Research Article

The effect of exercise training with Nano selenium supplementation on LDHA, LDHB genes and LDHA/LDHB ratio at breast tumor tissue of mouse model

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Abstract

This study investigated the effects of high intensity interval training (HIIT), Nano-selenium supplementation, and their combination on the expression of LDHA, LDHB, and the LDHA/LDHB ratio in mice breast tumor tissue. Female mice (n=32) were inoculated with mammary adenocarcinoma cells (4T1) and randomly assigned to four groups (n=8 each): tumor control (Tu), tumor+HIIT (Tu+Ex), tumor + Nano-selenium (Tu+Nsel, 2 mg/kg/day orally), and tumor+HIIT+Nano-selenium (Tu+Ex+Nsel). HIIT was performed on a treadmill (30 min/day, 5 days/week) for four weeks. One-way ANOVA revealed significant differences among groups for LDHA expression ($F=38.66$, $p<0.0001$). Compared to the Tu group, all intervention groups (Tu+Ex,Tu+Nsel, and Tu+Ex+Nsel) showed a significant increase in LDHA expression ($p<0.05$). The greatest increase was observed in the combined treatment group (Tu+Ex+Nsel), which was significantly higher than both Tu+Ex and Tu+Nsel ($p<0.001$). For the LDHA/LDHB ratio, a significant overall effect was found ($F=163.87$, $p<0.0001$). The Tu+Nsel group exhibited a significant increase in the ratio compared to the Tu group ($p<0.05$), whereas both Tu+Ex and Tu+Ex+Nsel showed a significant decrease in the ratio ($p<0.05$). The ratio in the Tu+Nsel group was also significantly higher than in the two exercise containing groups ($p<0.05$). HIIT and Nano-selenium independently upregulate LDHA expression in breast tumor tissue, with an additive effect when combined. However, only Nano-selenium alone increased the LDHA/LDHB ratio, while exercise-based interventions (with or without Nano-selenium) decreased this ratio. These findings suggest that exercise and Nano-selenium differentially shift the balance between LDHA and LDHB, potentially influencing tumor lactate metabolism and the tumor microenvironment.

Key Words: Breast cancer, LDHA, LDHB, Nano-selenium, Exercise

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Introduction

Breast cancer remains the most frequently diagnosed malignancy and the leading cause of cancer-related mortality among women worldwide, accounting for approximately 2.3 million new cases and 685,000 deaths in 2020 (Sung et al., 2021). Despite significant advances in early detection and targeted therapies, the aggressive nature of certain breast cancer subtypes, particularly triple-negative breast cancer, necessitates the exploration of novel adjuvant strategies that can modulate tumor metabolism and enhance treatment efficacy (Schmid et al., 2018). One of the fundamental hallmarks of cancer is the reprogramming of energy metabolism, famously described by Otto Warburg as aerobic glycolysis, where cancer cells preferentially convert glucose to lactate even in the presence of ample oxygen (Warburg, 1956). This metabolic shift, known as the Warburg effect, provides rapidly proliferating tumor cells with essential biosynthetic precursors and creates an acidic tumor microenvironment that promotes invasion, metastasis, and immune evasion (Liberti & Locasale, 2016).

Lactate dehydrogenase (LDH) is a critical enzyme in this metabolic rewiring, existing as two major isoforms: LDHA and LDHB. LDHA catalyzes the conversion of pyruvate to lactate with concomitant regeneration of NAD^+, thereby sustaining glycolysis under hypoxic conditions, while LDHB preferentially converts lactate back to pyruvate, facilitating lactate oxidation and utilization (Doherty & Cleveland, 2013). The LDHA/LDHB ratio is increasingly recognized as a key determinant of the glycolytic versus oxidative phenotype within tumors. A high LDHA/LDHB ratio, often driven by oncogenic signaling through c-Myc and HIF-1α, is associated with aggressive tumor behavior, poor patient prognosis, and resistance to conventional therapies in various cancers, including breast cancer (Dong et al., 2017; Jang et al., 2025). Consequently, therapeutic strategies aimed at modulating the LDHA/LDHB balance have emerged as promising approaches to disrupt tumor metabolic plasticity.

