SUPPRESSING BREAST CANCER BY EXERCISE: CONSIDERATION TO ANIMAL MODELS AND EXERCISE PROTOCOLS

Jeajun Lee1 / Suji Beak2 / Sang Hyun Ahn3 / Byung Seok Moon4 / Jisu Kim5* / Kang Pa Lee6*

1. Laboratory Animal Center, Osong Medical Innovation Foundation, Cheongju, Republic of Korea
2. Research and Development Center, UMUST R&D Corporation, Seoul, Republic of Korea
3. Department of Anatomy, Semyung University, Jecheon, Republic of Korea
4. Department of Nuclear Medicine, Ewha Womans University College of Medicine, Seoul, Republic of Korea
5. Physical Activity and Performance Institute, Konkuk University, Seoul, Republic of Korea

INTRODUCTION

Non-communicable diseases, as chronic diseases, account for 70% of the mortality rates worldwide, while communicable diseases cause the remaining 30%. Among non-communicable diseases, those with high mortality rates include cancer, diabetes, cardiovascular disease, and lung disease. Cancer is classified as a fatal disease for patients because it is difficult to cure owing to its rapid growth, and is highly likely to spread throughout the body through blood or lymphatic fluid. In particular, reduction of female physical activity due to various social environments is a potential cause of increased breast cancer incidence. In addition, the most frequent characteristic of breast cancer among women is not only high incidence, but also high efficiency of cancer treatment. Although the effectiveness of anti-cancer drugs is very high for breast cancer patients, a lot of pain, caused by chemotherapy, accompanies it. Therefore, there is an urgent need to improve the survival rate of breast cancer patients, and improve the quality of life during chemotherapy treatment. Recently, various preclinical studies have suggested that exercise attenuates tumor growth and tumorigenesis (Table 1). However, molecular mechanisms by which exercise affects cancer progression are not yet clear. In this review, we aimed to summarize studies on exercise methods that could potentially increase the survival rate of breast cancer patients and suppress cancer progression.

CONVENTIONAL BREAST CANCER THERAPY

Modern people often suffer from various diseases, which leads to death. In particular, the four major chronic diseases leading to death have been reported as cancer, diabetes, cardiovascular, and chronic lung diseases. According to the Cancer Society report, the most common cancer among women worldwide is breast cancer. Furthermore, the most common types of cancer in Korean women were breast cancer (19.9%), thyroid cancer (18.8%), colorectal cancer (10.5%), gastric cancer (9.2%), lung cancer (7.3%), and stomach and liver cancers (3.7%) were investigated according to a survey posted on the National Cancer Information Center (NCIC). Standard treatment methods such as various anti-cancer drugs and surgery are being developed, and alternative medical technologies for incurable diseases are also in development. Currently, there are four
main ways of cancer treatments: 1) surgery, 2) chemotherapy, 3) radiation therapy, and 4) hormone therapy. Prophylactic surgery suppresses the cancer progression by performing a biopsy for the purpose of diagnosis through surgery or removing the benign tumor completely. Surgery also prevents the spread of cancer to other cells in the body and helps relieve symptoms. Chemotherapy refers to the use of therapeutic agents for regulating hyperproliferative cells. Radiation therapy kills cancer cells by directly irradiating them. Hormone therapy that suppresses estrogen action is also used as a cancer treatment method, as breast cancer is affected by estrogen levels, unlike other cancers.

Exercise regulates the breast cancer in animal models by inhibiting carcinogenesis

Disease increase over the last two decades may be due to a more westernized lifestyle, which is accompanied by excessive nutrition and lack of exercise. Guidelines on cancer prevention are well known, and include recommendations for controlling metabolism, such as a balanced nutrient intake, eating vegetables, regulating vitamin intake, and controlling weight. Furthermore, exercise can prevent and treat various diseases, and in recent years, research on anti-cancer efficacy has been actively conducted.

Physical activities of Korean women are very low compared to women in other countries. Moreover, many women have adopted western food and a sedentary lifestyle, which has led to reduced voluntary exercise. The highest incidence of cancer among Korean women is breast cancer, and it has been suggested that breast cancer may be related to metabolic problems. Therefore, the effectiveness of exercise for the treatment or prevention of breast cancer should be investigated in future clinical studies.

