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The Impact of Exercise Training on Breast Cancer

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Abstract. *Background/Aim: Physical exercise is increasingly considered by many authors to be a factor reducing the risk of cancer development and premature cancer-related death. Data indicate higher cure rates and longer times of survival in cancer patients who regularly exercise. Materials and Methods: A total of 50 female Sprague-Dawley rats were used in the experiment. Animals at 1 month of age were intraperitoneally injected with N-methyl-N-nitrosourea. Three months following drug administration, rats underwent supervised physical training. The animals were divided into four groups: control untrained group and 3 groups trained with different intensities – i.e. low, moderate and high. Routine histopathological examination of tumors was performed and mitotic activity was assessed by immunohistochemical expression of the Ki-67 antigen. Results: Ki-67 antigen expression was observed in all analyzed tumors. The increase in Ki-67 antigen expression correlated positively with the increase in training intensity. Conclusion: It can be assumed that low-intensity physical training is safe for patients with breast cancer. However, moderate- and high-intensity training may induce tumor cell proliferation worsening patients' prognosis.*

Breast cancer is still a major medical, social and economic problem due to the increasing prevalence and unsatisfactory treatment results. In 2013, nearly 235,000 new cases of breast cancer were reported in the United States, which accounted for 29% of all malignant neoplasms and was related to 14% risk of death. Based on current trends, over 23 million of new cases will be diagnosed by the year 2030 (1).

Numerous scientific reports indicate the impact of physical activity on the development of breast cancer. However, the results are not conclusive (2-9). Westerlind *et al*. found that in girls aged 10-15 who were more active than their peers with a sedentary lifestyle the risk for developing breast cancer decreased by 30-50% (2). Studies on animal models also confirm this thesis. Malicka *et al*. demonstrated that moderate intensity training reduced the number of induced tumors in rats (4). Other studies showed that in animals physically active at puberty (rodent treadmill, tunnels and ladders), the risk of developing the disease is decreased, the possible tumor development is delayed and smaller-sized tumors are observed (5-11). Currently, a longer survival time is observed in individuals after cancer treatment who regularly exercise (12-17). Holmes *et al*. demonstrated a reduction in the risk of death due to breast cancer in women who exercised (3-5 hours per week) after treatment compared to women with a sedentary lifestyle (18). Other research groups have confirmed these results (19-22). Walsh *et al*. and Daroux-Cole *et al*. suggested that exercise stimulated the immune system in cancer patients (23, 24). Fairey *et al*. found an increase in the number of NK cells in the blood of women who trained and had previously undergone breast cancer treatment (13). It should also be noted that Demarzo and Garcia demonstrated an increase of breast tumor incidence in rats after intense exercise compared to untrained rats (25). Experiments on rats are a recognized model of experimental breast cancer research. The mammary gland model of rodents shows a significant similarity to the human mammary gland. In rodents, terminal end buds are the basic structures forming the mammary ridge while in humans it is the duct lobular unit which forms the

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mammary gland (26). Both structures are similar in respect to development, architecture, function and sensitivity to carcinogens (9). It was also demonstrated that the body's response to physical exercise in the biochemical profile of blood in rats is adequate to the human profile (27).

The aim of the project entitled "Impact of physical training on the carcinogenesis and progression of rat mammary glands" was the assessment of the impact of physical training on the course of cancer, depending on the intensity of exercise training and the examined prevention model. The results related to the primary prevention were presented in Malicka *et al*. (4). In the present paper, the results related to the secondary prevention model will be provided.

Materials and Methods

Animals. Fifty female Sprague-Dawley rats (Medical University of Silesia, Katowice, Poland) were used in the experiment. All the conducted procedures were described in Malicka *et al*. (4). They were consistent with the European Union standards and the consent was issued by the Bioethics Committee of the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences (consent no 37/2010).

Induction of tumorigenesis. Animals at 1 month of age were intraperitoneally injected with 180 mg/kg N-methyl-N-nitrosourea (MNU) (Sigma-Aldrich, Darmstadt, Germany) as previously described (4, 28).

Physical training. Three months after the administration of MNU the rats were subjected to supervised physical training. The animals were divided into four groups *i.e.*: low intensity training (LIT; n=12), moderate intensity training (MIT; n=12) and high intensity training (HIT; n=12) groups and an untrained group *i.e.* sedentary control (SC; n=14). Physical training was conducted for five days per week on a 3-position treadmill (Exer 3/6, Columbus, OH, USA). The speed of the treadmill and the training duration gradually increased for each group, depending on the intensity of the training (Table I). The LIT parameters were reduced by 20% and the HIT parameters were increased by 20% compared to the MIT parameters.