Regular physical exercise has gained considerable attention as a non-pharmacological intervention that can influence tumor biology and improve clinical outcomes in cancer patients (Hojman et al., 2018). High-intensity interval training (HIIT), in particular, has been shown to elicit profound systemic and intratumoral metabolic adaptations, including alterations in glucose uptake, lactate flux, and redox status (Ashcraft et al., 2016; Ruiz-Casado et al., 2017). Selenium, an essential trace element with potent antioxidant and chemopreventive properties, has also been investigated for its anticancer effects. Nano-selenium, due to its enhanced bioavailability and lower toxicity compared to inorganic selenium compounds, exhibits selective cytotoxicity against cancer cells while protecting normal tissues (Hosnedlova et al., 2018; Hughes et al., 2015). However, the combined effects of exercise training and nano selenium on the expression of LDHA and LDHB, and particularly on the LDHA/LDHB ratio within breast tumor tissue, remain unexplored. Therefore, the present study aimed to investigate the individual and combined effects of four weeks of HIIT and nano selenium supplementation on LDHA, LDHB, and their ratio in a mice model of breast cancer.

Materials and Methods

Animals and ethical statement

Thirty two female BALB/c mice (aged 6–8 weeks, body weight 17–20 g) were obtained from the animal house of Pasteur Institute, Tehran, Iran. The animals were housed under controlled environmental conditions (temperature 22 ± 2 °C, relative humidity 50 ± 10 %, 12 h light/dark cycle) with free access to standard rodent chow and tap water. All experimental procedures were approved by the Institutional Animal Ethics Committee and conformed to the ARRIVE guidelines and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th edition, 2011). Every effort was made to minimize animal suffering and to reduce the number of animals used.

Experimental design

One week after tumor cell inoculation, the mice were randomly allocated into four experimental groups (n = 8 per group):

1. Tumor group (Tu): tumor bearing mice receiving no intervention.

2. Tumor + Exercise group (Tu + Ex): tumor bearing mice's subjected to a treadmill exercise protocol.

3. Tumor + Nano-selenium group (Tu + Nsel): tumor bearing mice treated with Nano-selenium.

4. Tumor + Exercise + Nano-selenium group (Tu +Ex + Nsel): tumor bearing mice receiving both exercise training and Nano-selenium.

The study duration was 5 weeks after tumor induction (one week for tumor establishment followed by four weeks of interventions).

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 5×10^5 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 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.

Nano-selenium administration

The supplement program was implemented for the supplement and supplement-exercise group. Selenium nanoparticle supplement with 99% purity (particle size 80-100nm) and concentration of 1000ppm was purchased commercially from Pardis Technology Park in Tehran and 100mg/kg equivalent was gavage to each mouse after the start of the lighting cycle. Taking into account the weight of 20 grams of mice, 2mg of nanoselenium supplement was given to each mouse.

Exercise training protocol

Animals in the Tu+Ex and Tu+Ex+Nsel groups underwent a

four-week treadmill running program (Tajhizgostar, Iran) performed five days per week (Saturday to Wednesday). Before the main protocol, all animals were familiarized with the treadmill for three days (10 min/day at 5 m/min, 0° inclination). Inclusion criteria during familiarization required that animals voluntarily run on the treadmill for at least 8 of the 10 minutes without repeated manual prodding (defined as more than three prodding events per minute). Animals that consistently stopped running, clung to the shock bar, or exhibited signs of distress (e.g., excessive freezing, vocalization, or attempted escape) for two consecutive familiarization sessions were excluded from the exercise groups. No animals met exclusion criteria during this study; all 16 mice assigned to exercise-containing groups successfully completed the familiarization protocol.

The high intensity interval training (HIIT) protocol consisted of:

• Warm up: 3 min at 5 m/min.

• Main set: six repetitions of 2 min high intensity running (estimated at 80–90 % of maximal aerobic speed based on published ranges for female BALB/c mice from pilot studies) followed by 3 min active recovery (50–60 % of estimated maximal speed). Individual maximal aerobic capacity was not empirically assessed; therefore, actual relative intensities may have varied between animals. Running speeds were increased weekly:

O Week 1: 18–19 m/min (high), 5–6 m/min (recovery)

O Week 2: 20–21 m/min (high), 6–7 m/min (recovery)

O Week 3: 22–23 m/min (high), 7–8 m/min (recovery)

O Week 4: 24–25 m/min (high), 8–9 m/min (recovery)

• Cool down: 3 min at 5 m/min.

