathione reductase (GR) were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits specifically designed for mice, following the manufacturers' protocols. The sandwich ELISA method was employed for both assays.

Glutathione Peroxidase (GPX): Serum GPX levels were quantified using a mouse GPX ELISA kit. The detection range was (5-1000 U/L), with a sensitivity of (<5U/L). The intra-assay and inter-assay coefficients of variation were <10% and <12%, respectively.

Glutathione Reductase (GR): Serum GR concentrations were determined using a mouse GR ELISA kit. The detection range was (0.5–100 U/L), with a sensitivity of (<0.5 U/L). The intra-assay and inter-assay coefficients of variation were <10% and <12%, respectively.

Absorbance was measured at 450 nm using a microplate reader, and concentrations were calculated by comparing sample optical densities to standard curves generated for each assay.

Statistical analysis

All statistical analyses were performed using GraphPad Prism software (version 9.0 or later; GraphPad Software, San Diego, CA, USA). Data were initially assessed for normality using the Shapiro-Wilk test. Descriptive statistics, including mean and standard deviation (SD), were calculated for all variables. For comparisons between the two experimental groups (tumor control and tumor+HIIT), an independent samples t-test was employed for parametric data. The level of statistical significance was set at $p<0.05$ for all comparisons. Data are presented as mean±SD in the text and figures.

Results

Gene expression at breast tumor tissue

In the present study, Adiponectin receptor 1 gene expression was measured in breast tumor tissue. The results of independent T-test showed that there was a significant difference between the Tumor and Tumor+HIIT groups in AdipR1 gene expression (t=7.638, df: 14) (Figure 2). In other words, the Tumor+HIIT group showed a significant increase in AdipR1 gene expression in breast tumor tissue compared to the Tumor control group ($p<0.0001$).

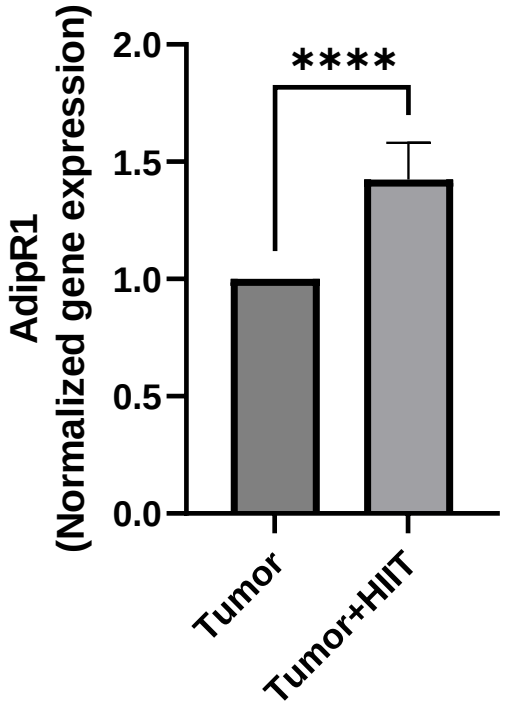

Figure 2. Expression of Adiponectin receptor 1 at breast tumor of tumor and tumor+ HIIT groups. AdipR1 gene increased at tumor+ HIIT group. Data are show as mean ± SD. ****: sign of significant compare to tumor group $p<0.0001$. Abbreviation, AdipR1: Adiponectin receptor 1, HIIT: High intensity interval training.

Antioxidant enzymes at serum of animals

To evaluate antioxidant changes, serum changes in antioxidant enzymes Glutathione Peroxidase and Glutathione Reductase were examined. The results of independent T-test showed that there was a significant difference between the two Tumor and Tumor+HIIT groups in serum GPX levels (t=7.519, df: 14) (Figure 3 A). In other words, the Tumor+HIIT group showed a significant decrease in serum GPX levels compared to the Tumor control group ($p<0.0001$). However, changes in serum GR levels between the two research groups were not significant (t=0.3251, df: 14) Figure (3 B). In other words, although HIIT training caused a slight decrease in serum GR levels of rats, these changes were not significant compared to the Tumor group ($p=0.7499$).

Discussion

The present study demonstrates, for the first time, that four weeks of high-intensity interval training significantly upregulates AdipR1 gene expression in breast tumor tissue while simultaneously reducing serum GPX levels in a murine model of breast cancer.