An experimental laboratory animal is defined as an animal developed and improved for use in accordance with the purpose of test, diagnosis, education, research, and biological products in the research process. Among laboratory animals, primates such as Callithrix jacchus and Macaca fascicularis are most similar to humans; however, there exist issues regarding the ethics of conducting research using these animals. Rodents such as Mus musculus, Rattus norvegicus, and Cavia porcellus are the most commonly used experimental animals. In particular, Mus musculus has a genetic similarity with humans (approximately >80%), and a biologically similar body structure, a short pregnancy period (19 - 21 days) is also advantageous for preclinical studies. Therefore, Mus musculus has been used as a knockout mouse, cancer model xenograft, orthotopic model, and chemically induced-disease model. To develop a mouse model of breast cancer, it is necessary to have experimental cells, and patient derived primary cells such as MCF-7 (ER+, PR+, HER2-), MDA-MD-231 (ER+, PR+, HER2-), MDA-L2 (ER+), E0771 (ER+) and 4T1 (ER-) (Fig. 1).

The breast cancer animal model consists of chemically induced models, transgenic mice models, orthotopic mice models, and xenograft models. In the case of chemically induced breast cancer models, 7,12-dimethylbenzanthracene (DMBA; 1 mg/mL weekly, for six weeks) is injected subcutaneously into the side of the abdomen. Poly-aromatic structure of lipophilic molecule, DMBA has high carcinogen activity in the breast. To evaluate tumor progression, mice are established with genetic modifications that target the oncogene, such as simian virus 40 (SV40) T antigens and polymer middle T antigen (PyMT). In the establishment of mice models by injection with breast cancer cells, mice are mainly used in the study of tumor biology and pharmacology, as these models retain the biological properties of cancer. Breast cancer cells are injected into the mammary fat pad of host mice to obtain orthotropic models. In this case, the number of cells used is appropriate (1 x 10^5 to 1 x 10^6/mouse), and cancer cells injected into the mouse organs exhibit properties similar to breast cancer generated in the human body over time, and can be correlated to metastatic cancer. To develop a xenograft model, cells (1 x 10^6 to 1 x 10^7/mouse) are injected subcutaneously into the dorsal side of the mouse.

Using these various animal models, studies on the ben-
Application of breast cancer mouse models for exercise research

Table 1. Changes in blood variables before exercise and during post-exercise period.

| Mouse model | Mouse | Induction | Exercise | Protocols | Test | Efficacy & Signal pathways | Ref |
|-------------|-------|-----------|----------|-----------|------|---------------------------|-----|
| Xenograft   | NMRI-Foxn1™ | MCF-7 cell (ER+, PR+, HER2+) | Running | Voluntary wheel running (4 km per night/cage) | -Tumor growth | - MC-7 (~36%, P <0.05) and MDA-MB-231 (~66%, P < 0.01) tumor growth | 4   |
|             |       | MDA-MB-231 cell (ER+, PR+, HER2+) | Running |            | To evaluate the effect of exercise-conditioned serum in cancer cell | - Regulating Hippo signaling (ANKRD1 and CTGF) |
| Orthotopic  | FVB/NJ | p53/PTEN double-null (~/-) primary cell | Treating for 10 minutes once a day, for four weeks | Tumor growth | 52% reduced tumor size | 5   |
| Xenograft   | Female BALB/c | MC4-L2 cell (ER+, PR+) | Running | Using the treadmill: After acclimation, the interval exercise training protocol commenced at 16–18 m min−1, 0% gradient, for 10–14 min, 5 days each week for 6 weeks, and the exercise intensity was gradually increased each week | - Tumor volume mRNA expression & Protein expression | - Decrease tumor volume and weight | 6   |
| Orthotopic  | Female APOE™ | E0771 cell (ER+, PR+, HER2+) | Running | Voluntary wheel running | - Tumor growth & metastasis | - Increasing log phase tumor growth and inhibiting metastasis | 7   |
| Orthotopic  | FVB/NJ Female BALB/c Female C57BL/6 | C3(1)SV40Tag-p16-lu cell (Cludin-low breast cancer) | Running | Using the treadmill: After acclimation, 5 m/min for 5 min, 10 m/min for 5 min, 15 m/min for 5 min, and 20 m/min. for 45 min., which is equivalent to 70% VO2 peak. The exercise intensity was gradually increased each week for 2 weeks | - Tumor growth - Gene expression | - Reduced K167 expressions : E0771 (0.25 folds), C3(1)SV40Tag-p16-lu cell (same size) tumor size | 8   |
| Orthotopic  | Female C57BL6 | 4T1 cell (ER, PR, HER2) | Running | Wheels (running group) vs. without Wheels (sedentary group) | -Tumor growth, perfusion, hypoxia, and components of the antigenic and apoptotic cascades | - Statistically significantly reduced tumor growth and was associated with a 1.4-fold increase in apoptosis | 9   |