Total session duration was 30 min. Training was carried out during the 8-10 AM. No electrical stimulation was used; manual prodding was applied only when necessary. Mice in the sedentary groups (Tu and Tu+Nsel) were placed on the stationary treadmill for the same duration to control for handling stress.

Tissue collection

Twenty four hours after the last exercise session, all animals were deeply anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally. Blood was drawn by cardiac puncture, and then the mice were euthanized by cervical dislocation. Breast tumor tissues were rapidly excised, cleaned of adhering fat and connective tissue, snap frozen in liquid nitrogen, and stored at −80 °C until molecular analysis.

Gene expression analysis of LDHA and LDHB

RNA extraction and cDNA synthesis

Total RNA was isolated from ~30 mg of frozen tumor tissue using a commercial RNA extraction kit (e.g., RNeasy Mini Kit, Qiagen) following the manufacturer's protocol. RNA concentration and purity were assessed with a NanoDrop spectrophotometer (A260/A280 ratio between 1.8 and 2.0). Subsequently, 1 µg of total RNA was reverse transcribed into complementary DNA (cDNA) using a high capacity cDNA synthesis kit (Thermo Fisher Scientific) with random hexamers, according to the supplier's instructions.

Quantitative real time PCR (qPCR)

The mRNA expression levels of LDHA and LDHB were quantified by qPCR using SYBR Green master mix (Ampliqon, Denmark) on a real time PCR detection system (LongGene, China). Glyceraldehyde 3 phosphate dehydrogenase (GAPDH) was used as the internal reference gene. Primer sequences were as follows ($5'\rightarrow3'$) (Table 1):

Each reaction (20 µL) contained 10 µL SYBR Green mix, 1 µL of each primer (10 pmol/µL), 2 µL cDNA (diluted 1:5), and 6 µL RNase free water. The thermal profile was: 95 °C for 2 min; 40 cycles of 95 °C for 5 s and 60 °C for 30 s; followed by a melt curve analysis (60–95 °C, 0.5 °C increments) to verify single product amplification. All samples were run in duplicate, and no template controls were included in each run.

Relative quantification and LDHA/LDHB ratio

The $2^{-\Delta\Delta Ct}$ method was used to calculate fold changes in LDHA and LDHB mRNA expression relative to the Tu control group. The ΔCt was calculated as Ct (target) – Ct (GAPDH). Then ΔΔCt = ΔCt (treatment group) – ΔCt (Tu group). The expression ratio LDHA/LDHB for each animal was obtained by dividing the normalized expression level of LDHA by that of LDHB (both expressed as $2^{-\Delta Ct}$ relative to GAPDH). Group means of the LDHA/LDHB ratio were then compared.

Statistical analysis

Table 1. Primer sequences

Gene	Primer
LDHA	F: AACTTGGCGCTCTACTTGCT
	R: GGACTTTGAATCTTTTGAGACCTTG
LDHB	F: TTCTGCTCGATTCCGCTACC
	R: ATGCCGTACATTCCCTGTCC
GAPDH	F: AAGAGGGATGCTGCCCTTAC
	R: TACGGCCAAATCCGTTCACA

Data are presented as mean ± standard deviation (SD). Normality was checked using the Shapiro–Wilk test. One way analysis of variance (ANOVA) was employed to compare LDHA expression, LDHB expression, and the LDHA/LDHB ratio among the four groups. When a significant overall effect was found, Tukey's post hoc test was applied for pairwise comparisons with family-wise error rate controlled at α=0.05 for six pairwise comparisons per outcome. Sample size (n=8 per group) was determined based on a power analysis using G*Power software (α=0.05, power=0.80, effect size f=0.60 from pilot data), which indicated a minimum of 7 mice per group. Eight mice were included per group to account for potential attrition. A p value <0.05 was considered statistically significant. All analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

Results

LDHA gene

Changes in LDHA gene expression in mice breast tumor tissue in different groups are shown in Figure 1. The results of one-way ANOVA statistical test showed that there was a significant difference in LDHA gene expression in breast tumor tissue between different research groups (F=38.66, p<0.0001). The results of Tukey's post hoc test showed that the treatment groups including exercise tumor (Tu+Ex), Nano-selenium tumor (Tu+Nsel), and exercise and Nano-selenium tumor (Tu+Ex+Nsel) showed a significant increase in LDHA gene expression in breast tumor tissue compared to the tumor group (Tu) (p<0.05). The greatest increase in LDHA gene expression in breast tumor tissue was related to the combined treatment, namely Tu+Ex+Nsel, which was significant compared to the Tu+Ex and Tu+Nsel groups (p<0.001).