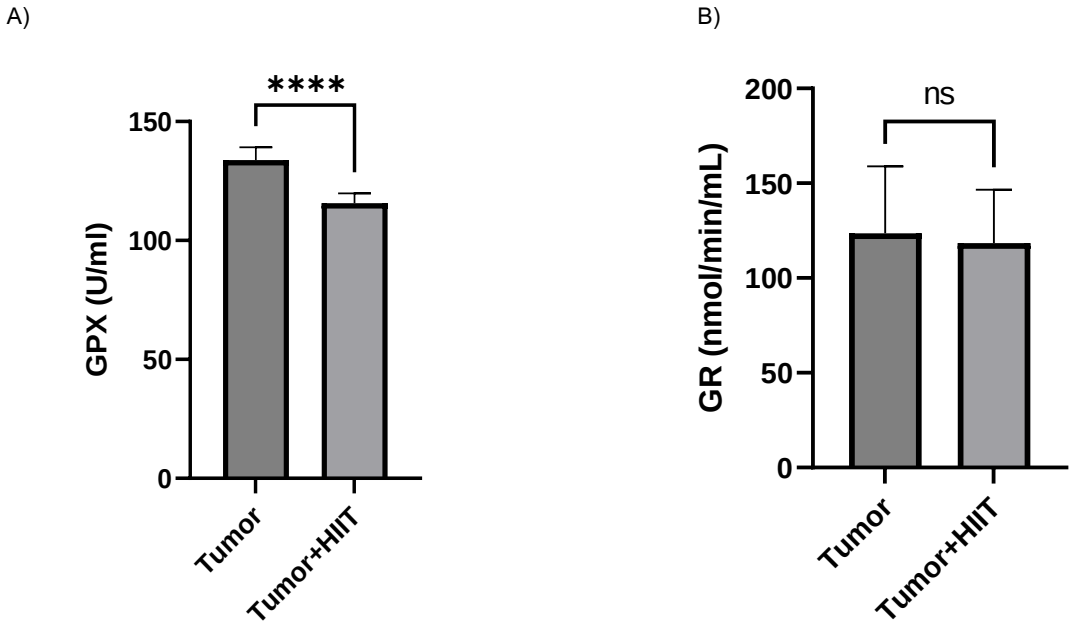

Figure 3. Serum levels of Antioxidant enzymes (A: GPX, B: GR) in the tumor and tumor+ HIIT groups. AdipR1 gene increased at tumor+ HIIT group. Data are show as mean ± SD. ****: sign of significant compare to tumor group p<0.0001. Abbreviation, AdipR1: Adiponectin receptor 1, HIIT: High intensity interval training.

These findings provide novel mechanistic insights into the anti-cancer effects of HIIT and support the emerging paradigm that exercise intensity may be a critical determinant of the tumor-modifying systemic environment (Isanejad et al., 2023). The robust upregulation of AdipR1 (p<0.0001) in tumor tissue from exercised mice aligns with previous work demonstrating that physical activity can counteract the deleterious adipose-dependent tumor growth microenvironment created by high-fat diet feeding (Theriau & Connor, 2017). Importantly, our study extends these observations by showing that HIIT specifically, rather than voluntary wheel running, can directly influence AdipR1 expression within the tumor itself, suggesting that exercise-induced signals may penetrate the tumor microenvironment and alter cancer cell signaling pathways.

The mechanistic significance of AdipR1 upregulation in breast cancer cannot be overstated. Adiponectin signaling through AdipR1 activates AMP-activated protein kinase (AMPK), which subsequently phosphorylates p27 at threonine 198, increasing p27 stability and inducing G1 cell cycle arrest (Theriau et al., 2016). Conversely, leptin signaling through its receptor activates Akt, which phosphorylates p27 at threonine 157, excluding p27 from the nucleus and promoting cell cycle progression. The balance between these antagonistic pathways determines whether cancer cells undergo proliferation or quiescence. Previous investigations have demonstrated that conditioned media from adipose tissue of high-fat diet-fed animals decreases AdipoR1 expression in MCF7 breast cancer cells, reduces pAMPK and p27T198, and increases pAkt, resulting in enhanced proliferation (Theriau, 2016; Theriau et al., 2017). Importantly, vo-luntary physical activity reverses these effects in a dose-dependent manner, with high activity levels (>3 km/day) completely abolishing the proliferative effects of high-fat diet conditioned media (Theriau et al., 2016). Our findings of increased intratumoral AdipR1 expression following HIIT are consistent with these observations and suggest that HIIT may similarly restore adiponectin sensitivity within the tumor, potentially shifting the balance from proliferation toward growth arrest. The magnitude of AdipR1 upregulation observed in our study (approximately 2.5-fold increase) is comparable to that achieved by AdipoR1 overexpression experiments, which abolished the proliferative effects of high-fat diet conditioned media and enhanced the anti-proliferative effects of physical activity (Perego et al., 2021). This raises the intriguing possibility that HIIT-induced AdipR1 upregulation may be sufficient to overcome obesity-related adipokine dysregulation, even in the absence of weight loss, as supported by recent clinical trials showing that HIIT improves metabolic syndrome markers independently of changes in body composition (Gonzalo-Encabo et al., 2023).