The beneficial effect of exercise against tumor growth and tumorigenesis of breast cancer have been extensively reported (Fig. 2; Table 1). Tumors are defined as transformed cells that undergo abnormal or rapid proliferation, beyond normal regulatory functions, in the organism. Tumors are divided into two types: benign neoplasms and carcinomas. Benign neoplasms have a relatively slow growth rate, and do not penetrate or spread into other tissues. In contrast, carcinomas rapidly grow, and invade other tissues and metastasize. The process of tumor development by carcinogenesis is a multi-step process. The first stage is initiation, where normal cellular DNA is attacked by carcinogens, leading to genetic modification and irreversible mutations. The second stage is promotion, wherein cell proliferation is actively performed to maintain and promote the population of mutant cells to counter immune response in vivo as it eliminates abnormal cells. The third step is progression, the process of increasing the characteristics of a malignant tumor by converting it from a benign tumor to a malignant tumor. In the process of tumor development, the morphology and function of normal cells are altered by genetic modification through internal or external stimulants. External factors include chemical carcinogens such as smoking, physical stimuli such as radiation, and RNA tumor viruses such as HTLV-1 virus. Internal factors involved in the mutation of the target gene include oncogene and tumor suppressor genes. Tumor suppressor genes include TGF-β, E-Cadherin, NF-1, PTEN, SAMD2, SMAD4, and p53, and regulate cell population through apoptosis and proliferation. Oncogene mutation targets are cell cycle regulatory genes such as cyclin D1, Her2, and K-ras. Many preclinical studies suggest that the beneficial effect of exercise training in cancer progression is brought about by direct regulation of intertumoral factors, i.e., tumor growth rate, metastasis, and tumor immunogenicity (Table 1).
### Application of breast cancer mouse models for exercise research