LDHB gene

Changes in LDHB gene expression in mice breast tumor tissue in different groups are shown in Figure 2. The results of one-way ANOVA statistical test showed that there was a significant difference in LDHB gene expression in breast tumor tissue between different research groups (F=116.3, p<0.0001). The results of Tukey's post hoc test showed that the treatment groups including exercise tumor (Tu+Ex) and exercise and Nano-selenium tumor (Tu+Ex+Nsel) showed a significant increase in LDHB gene expression in breast tumor tissue compared to the tumor group (Tu) (p<0.0001). The greatest increase in LDHB gene expression in breast tumor tissue was related to the combined treatment, namely Tu+Ex+Nsel, which was significant compared to the Tu+Ex and Tu+Nsel groups (p<0.0001).

LDHA/LDHB ratio

In the present study, the ratio of LDHA/LDHB gene expression in mice breast tumor tissue was also examined (Figure 3). The results of one-way ANOVA statistical test showed that there was

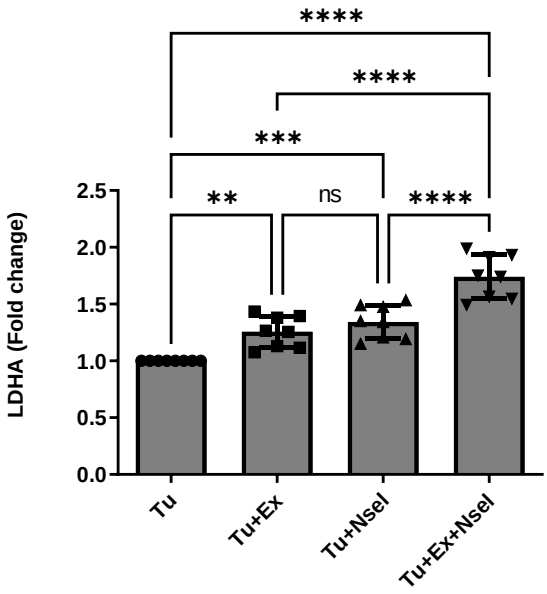

Figure 1. Expression of LDHA gene at breast tissue of mice at different groups of study. Data are show as means ± standard deviation. Sign of significant difference * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Abbreviation, Tu: Tumor, Ex: Exercise, Nsel: Nano selenium.

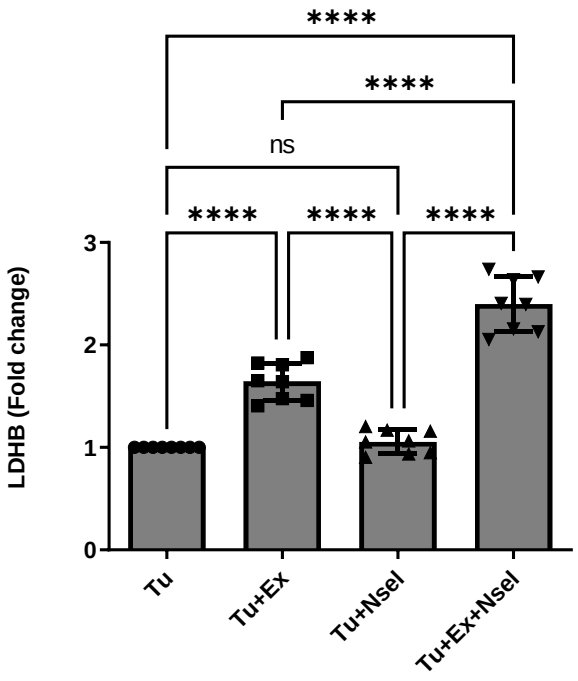

Figure 2. Expression of LDHB gene at breast tissue of mice at different groups of study. Data are show as means ± standard deviation. Sign of significant difference * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Abbreviation, Tu: Tumor, Ex: Exercise, Nsel: Nano selenium.