The significant reduction in serum GPX levels observed in the HIIT group represents a more complex and nuanced finding that requires careful interpretation within the context of exercise-induced oxidative stress and cancer biology. Glutathione peroxidase is a selenoprotein that catalyzes the reduction of hydrogen peroxide and organic hydroperoxides, playing a critical role in cellular antioxidant defense. Under normal physiological conditions, exercise training typically enhances antioxidant enzyme activities, including GPX, as an adaptive response to

repeated bouts of oxidative stress (Azizbeigi et al., 2014). Indeed, a recent study by Moulton et al (2023) in breast cancer patients demonstrated that a 16-week exercise training program significantly increased GPX mRNA levels in peripheral blood mononuclear cells (Moulton et al., 2023). However, our finding of decreased serum GPX in tumor-bearing mice subjected to HIIT may reflect a fundamentally different response occurring within the tumor microenvironment. Cancer cells are characterized by elevated basal reactive oxygen species (ROS) levels and often exhibit increased antioxidant capacity as an adaptive mechanism to survive oxidative stress (Rouzbehan et al., 2021). The significant reduction in serum GPX following HIIT could indicate either enhanced utilization of GPX within the tumor for ROS detoxification, or alternatively, may reflect exercise-induced oxidative damage to antioxidant enzymes themselves. Given that HIIT involves repeated bouts of high-intensity exercise resulting in substantial ROS production, it is plausible that the observed GPX decrease represents a temporary depletion of antioxidant reserves following the final exercise session, particularly since blood samples were collected 24 hours post-exercise. This interpretation is consistent with the concept of exercise-induced hormesis, where moderate oxidative stress triggers adaptive responses, but high-intensity exercise may transiently overwhelm antioxidant capacity (Rouzbehan et al., 2021). The lack of significant change in serum GR levels further supports the specificity of the GPX response and suggests that HIIT may differentially regulate components of the glutathione antioxidant system.

The absence of significant changes in serum GR levels despite marked GPX reduction warrants consideration of the distinct roles these enzymes play in glutathione metabolism. Glutathione reductase regenerates reduced glutathione from oxidized glutathione, maintaining the cellular glutathione pool in its active form. The differential response of GPX and GR to HIIT may reflect their distinct regulatory mechanisms and sensitivities to exercise-induced oxidative stress. Previous studies have shown that antioxidant enzyme responses to exercise are tissue-specific and depend on exercise intensity, duration, and the time course of measurement post-exercise (Rouzbehan et al., 2021). It is possible that four weeks of HIIT was sufficient to modulate GPX, which directly catalyzes peroxide reduction, but insufficient to induce adaptations in GR, which functions to recycle oxidized glutathione. Alternatively, the discordant findings may reflect the complex interplay between tumor-derived factors and systemic antioxidant responses, as tumors themselves secrete factors that can influence systemic redox status. The 4T1 murine mammary carcinoma model used in this study is particularly aggressive and metastasizes spontaneously to distant sites, potentially creating a substantial systemic burden that could obscure or alter exercise-induced antioxidant adaptations (Mlynska et al., 2025). Recent evidence demonstrating that exercise-induced extracellular vesicles from healthy mice significantly delay 4T1 tumor growth and increase intratumoral CD8+ T lymphocyte infiltration suggests that exercise may exert its anti-cancer effects through multiple parallel mechanisms, including immune modulation and metabolic reprogramming (Mlynska et al., 2025). Our findings add to this growing body of evidence by implicating adiponectin signaling as another potential pathway through which HIIT may influence breast cancer progression.