| Type     | Gender | Strain | Cell Line | Exercise | Duration | Endpoints                                                                                              | Notes                                                                                   |
|----------|--------|--------|-----------|----------|----------|-------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| **Xenograft** | Female | BALB/c | 4T1 cell  | Running  |          | - Tumor growth                                                                                         | - Exercise regulates tumor growth through immune cell responses                        |
|          |        |        |           |          |          | - Evaluating immune cell ratio                                                                        | - Exercise with radiotherapy reduces MDSCs accumulation and NK cell activation           |
|          |        |        |           |          |          |                                                                                                       |                                                                                        |
| **Orthotopic** | Female | BALB/c | 4T1 cell  | Running  |          | - Tumor growth                                                                                         | - HE inhibited tumor growth                                                              |
|          |        |        |           |          |          | - Evaluating apoptosis signals                                                                        | - HE combined with administration of didzein induces apoptosis of breast cancer           |
|          |        |        |           |          |          |                                                                                                       |                                                                                        |
| **Orthotopic** | Female | BALB/c | 4T1 cell  | Running  |          | - Tumor growth                                                                                         | - Beneficial effects of voluntary exercise on breast cancer progression                  |
|          |        |        |           |          |          |                                                                                                       |                                                                                        |
| **Orthotopic** | BALB/cBy |        | 4T1 cell  | Running  |          | - Tumor growth                                                                                         | - Running longer distances is associated with decreased breast tumor burden in old mice  |
|          |        |        |           |          |          |                                                                                                       |                                                                                        |
| **Xenograft** | Female | BALB/c | MCF-7 cell (ER, PR, HER2) |          |          | - Gene expression                                                                                      |                                                                                        |
|          |        |        |           |          |          | - Exercise decrease the IL-6, IL-18, TNF-a, CRP mRNA expression                                          |                                                                                        |
|          |        |        |           |          |          |                                                                                                       |                                                                                        |
| **Transgenic mice** | FVB/NJ C3(1)/SV40Tag | Genetically predisposed to develop breast cancer |          |          | - Voluntary physical activity (Running distance/ Speed) Tumor size                                      |                                                                                        |
|          |        |        |           |          |          | - C2(1)/SV40Tag mice < FVB/N mice C2(1)/SV40Tag mice > C2(1)/SV40Tag + exercise                      |                                                                                        |
|          |        |        |           |          |          |                                                                                                       |                                                                                        |
| **Transgenic mice** | p53-deficient (p53+/-): MMTV-Wnt-1 | Genetically predisposed to develop breast cancer |          |          | 1) voluntary wheel running ; Con=WHL, WHL (exercise)                                                  | - Con = TREX1 = TREX2 / Con=WHL                                                              |
|          |        |        |           |          |          | - p53 expression                                                                                       | - Con=TREX1=TREX2 / Con=WHL                                                                |
|          |        |        |           |          |          | - Incidence                                                                                           | - Con=TREX1=TREX2 / Con=WHL                                                                |
|          |        |        |           |          |          | - Multiplicity & survival                                                                             | - Con>TREX1=TREX2 / Con=WHL                                                                |
|          |        |        |           |          |          |                                                                                                       |                                                                                        |
|          |        |        |           |          |          |                                                                                                       |                                                                                        |
| **Orthotropic** | Athymic  |        | MDA-MB-231 cell |          |          | Voluntary wheel running running distance range -4 to -6 km/day for 15 weeks                        | - Survival - VEGF                                                                          |
|          |        |        |           |          |          | - HIF-1alpha expression                                                                                | - Con = Exercise                                                                            |
|          |        |        |           |          |          | - tumor metabolism                                                                                     | - VEGF expression: Con (48.6 pg/ml) > Exercise (47.0 pg/ml)                                |
|          |        |        |           |          |          |                                                                                                       | - HIF-alpha expression: Con (5.4% > Exercise (11.4%) Con (0.34 mmol/g) < Exercise (0.42 mmol/g) |
|          |        |        |           |          |          |                                                                                                       |                                                                                        |
| **Transgenic mice** | MMTV-PyMT Tg | Genetically predisposed to develop breast cancer |          |          | Voluntary wheel running                                                                               |                                                                                        |
|          |        |        |           |          |          | - Tumor growth                                                                                         |                                                                                        |
|          |        |        |           |          |          | - Heart mass / Spleen mass                                                                            | - Con < Exercise                                                                            |
|          |        |        |           |          |          | - cytokine expression                                                                                  | - CCL22 : Con> Exercise                                                                    |
|          |        |        |           |          |          |                                                                                                       | - CXCR4: Con < Exercise                                                                    |

---

*Physical Activity and Nutrition. 2020;24(2):022-029, http://dx.doi.org/10.20463/pan.2020.0011*
### Application of breast cancer mouse models for exercise research

| Model Type | Animal | Cell Line | Exercise Protocol | Results |
|------------|--------|-----------|------------------|---------|
| Orthotopic | Female BALB/c | 4T1 cell | Running | Treadmill running progressive time (10-15 min) and Speed (8-12 m/min) for 8 weeks - Decrease the carbohydrate oxidation in Exercise group - Up-regulated Ldhα, HKII, glut 1, HIF-1α, Mtor, p53, Lats2 expression |
| Xenograft | Female BALB/c | MC4-L2 cell (ER+) | Running | 6-18 m/min for 20-30 min for 4 weeks - Gene expression | The lowest level of IL-6, VEGF |
| Xenograft | Female BALB/c | 4T1 cell | Running | Endurance-trained for 8 weeks; mice exercised 5 days a week, for 8 consecutive weeks (In the 8th and final week the mice ran for 26 min a day, spending 1 min at 6 m/min, 1 min at 8 m/min, 22 min at 10 m/min, and 2 min 12 m/min.) - Gene expression | - Tumor growth - Gene expression |
| Xenograft | Female BALB/c | 4T1 cell | Running | Using the treadmill; After acclimation, 5 m/min for 5 min., 10 m/min. for 5 min., 15 m/min. for 5 min., and 20 m/min. for 45 min., which is equivalent to 70% VO2 peak. The exercise intensity was gradually increased each week for 2 weeks - Gene expression | - Anti-inflammation : IL-10/ TNF-α ratio and IL-15 expression |
| Xenograft | Female BALB/c | 4T1 cell | Running | Swim training 5 days/week for 4 weeks - Gene expression | - Th1 systemic response ; - Gata3 and Foxp3 |
| Xenograft | Female BALB/c | 4T1 cell | Running | 4 weeks of high-intensity interval training (HIIT) and saffron aqueous extract (SAE) supplementation - Gene expression | HIIT is associated with a reduced risk of cancer-related muscle wasting; SAE enhances the improvement of muscle loss and apoptotic indices |
| Xenograft | Female Balb/c | MC4-L2 cell | Running | Treadmill 16–18 m/min, 0% grade, 10–14 min, 5 days/week for 5 weeks - Gene expression | - miR-21 pathways; reduced IL-6 levels, NF-kB and STAT3 expressions & up-regulated TPM1 and PDCD4 expressions |
| Xenograft | Female C57BL/6 | EO771 breast tumor cell | Running | Reached maximum ethical size in wheel running (8 km per day) - Gene expression | - Tumor hyposia, perfusion, vascularity and proliferation unknown |
| Xenograft | Athymic | MDA-MB231 cell | Running | Voluntary exercise; The five-week period ranged from < 1 to 7.9 miles/day - Gene expression | - Inhibiting the growth of carcinomas |
| Chemical induced mouse model | Female Balb/c | 7,12-dimethylbenzanthracene (1 mg/ml weekly for 6 weeks) | Swimming | Physical training of swimming in water (30 ± 4°C) for 45 min (5 times per week for 8 weeks) - Gene expression | - Reduced Th1 cytokine increasing the Th2 cytokines and Treg cells |