Obtaining material and routine pathomorphological examination. At 6 months after the administration of MNU, the animals were sacrificed by intraperitoneal administration of 200 mg/kg pentobarbital, 60 mg/kg ketamine and 0.5 mg/kg medetomidine. All tumors detected on palpation in the animals were collected and measured. The obtained tissues were fixed in 4% buffered formalin and then embedded in paraffin blocks. Routine histopathological examination was performed on 6-μm-thick paraffin sections stained with hematoxylin and eosin (HE). Representative areas were selected by two independent researchers utilizing a double-headed BX41 light microscope (Olympus, Tokyo, Japan). The lesions were initially classified as benign or malignant, and then 6 major malignant tumors were identified, *i.e.*: papillar, tubular, planoepithelial, solid, cribriform and carcinosarcoma.

Table I. *Protocol of moderate-intensity training*.

Training phases [weeks]	1	2	3	4	5	6	7	8	9-12
Speed [km/h]	0.60	0.96	1.20	1.44	1.68	1.68	1.68	1.68	1.68
Duration [min.]	10	20	30	40	50	55	60	65	30

Table II. *Clinicopathological parameters of the study animals*.

	SC group (n=8)	LIT group (n=9)	MIT group (n=9)	HIT group (n=6)	*p*-Value
Mean body weight at the administration of MNU [g]	133.87 ±11.67	118.88 ±32.57	118.88 ±39.19	97.50 ±5.68	0.15
Mean dose of MNU [ml/kg]	2.41 ±0.19	2.15 ±0.49	2.10 ±0.55	1.76 ±0.10	0.12
Mean body weight at euthanasia [g]	311.00 ±35.58	307.77 ±30.31	292.77 ±25.49	319.83 ±52.36	0.6
Total number of tumors	24	10	12	21	0.54
Histological type					
Cribriform	12	3	6	15	
Papillar	1	4	3	1	
Tubular	8	2	2	4	
Solid	3	0	0	1	
Carcinosarcoma	0	0	1	0	
Planoepithelial	0	1	0	0	
Total number of rats with tumors	7 (87.5%)	6 (66.6%)	9 (100%)	4 (66.6%)	
Total number of tumors per rat	3.00	1.22	1.44	3.5	
Total volume of tumors [mm^3]	2853.81± 4698.39	907.83± 1287.19	1664.16± 2024.16	3107.67± 5503.22	0.8
Total mass of tumors (per rat) [mg]	356.70	100.87	184.90	517.94	
Mean volume of tumors [mm^3]	849.37± 1138.61	670.69± 1130.41	1193.27± 1581.44	453.57 ±519.68	0.75
Mean volume of tumors (per rat) [mm^3]	106.17	74.52	132.58	75.59	

Tissue microarray (TMA) preparation. Tissue microarrays were prepared using a 2-mm-gauge needle and Manual Tissue Arrayer I (MTA, Beecher Instruments Inc., Sun Prairie, WI, USA) as previously described (4).

Immunohistochemistry (IHC). All reactions were performed, as previously described, on 4-μm-thick TMA sections in an automated Autostainer Link48 staining platform (Dako, Glostrup, Denmark) in order to ensure constant reaction conditions. Deparaffinization, rehydration, and antigen retrieval were performed by boiling the sections in Target Retrieval Solution buffer (Dako, Glostrup, Denmark) using a Pre-Treatment Link Platform (Dako, Glostrup, Denmark). The sections were then washed in a TBS/0.05% Tween buffer followed by 5-min incubation with the EnVision FLEX