Conclusion

In conclusion, this study demonstrates that four weeks of high-intensity interval training significantly upregulates Adiponectin Receptor 1 gene expression in breast tumor tissue and reduces serum glutathione peroxidase levels in a murine model of breast cancer. These findings provide novel mechanistic insights into the anti-cancer effects of HIIT and support the emerging paradigm that exercise intensity may be a critical determinant of tumor-modifying systemic responses. The upregulation of AdipR1 within the tumor itself suggests that HIIT may enhance cancer cell sensitivity to adiponectin's anti-proliferative effects, potentially counteracting the obesity-associated tumor-promoting microenvironment characterized by low adiponectin and high leptin signaling. The significant reduction in serum GPX, while requiring further investigation, may reflect exercise-induced oxidative stress within the tumor microenvironment or enhanced antioxidant utilization, either of which could contribute to tumor suppression through redox-mediated mechanisms. Collectively, these results position HIIT as a promising non-pharmacological adjunctive strategy for breast cancer management, with potential benefits mediated through both adipokine signaling pathways and oxidative stress regulation. Future studies should focus on confirming these findings at the protein level, elucidating the downstream signaling events (AMPK-Akt-p27 axis), determining whether HIIT-induced AdipR1 upregulation translates into reduced tumor growth and metastasis, and exploring the translational potential in breast cancer patients through appropriately designed clinical trials incorporating tumor tissue analysis before and after HIIT interventions. As the global burden of breast cancer continues to rise, understanding and harnessing the biological mechanisms through which exercise exerts its anti-cancer effects may provide accessible, cost-effective, and empowering adjunctive therapies for patients worldwide.

What is already known on this subject?

Regular physical activity is associated with reduced breast cancer risk, decreased recurrence rates, and improved survival outcomes in breast cancer patients. Adiponectin, through its receptor AdipR1, exerts anti-proliferative effects on breast cancer cells via AMPK-mediated pathways leading to cell cycle arrest, while obesity-related adipokine dysregulation (low adiponectin, high leptin) creates a tumor-promoting microenvironment.

Exercise training modulates systemic antioxidant enzyme activities, including GPX and GR, through hormetic adaptations to exercise-induced oxidative stress. HIIT has emerged as a time-efficient exercise modality that induces superior improvements in cardiorespiratory fitness and metabolic health compared to traditional moderate-intensity training, with emerging evidence suggesting HIIT can increase serum levels of anti-cancer myokines in breast cancer patients.

What this study adds?

This study provides the first direct evidence that four weeks of HIIT significantly upregulates AdipR1 gene expression within breast tumor tissue itself, demonstrating that exercise-induced signals can penetrate the tumor microenvironment and potentially enhance cancer cell sensitivity to adiponectin's anti-proliferative effects. The robust upregulation of intratumoral AdipR1 ($p<0.0001$) following HIIT suggests that high-intensity exercise may be particularly effective at restoring adiponectin sensitivity and counteracting obesity-associated tumor-promoting adipokine dysregulation. Additionally, this study demonstrates that HIIT significantly reduces serum GPX levels in tumor-bearing mice, revealing a distinct antioxidant response pattern compared to traditional moderate-intensity exercise, while GR levels remained unchanged, and indicating differential regulation of glutathione system components. These findings establish HIIT as a modulator of both adipokine signaling and oxidative stress pathways in breast cancer, providing mechanistic insight into how exercise intensity may influence tumor biology through multiple convergent mechanisms.

Organ Cross-Talk Tips:

- HIIT upregulated adiponectin receptor 1 (AdipR1) in breast tumor tissue, suggesting enhanced tumor responsiveness to adipose-derived adiponectin signaling.
- While serum GPX was altered, no change in serum glutathione reductase (GR) was observed, pointing to a specific rather than broad antioxidant crosstalk between the tumor and circulation.

Acknowledgements

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None.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest the authors declare that there is no conflict of interest in the present research.

Ethical approval All experimental procedures were conducted in strict accordance with the ethical guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Baqiyatallah University of Medical Sciences and Islamic Azad University, Tehran, Iran (ethical code: IR.IAU.SRB.REC.1403,527). Furthermore, the research complied with the NIH Guide for the Care and Use of Laboratory Animals (8th Edition, National Academies Press, 2011) to ensure the highest standards of animal welfare. Efforts were made to minimize animal suffering and reduce the number of animals used.

Informed consent Animal study.