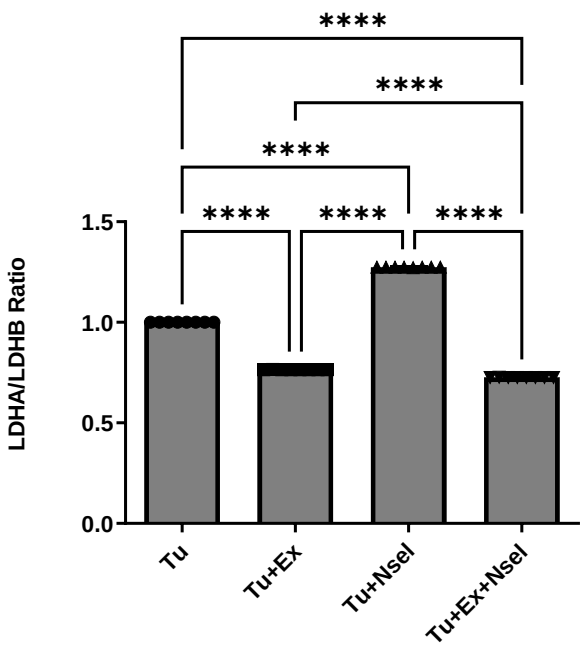

Figure 3. The ratio of LDHA/LDHB gene at breast tissue of mice at different groups of study. Data are show as means ± standard deviation. Sign of significant difference * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Abbreviation, Tu: Tumor, Ex: Exercise, Nsel: Nano selenium.

a significant difference between the different research groups in the ratio of LDHA/LDHB gene expression in mice breast tumor tissue ($F=163.87$, $p<0.0001$). The results of the Tukey post hoc test showed that only the Tu+Nsel group showed an increase in this ratio, and the exercise tumor (Tu+Ex) and exercise tumor and Nano-selenium (Tu+Ex+Nsel) groups showed a significant decrease in the ratio of LDHA/LDHB gene expression in breast tumor tissue compared to the Tu group ($p<0.0001$). The increase in this ratio in the Tu+Nsel group compared to the exercise tumor (Tu+Ex) and exercise tumor and Nano-selenium (Tu+Ex+Nsel) groups was also significant ($p<0.0001$).

Discussion

The primary finding of this study was that both HIIT and nano selenium supplementation independently upregulated LDHA mRNA expression in breast tumor tissue, with an additive effect observed when the two interventions were combined. However, despite this coordinated increase in LDHA, the LDHA/LDHB ratio responded differentially: nano selenium alone significantly increased the ratio, whereas both exercise containing groups (Tu+Ex and Tu+Ex+Nsel) exhibited a significant decrease in the LDHA/LDHB ratio. These results reveal a complex, context dependent regulation of the LDH isoenzyme balance and suggest that exercise and nano selenium may exert opposing effects on the directionality of lactate metabolism within the tumor microenvironment.

The observed upregulation of LDHA following HIIT is consistent with previous reports demonstrating that acute and chronic exercise can enhance glycolytic enzyme expression in both skeletal muscle and tumor tissue. For instance, Aveseh et al. (2020) reported that eight weeks of treadmill running increased LDHA activity in breast tumors of rats, an effect they attributed to exercise-induced activation of HIF-1α and AMPK signaling pathways, although these were not directly measured in their study. Similarly, Jones et al. (2019) found that voluntary wheel running upregulated LDHA in murine melanoma, proposing that exercise-mediated metabolic stress forces tumors to adopt a more glycolytic phenotype. While we did not measure HIF-1α, AMPK, or ROS in the present study, it is plausible that similar mechanisms contributed to our findings. The additive effect of nano selenium with exercise in our study is novel and may be hypothetically explained by selenium-induced oxidative stress at the tumor site. Previous studies have suggested that nano selenium can generate reactive oxygen species (ROS) in cancer cells, potentially leading to HIF-1α stabilization and subsequent transcriptional activation of LDHA (Ali et al., 2024; Wang et al., 2022). However, we emphasize that these mechanisms remain speculative, as we did not directly assess ROS levels, HIF-1α protein, or downstream signaling molecules. It is possible that exercise-induced systemic adaptations (e.g., catecholamine release, increased perfusion) synergize with local pro-oxidant effects of nano selenium to maximize LDHA expression, but confirmation of these pathways requires future mechanistic studies.