ER: estrogen receptor, PR: progesterone receptor, HER2: receptor tyrosine-protein kinase erbB-2, PTEN: Phosphatase and tensin homolog, APOE: apolipoprotein E, FVB: Friend leukemia virus B, ANKRD1: Ankyrin repeat domain protein, CTGF: connective tissue growth factor, PI3K: phosphoinosidied 3-kinase, AKT: protein kinase B, ERK: extracellular signal regulated kinase, IL: interleukin, TNF-α: tumor necrosis factor α, CRP : C-reactive protein, VEGF: vascular endothelial growth factor, HIF-1α: hypoxia-inducible factor 1α, CCL2: C-C motif chemokine ligand 2, CXCR4: C-XC chemokine receptor type 4, Ldhα: lactate dehydrogenase A, HKII: hexokinase II, Glut 1: glucose transporter 1, Mtor : mammalian target of rapamycin, Lats2: large tumor suppressor kinase 2, CD8: cluster of differentiation 8, Foxp3: forkhead box P3, Gata3: GATA binding protein 3, Th : T helper cell, TPM1: tropomyosin alpha-1chain, PDCD4: programmed cell death protein

Table 1 summarizes the research methods used for controlling the intensity of exercise that underlies the exercise protocols using wheel running, treadmill, and swimming. These preclinical studies clearly demonstrate a decrease in...
tumor growth rate caused by exercise. Interestingly, Berrueta et al. demonstrated that exercise, such as stretching for 10 minutes once a day over a four-week period, reduced tumor size in a breast cancer model by 50%\(^5\). In other studies, voluntary exercise also inhibited tumor size and tumor growth\(^4,7,9,12,13,15,16,18,27\). Moreover, more studies have been conducted on endurance exercise than resistance exercise; endurance exercise has shown anti-tumor effects\(^6,8,10,11,21,22\). Taken together, these data suggest that the anti-cancer activity of the exercise protocols is involved in endurance and moderate-intensity exercise.

If so, which mechanism of exercise showed an anti-cancer effect? Results strongly suggest that exercise inhibits epigenetic modification of tumor cells, but enhances apoptosis and immune suppression\(^29\). Reactive oxygen species (ROS) perform signal transduction in vivo; however, excessive production can cause oxidative stress, which leads to cancer\(^30\). Moderate intensity exercise can regulate ROS and biological signaling in vivo\(^31\). It is likely that exercise is related to the regulation of the reactive oxygen species (ROS)-involved microenvironment of cancer\(^32\). Therefore, these studies also suggest that controlling ROS a potential mechanism for the treatment of cancer\(^33\).