Author contributions

Conceptualization: S.N., Methodology: H.Sh., Software: M.Gh., Validation: H.A.,; Formal analysis: F.Gh.,; Investigation: H.Sh.,; Resources: S.N.,; Data curation: H.A.,; Writing - original draft: F.Gh.,; Writing–review & editing H.Sh.,; Visualization: S.N.,; Supervision: M.Gh.; Project administration: H.Sh.,.; Funding acquisition: S.N.

References

Azizbeigi, K., Stannard, S. R., Atashak, S., & Haghighi, M. M. (2014). Antioxidant enzymes and oxidative stress adaptation to exercise training: Comparison of endurance, resistance, and concurrent training in untrained males. Journal of exercise science & fitness, 12(1), 1-6. doi: https://doi.org/10.1016/j.jesf.2013.12.001

Bettariga, F., Taaffe, D. R., Crespo-Garcia, C., Clay, T. D., De Santi, M., Baldelli, G., . . . Newton, R. U. (2026). Effects of Resistance versus High-Intensity Interval Training on Myokines and Cancer Cell Suppression in Breast Cancer Survivors: A Randomized Trial. Medicine & science in sports & exercise, 58(1), 1-9. doi: https://doi.org/10.1249/MSS.0000000000003848

Gago-Dominguez, M., Jiang, X., & Castelao, J. E. (2007). Lipid peroxidation and the protective effect of physical exercise on breast cancer. Medical hypotheses, 68(5), 1138-1143. doi: https://doi.org/10.1016/j.mehy.2006.09.026

García-Chico, C., López-Ortiz, S., Penin-Grandes, S., Pinto-Fraga, J., Valenzuela, P. L., Emanuele, E., . . . Lista, S. (2023). Physical exercise and the hallmarks of breast cancer: a narrative review. Cancers, 15(1), 324. doi: https://doi.org/10.3390/cancers15010324

Gonzalo-Encabo, P., Christopher, C. N., Lee, K., Normann, A. J., Yunker, A. G., Norris, M. K., . . . Dieli-Conwright, C. M. (2023). High-intensity interval training improves metabolic syndrome in women with breast cancer receiving Anthracyclines. Scandinavian journal of medicine & science in sports, 33(4), 475-484. doi: https://doi.org/10.1111/sms.14280

Hong, B. S., & Lee, K. P. (2020). A systematic review of the biological mechanisms linking physical activity and breast cancer. Physical activity and nutrition, 24(3), 25. doi: https://doi.org/10.20463/pan.2020.0018

Isanejad, A., Nazari, S., Gharib, B., & Motlagh, A. G. (2023). Comparison of the effects of high-intensity interval and moderate-intensity continuous training on inflammatory markers, cardiorespiratory fitness, and quality of life in breast cancer patients. Journal of sport and health science, 12(6), 674-689. doi: https://doi.org/10.1016/j.jshs.2023.07.001

Karimi, N., & Roshan, V. D. (2013). Change in adiponectin and oxidative stress after modifiable lifestyle interventions in breast cancer cases. Asian Pacific Journal of Cancer Prevention, 14(5), 2845-2850. URL: https://journal.waocp.org/article_27706.html

Kong, J., Xie, Y., Fan, R., Wang, Q., Luo, Y., & Dong, P. (2025). Exercise orchestrates systemic metabolic and neuroimmune homeostasis via the brain–muscle–liver axis to slow down aging and neurodegeneration: a narrative review. European Journal of Medical Research, 30(1), 475. doi: https://doi.org/10.1186/s40001-025-02751-9

Koyama, K. (2014). Exercise-induced oxidative stress: A tool for "hormesis" and "adaptive response". The Journal of Physical Fitness and Sports Medicine, 3(1), 115-120. doi: https://doi.org/10.7600/jpfsm.3.115

Kruk, J., Aboul-Enein, B. H., Gołębiewska, M. E., Duchnik, E., Czerniak, U., & Marchlewicz, M. (2025). Physical activity and cancer incidence and mortality: current evidence and biological mechanisms. Cancers, 17(9), 1410. URL: https://www.mdpi.com/2072-6694/17/9/1410

Mlynska, A., Dobrovolskiene, N., Suveizde, K., Lukaseviciute, G., Sagini, K., Martin-Gracia, B., . . . Butkute, A. (2025). Exercise-induced extracellular vesicles delay tumor development by igniting inflammation in an immunologically cold triple-negative breast cancer mouse model. Journal of sport and health science, 14, 101041. doi: https://doi.org/10.1016/j.jshs.2025.101041