The differential effect on the LDHA/LDHB ratio is particularly noteworthy. Nano selenium alone increased the ratio, indicating a shift toward a more glycolytic, LDHA dominant state. This observation aligns with the well-established pro-oxidant and pro-apoptotic mechanisms of selenium in cancer cells reported by others. Elevated LDHA/LDHB ratio favors lactate production, which not only fuels tumor growth but also creates an acidic microenvironment that suppresses immune effector cells while recruiting immunosuppressive cells such as myeloid derived suppressor cells (MDSCs) and tumor associated macrophages (TAMs) (Colegio et al., 2014; Zhang & Romero, 2018). Nevertheless, it is important to note that we did not measure lactate levels, pH, or immune cell populations; these functional consequences remain speculative based on published literature. Many studies have shown that nano selenium ultimately inhibits tumor progression despite increasing glycolysis, possibly because the net effect of ROS-mediated damage outweighs any metabolic advantage conferred by LDHA upregulation (Ullah et al., 2022; Zhao et al., 2018). In contrast, exercise containing groups (with or without nano selenium) significantly decreased the LDHA/LDHB ratio, suggesting a metabolic shift toward lactate oxidation and a less aggressive phenotype. This finding is supp-

-orted by recent work from Wennerberg et al. (2020), who demonstrated that 12 weeks of moderate intensity exercise reduced the LDHA/LDHB ratio in patient derived breast cancer xenografts, correlating with reduced tumor proliferation and increased intratumoral $CD8^+$ T cell infiltration. Mechanistically, it has been hypothesized that exercise lowers circulating insulin and insulin-like growth factor 1 (IGF-1) levels, which are potent inducers of LDHA via the PI3K/Akt/mTOR pathway (Swain et al., 2022). Furthermore, some authors have proposed that exercise-induced increases in catecholamines and interleukin-6 (IL-6) may promote LDHB expression through activation of AMPK and PGC-1α, thereby facilitating lactate clearance and oxidative metabolism (Locasale, 2022). However, we did not measure any of these circulating factors or signaling molecules; therefore, these proposed mechanisms are speculative and require direct testing in future studies. Thus, even when LDHA is elevated (as in the Tu+Ex group), the concurrent or greater elevation of LDHB leads to a net reduction in the LDHA/LDHB ratio.

The clinical implications of these findings are twofold. First, the decrease in LDHA/LDHB ratio following exercise suggests that regular HIIT could rewire tumor metabolism away from aggressive glycolysis toward a more favorable, lactate oxidizing phenotype. This aligns with epidemiological evidence showing that physically active breast cancer survivors have lower recurrence rates and improved survival (Cormie et al., 2017; Friedenreich et al., 2020). Second, the divergent effect of nano selenium when combined with exercise versus alone raises caution. Although nano selenium alone increased the LDHA/LDHB ratio (potentially undesirable), it simultaneously upregulated LDHA in combination with exercise, yet the ratio still decreased. This indicates that exercise dominates the regulation of LDHB, overriding the ratio increasing effect of nano selenium. Future studies should directly measure lactate levels, pyruvate, NAD^+/NADH ratios, HIF-1α protein, ROS, and immune cell populations within tumors to confirm the functional consequences of these transcriptional changes and to validate the proposed mechanisms.