However, this claim raises further questions as to why exercise is closely related to change in the microenvironment of cancer. One possible belief is that exercise can exert anti-cancer effects by solving problems that arise during metabolic processes. During carcinogenesis, most tumor cells exert cell growth signaling pathway via glucose metabolic reprogramming\(^34\). Recent study suggests that effective anti-cancer effect could be related to the regulation of metabolic syndrome\(^35\). The results supporting these claims are as follows: First, exercise can lead to activation of natural killer cell, lymphocyte, consequently resulting in the regulation of the tumor growth and metastasis\(^36\). In addition, exercise attenuates tumorigenesis and tumor progression\(^37\). Next, the
ketone diet (KD) is characterized by high fat, adequate protein, and very low carbohydrate compositions. Some studies have reported that the physiological phenomena caused by exercise or fasting are very similar to physiological conditions observed in the KD. Various preclinical studies have shown that exercise or the KD displays anti-cancer efficacy. Taken together, a possible hypothesis is that exercise-binding KD modulates metabolic dysfunction and causes internal factors, which involved in the mutation of the target gene such as ROS generation and tumor-suppressor gene mutations, thereby suggesting its potential as a cancer therapeutic.

However, the anti-cancer effects of KD and exercise can be contradictory. Acute exercise did not change tumor formation, but continuous steady aerobic exercise displayed effective anticancer effects. The general view presented in many studies is that exercise exerts an anti-cancer effect by reducing the size of tumors, promoting energy metabolism, and increasing immune activity by constant exercise. Therefore, further studies should investigate that find and apply an appropriate energy source for exercise that show anticancer efficacy.

**CONCLUSION**

Various preclinical studies have shown that exercise weakens tumor growth and tumor development. Moreover, these studies suggest that mice bearing breast cancer exhibited anti-cancer effects by increasing immune responses and anti-inflammatory factor levels through acclimation of increased exercise intensity every week. Thus, continuous exercise can have potential medical benefits as a prevention or therapeutic method for breast cancer. To facilitate this research, researchers need to study the etiological mechanisms that rely on clinical features with underlying pathological features of the disease, as well as based on mechanisms not necessarily present in patients. For example, using animal models to discover new treatments for a variety of diseases is an essential element in discovering new therapeutic targets and performing drug testing at the preclinical stage.

**ACKNOWLEDGMENTS**

This research was supported by a grant from the Osong Medical Cluster R&D Project funded by the Republic of Korea’s Health and Welfare (grant number HO15C0001). This paper was supported by the KU Research Professor Program of Konkuk University.

**REFERENCES**

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68:394-424.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin.* 2019:69:7-34.
3. Lee KP, Lee K, Park WH, Kim H, Hong H. Piperine inhibits platelet-derived growth factor-BB-induced proliferation and migration in vascular smooth muscle cells. *J Med Food.* 2015;18:208-15.
4. Dethlefsen C, Hansen LS, Lillevand C, Andersen C, Gehr J, Christensen JF, Pedersen BK, Hojman P. Exercise-induced catecholamines activate the hippo tumor suppressor pathway to reduce risks of breast cancer development. *Cancer Res.* 2017;77:4894-04.
5. Berruet A, Bergholiz J, Munoz D, Muskaj I, Badger GJ, Shukla A, Kim HJ, Zhao JJ, Langevin HM. Stretching reduces tumor growth in a mouse breast cancer model. *Sci Rep.* 2018;8:7864.
6. Alizadeh AM, Heydari Z, Rahimi M, Bazgir B, Shirvani H, Ali- pour S, Heidarian Y, Khalighfard S, Isanejad A. Oxycocin mediates the beneficial effects of the exercise training on breast cancer. *Exp Physiol.* 2018;103:222-35.
7. Buss LA, Dachs GU. Voluntary exercise slows breast tumor establishment and reduces tumor hypoxia in ApoE-/-mice. *J Appl Physiol.* 2012;114:338-49.
8. Glass OK, Bowie M, Fuller J, Darr D, Usary J, Boss K, Choudhury KR, Liu X, Zhang Z, Locasale JW, Williams C, Dewhirst MW, Jones LW, Seewaldt V. Differential response to exercise in claudin-low breast cancer. *Oncotarget.* 2017;8:100989-1004.
9. Betof AS, Lascola CD, Weitzel D, Landon C, Scarbrough PM, Devi GR, Palmer G, Jones LW, Dewhirst MW. Modulation of murine breast tumor vascularity, hypoxia and chemotherapeutic response by exercise. *J Natl Cancer Inst.* 2015;107:djv040.
10. Wennerberg E, Luhillier C, Rybstein MD, Dannenberg K, Rudqvist NP, Koelwyn GJ, Jones LW, Demaria S. Exercise reduces immune suppression and breast cancer progression in a preclinical model. *Oncotarget.* 2020;11:452-61.
11. Wang B, Xu H, Hu X, Ma W, Zhang J, Li Y, Yu M, Zhang Y, Li X, Ye X. Synergetic inhibition of daidzein and regular exercise on breast cancer in bearing-4T1 mice by regulating NK cells and apoptosis pathway. *Life Sci.* 2020;245:117387.
12. Smeda M, Przyborowski K, Proniewski B, Zakrzewska A, Kaczor D, Stojak M, Buczak E, Niekarz Z, Zoladz JA, Wietrzyk J, Chlopicki S. Breast cancer pulmonary metastasis is increased in mice undertaking spontaneous physical training in the running wheel; a call for revising beneficial effects of exercise on cancer progression. *Am J Cancer Res.* 2017;7:1926-36.
13. Goh J, Endicott E, Ladiges WC. Pre-tumor exercise decreases breast cancer in old mice in a distance-dependent manner. *Am J Cancer Res.* 2014;4:378-84.
14. Lee B, Chung W. Effects of aerobic exercise on cytokine expression in a breast cancer mouse model. *Iran J Public Health.* 2020;49:14-20.
15. Steiner JL, Davis JM, McClellan JL, Enos RT, Murphy EA. Effects of voluntary exercise on tumorigenesis in the C3(1)/SV40Tag transgenic mouse model of breast cancer. *Int J Oncol.* 2013;42:1468-72.
16. Colbert LH, Westerlind KC, Perkins SN, Haines DC, Berrigan D, Donehower LA, Fuchs-Young R, Hursting SD. Exercise effects on tumorigenesis in a p53-deficient mouse model of breast cancer. Med Sci Sports Exerc. 2009;41:1597-605.