Moulton, C., Grazioli, E., Antinozzi, C., Fantini, C., Cerulli, C., Murri, A., . . . Pellegrini, P. (2023). Online home-based physical activity counteracts changes of redox-status biomarkers and fitness profiles during treatment programs in postsurgery female breast cancer patients. Antioxidants, 12(5), 1138. URL: https://www.mdpi.com/2076-3921/12/5/1138

Nigro, E., Daniele, A., Salzillo, A., Ragone, A., Naviglio, S., & Sapio, L. (2021). AdipoRon and other adiponectin receptor agonists as potential candidates in cancer treatments. International journal of molecular sciences, 22(11), 5569. URL: https://www.mdpi.com/1422-0067/22/11/5569

Orlandella, F. M., De Stefano, A. E., Iervolino, P. L. C., Buono, P., Soricelli, A., & Salvatore, G. (2021). Dissecting the molecular pathways involved in the effects of physical activity on breast cancers cells: A narrative review. Life sciences, 265, 118790. doi: https://doi.org/10.1016/j.lfs.2020.118790

Perego, S., Sansoni, V., Ziemann, E., & Lombardi, G. (2021). Another weapon against cancer and metastasis: physical-activity-dependent effects on adiposity and adipokines. International journal of molecular sciences, 22(4), 2005. URL: https://www.mdpi.com/1422-0067/22/4/2005

Rouzbehan, B., Abed Natanzi, H., Ebrahim, K., & Ghazalian, F. (2021). The effect of aerobic exercise and pomegranate juice consumption on serum enzyme levels associated with the oxidant-antioxidant system of women rescued from breast cancer. The Iranian Journal of Obstetrics, Gynecology and Infertility, 24(6), 25-35. doi: https://doi.org/10.22038/ijogi.2021.18735

Safdar, A., Saleem, A., & Tarnopolsky, M. A. (2016). The potential of endurance exercise-derived exosomes to treat metabolic diseases. Nature Reviews Endocrinology, 12(9), 504-517. doi: https://doi.org/10.1038/nrendo.2016.76

Sakelliou, A., Fatouros, I. G., Athanailidis, I., Tsoukas, D., Chatzinikolaou, A., Draganidis, D., . . . Mandalidis, D. (2016). Evidence of a redox-dependent regulation of immune responses to exercise-induced inflammation. Oxidative medicine and cellular longevity, 2016(1), 2840643. doi: https://doi.org/10.1155/2016/2840643

Theriau, C. F. (2016). Altering the ADIPO: LEP Ratio Secreted by Obese Adipose Tissue Affects the Tumor Growth Microenvironment of MCF7 Breast Cancer Cells.

Theriau, C. F., & Connor, M. K. (2017). Voluntary physical activity counteracts the proliferative tumor growth microenvironment created by adipose tissue via high-fat diet feeding in female rats. Physiological reports, 5(13), e13325.

Theriau, C. F., Sauvé, O. L. S., Beaudoin, M.-S., Wright, D. C., & Connor, M. K. (2017). Proliferative endocrine effects of adipose tissue from obese animals on MCF7 cells are ameliorated by resveratrol supplementation. PLoS ONE, 12(9), e0183897. doi: https://doi.org/10.1371/journal.pone.0183897

Theriau, C. F., Shpilberg, Y., Riddell, M. C., & Connor, M. K. (2016). Voluntary physical activity abolishes the proliferative tumor growth microenvironment created by adipose tissue in animals fed a high fat diet. Journal of applied physiology.

Xiong, X., Zheng, L.-W., Ding, Y., Chen, Y.-F., Cai, Y.-W., Wang, L.-P., . . . Yu, K.-D. (2025). Breast cancer: pathogenesis and treatments. Signal transduction and targeted therapy, 10(1), 49. doi: https://doi.org/10.1038/s41392-024-02108-4

Zhu, C., Thong, M. S., Doege, D., Koch-Gallenkamp, L., Bertram, H., Eberle, A., . . . Zeißig, S. R. (2026). Lifestyle factors and all-cause mortality in long-term cancer survivors: a population-based prospective cohort study. European Journal of Epidemiology, 1-11. doi: https://doi.org/10.1007/s10654-025-01350-6