Several limitations of this study should be acknowledged. First, we only measured mRNA expression of LDHA and LDHB; protein levels and enzymatic activities were not assessed. Post translational modifications and allosteric regulation could alter the actual LDH isoenzyme activity ratio independently of transcript abundance. Second, we did not measure any of the proposed upstream signaling intermediates (e.g., HIF-1α, AMPK, ROS, catecholamines, IL-6, insulin/IGF-1). Therefore, all mechanistic interpretations presented in the Discussion are hypothetical and based on previously published studies rather than direct evidence from our experiments. Third, the use of a single tumor model (4T1), which is a triple negative breast cancer model, may limit generalizability to other breast cancer subtypes, particularly estrogen receptor positive or HER2 positive tumors that exhibit different metabolic dependencies. Fourth, we did not measure systemic (plasma) or intratumoral selenium levels. Such measurements are critical to confirm adequate absorption, tissue accumulation, and dose-response relationships of nano-selenium. Without these data, we cannot rule out inter-animal variability in selenium bioavailability, which may have influenced the observed additive effects with exercise. Future studies should include inductively coupled plasma mass spectrometry (ICP-MS) analysis of selenium concentrations in tumor tissue and blood. Fifth, the exercise protocol was not individualized based on maximal aerobic capacity; relative intensities were estimated from literature values and pilot data rather than empirically determined for each animal. This lack of individualized assessment may have introduced variability in the actual training stimulus and is a significant limitation of the study. Finally, the absence of a non-tumor control group prevents us from determining whether the observed changes are tumor specific or reflect a general metabolic response of mammary tissue.

Despite these limitations, this study provides the first evidence that HIIT and nano selenium differentially modulate the LDHA/LDHB ratio in breast tumor tissue. The finding that exercise reverses the ratio-increasing effect of nano selenium and promotes a lower LDHA/LDHB ratio is clinically promising. Future research should investigate the optimal timing, dose, and sequence of exercise and nano selenium administration, as well as explore the underlying mechanisms involving transcriptional factors (HIF-1α, c-Myc, p53) and epigenetic regulators using appropriate biochemical and molecular assays (e.g., Western blotting for protein expression, ELISA for cytokine levels, fluorescence-based ROS detection, and chromatin immunoprecipitation for transcription factor binding). Ultimately, randomized controlled trials in breast cancer patients are needed to determine whether exercise-induced reduction in the LDHA/LDHB ratio correlates with improved treatment responses to chemotherapy, immunotherapy, or radiotherapy.

Conclusion

In conclusion, this study demonstrates that four weeks of high-intensity interval training and nano-selenium supplementation each increase LDHA mRNA expression in breast tumor tissue, with an additive effect when combined. However, only nano-selenium alone elevates the LDHA/LDHB ratio, whereas exercise-based interventions (with or without nano-selenium) significantly decrease this ratio. These findings indicate that exercise and nano-selenium differentially shift the balance between LDHA and LDHB, potentially redirecting tumor lactate metabolism from a purely glycolytic toward a more oxidative phe-

-notype when exercise is involved. From a translational perspective, regular HIIT may counteract the unfavorable metabolic effects of Nano-selenium alone, suggesting that exercise training could serve as a safe and effective adjuvant strategy to modulate the metabolic landscape of breast tumors. Future studies should confirm these transcriptional changes at the protein and enzymatic activity levels and evaluate long-term tumor growth and metastasis outcomes.

What is already known on this subject?

It is already known that LDHA and LDHB play key roles in tumor lactate metabolism, and a high LDHA/LDHB ratio is associated with increased cancer aggressiveness and poor prognosis. Exercise and selenium supplements have each been shown to modulate tumor metabolism individually, but the combined effect of high-intensity interval training (HIIT) and nano-selenium on the LDHA/LDHB ratio in breast tumor tissue remained unknown.

What this study adds?

This study shows for the first time that both HIIT and nano-selenium upregulate LDHA mRNA expression in breast tumor tissue, but they exert opposite effects on the LDHA/LDHB ratio: Nano-selenium alone increases the ratio, whereas exercise -based interventions (with or without nano-selenium) decrease it. These findings suggest that exercise can shift the lactate dehydrogenase balance toward lactate oxidation and may serve as an adjunctive strategy to modulate breast tumor metabolism.

Organ Cross-Talk Tips:

- The study suggests that exercise reduces circulating insulin and insulin-like growth factor-1 (IGF-1), factors known to activate the PI3K/Akt/mTOR pathway and induce LDHA. By lowering these systemic signals, exercise may counteract the glycolytic drive in tumors, representing a liver-pancreas-tumor crosstalk axis.

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 approved by the Institutional Animal Ethics Committee and conformed to the ARRIVE guidelines and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th edition, 2011).

Informed consent Animal study.

Author contributions

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

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