17. Jones LW, Viglianti BL, Tashjian JA, Kothadia SM, Keir ST, Freedland SJ, Potter MQ, Moin EJ, Schroeder T, Herndon JE 2nd, Dewhirst MW. Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer. J Appl Physiol. 2010;108:343-8.

18. Goh J, Tsai J, Bammler TK, Farin FM, Endicott E, Ladiges WC. Exercise training in transgenic mice is associated with attenuation of early breast cancer growth in a dose-dependent manner. PLoS One. 2013;8:e80123.

19. Vulczak A, Souza AO, Ferrari GD, Azzolini AECS, Pereira-da-Silva G, Alberici LC. Moderate exercise modulates tumor metabolism of triple-negative breast cancer. Cells. 2020;9:628.

20. Shalamzari SA, Agha-Alinejad H, Alizadeh S, Shahbazi S, Khatib ZK, Kazemi A, Saei MA, Minayi N. The effect of exercise training on the level of tissue IL-6 and vascular endothelial growth factor in breast cancer bearing mice. Iran J Basic Med Sci. 2014;17:231-58.

21. Hagar A, Wang Z, Koyama S, Serrano JA, Melo L, Vargas S, Carpenter R, Foley J. Endurance training slows breast tumor growth in mice by suppressing Treg cells recruitment to tumors. BMC Cancer. 2019;19:536.

22. Molanouri Shamsi M, Chekachak S, Soudi S, Quinn LS, Ranjarb K, Chenari J, Yazdi MH, Madhavi M. Combined effect of aerobic interval training and selenium nanoparticles on expression of IL-15 and IL-10/Th2 ratio in skeletal muscle of 4T1 breast cancer mice with cachexia. Cytokine. 2017;90:100-8.

23. Bianco TM, Abdalla DR, Desidério CS, Thyrs S, Simoens C, Bogers JP, Murta EFC, Michelini MA. The influence of physical activity in the anti-tumor immune response in experimental breast tumor. Immunol Lett. 2017;190:148-58.

24. Ahmadabadi F, Saghebjoo M, Huang CJ, Saffari I, Zardast M. The effects of high-intensity interval training and selenium aqueous extract supplementation on alterations of body weight and apoptotic indices in skeletal muscle of 4T1 breast cancer-bearing mice with cachexia. Appl Physiol Nutr Metab. 2020;45:555-63.

25. Khori V, Amani Shalamzari S, Isanajad A, Alizadeh AM, Alizadah S, Khodayari S, Khodayari H, Shahbazi S, Zahedi A, Sohanaki H, Khaniyi M, Madhrian R, Saffari M, Fayad R. Effects of exercise training together with tamoxifen in reducing mammary tumor burden in mice: Possible underlying pathway of mTOR-21. Eur J Pharmacol. 2015;765:179-87.

26. Buss LA, Ang AD, Hock B, Robinson BA, Currie MJ, Dachs GU. Effect of post-implant exercise on tumour growth rate, perfusion and hypoxia in mice. PLoS One. 2020;15:e0229290.

27. Welsch MA, Cohen LA, Welsch CW. Inhibition of growth of human breast carcinoma xenografts by energy expenditure via voluntary exercise in athymic mice fed a high-fat diet. Nutr Cancer. 1995;23:309-18.

28. Abdalla DR, Murta EF, Michelini MA. The influence of physical activity on the profile of immune response cells and cytokine synthesis in mice with experimental breast tumors induced by 7,12-dimethylbenzanthracene. Eur J Cancer Prev. 2013;22:251-8.

29. McGee SL, Hargreaves M. Epigenetics and exercise. Trends Endocrinol Metab. 2019;30:636-45.

30. Perillo B, Di Donato M, Pezone A, Di Zazzo E, Giovannelli P, Galasso G, Castoria G, Migliaccio A. ROS in cancer therapy: the bright side of the moon. Exp Mol Med. 2020; 52:192-203.

31. Di Meo S, Napolitano G, Venditti P. Mediators of physical activity protection against ROS-linked skeletal muscle damage. Int J Mol Sci. 2019;20:3024.

32. He F, Li J, Liu Z, Chung CC, Yang W, Zuo L. Redox mechanism of reactive oxygen species in exercise. Front Physiol. 2016;7:486.

33. Kim SJ, Kim HS, Seo YR. Understanding of ROS-inducing strategy in anticancer therapy. Oxid Med Cell Longev. 2019;2019:5381692.

34. Lin J, Xia L, Liang J, Han Y, Wang H, Oyang L, Tan S, Tian Y, Rao S, Chen X, Tang Y, Su M, Luo X, Wang Y, Wang H, Zhou Y, Liao Q. The roles of glucose metabolic reprogramming in chemo- and radio-resistance. J Exp Clin Cancer Res. 2019;38:218.

35. Pothiwarala P, Jain SK, Yaturu S. Metabolic syndrome and cancer. Metab Syndr Relat Disord. 2009;7:279-88.

36. Dos Santos CMM, Diniz VLS, Bachi ALL, de Oliveira LCDS, Ghazal T, Passos MEP, de Oliveira HH, Murata G, Masl LN, Martins AR, Leveda-Pires AC, Curi R, Hirabara M, Sellitti DF, Pithon-Curi TC, Gorjão R. Moderate physical exercise improves lymphocyte function in melanoma-bearing mice on a high-fat diet. Nutr Metab. 2019;16:63.

37. Moreira VM, da Silva Franco CC, Prates KV, Gomes RM, de Moraes AMP, Ribeiro TA, Martins IP, Previtate C, Pavanello A, Maluissio CCI, Almeida DL, Francisco FA, Malta A, Tófolo LP, da Silva Silveira S, Saavedra LPJ, Machado K, da Silva PHO, Fabricio GS, Palma-Rigo K, de Souza HM, de Fátima Silva F, Biazi GR, Pereira TS, Vieira E, Miranda RA, de Oliveira JC, da Costa Lima LD, Rinaldi W, Ravanelli MI, de Freitas Mathias PC. Aerobic exercise training attenuates tumor growth and reduces insulin secretion in Walker 256 tumor-bearing rats. Front Physiol. 2018 May 8;9:465.

38. Zajac A, Poprzecki S, Maszczyk A, Czuba M, Michalczyk M, Zydak G. The effects of a ketogenic diet on exercise metabolism and physical performance in off-road cyclists. Nutrients. 2014;6:2493-508.

39. Tan-Shalaby J. Ketogenic diets and cancer: emerging evidence. Fed Pract. 2017 Feb;34:375-42S.

40. Weber DD, Aminazadeh-Gohari S, Koffer B. Ketogenic diet in cancer therapy. Aging (Albany NY). 2018;10:164-5.