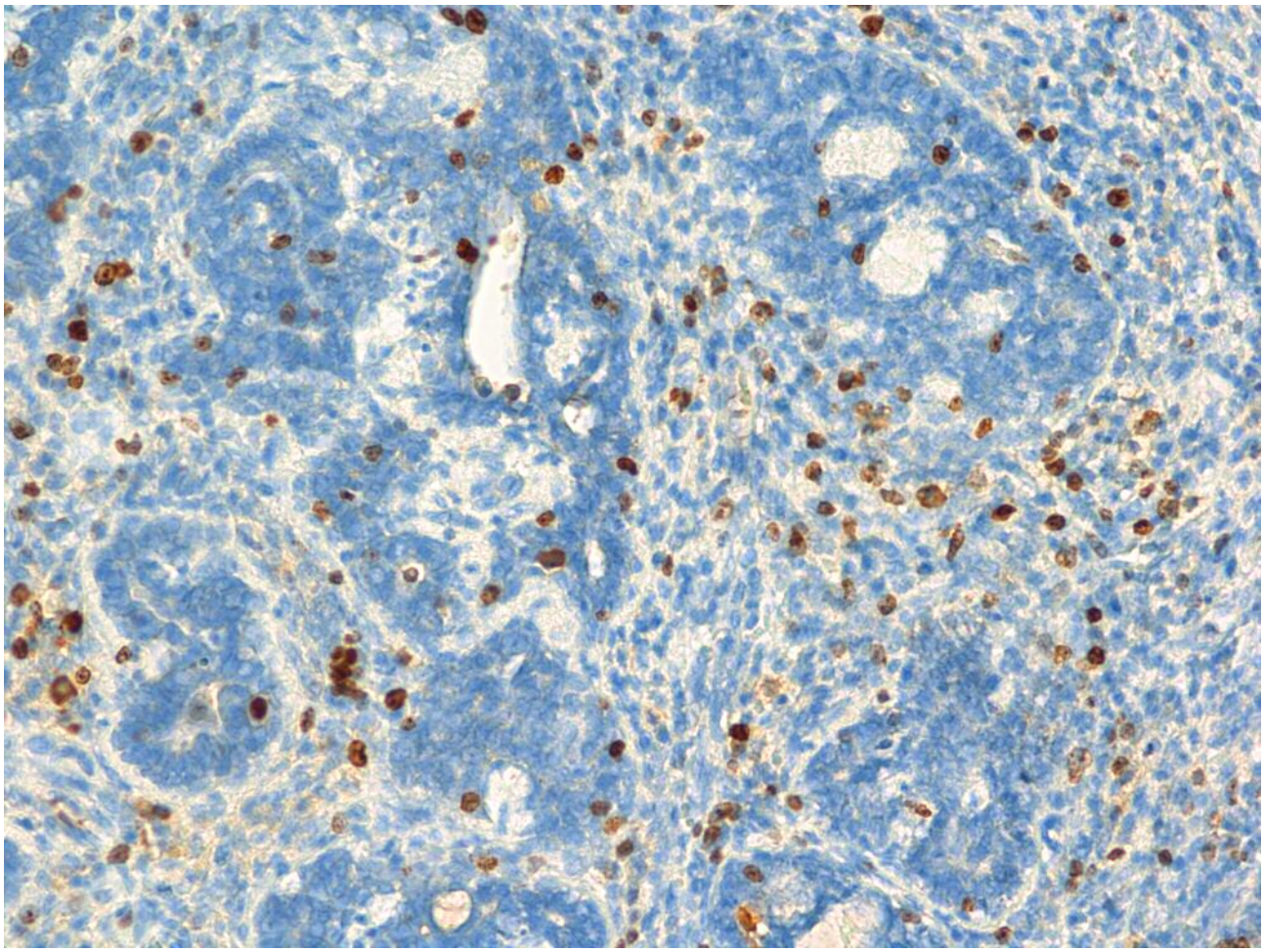

Figure 1. *Immunohistochemical expression of Ki-67 proliferation antigen. Magnification ×200.*

Peroxidase-Blocking Reagent to block the endogenous peroxidase activity. Subsequently, the sections were rinsed in the TBS/0.05% Tween buffer and incubated with a primary antibody directed against the Ki-67 antigen (MIB-5, Dako Glostrup, Denmark). The sections were then washed in the TBS/0.05% Tween, followed by incubation (20 min at room temperature; RT) with EnVision FLEX/horseradish peroxidase (HRP)-conjugated secondary antibodies (Dako, Glostrup, Denmark). The substrate for peroxidase, diaminobenzidine (Dako, Glostrup, Denmark), was then applied and the sections incubated at RT for 10 min. Finally, the sections were rinsed and counterstained with Mayer's hematoxylin, dehydrated in alcohol (70%, 96%, 99.8%) and xylene, and mounted using the SUB-X Mounting Medium (Dako, Glostrup, Denmark).

Evaluation of IHC reactions. The IHC sections were evaluated under a BX41 light microscope equipped with Cell^{D} software for computer-assisted image analysis (Olympus). For the evaluation of Ki-67 antigen in the TMA sections, three fields with the highest number of tumor cells yielding a positive reaction were selected (hot spots). The percentage of positive cells (brown-labeled nuclei) was evaluated by scoring labeled cell in relation to all cancer cells under ×400 magnification. The final score consisted of three hot spot values for every tumor.

Statistical analysis. Statistical analysis was performed using Statistica 10.0 (Statsoft, Cracow, Poland) and Prism 7.0 (GraphPad, La Jolla, CA, USA). Shapiro-Wilk, Levene's, Mann-Whitney and Kruskal-Wallis tests were used for the calculations.

Results

Pathomorphology. In both, the LIT and MIT groups the training was completed by 9/12 rats while 6/12 and 8/14 animals completed the training in the HIT and SC groups, respectively. After the experiment, 24 tumors were detected in 7/8 rats in SC, 10 tumors in LIT in 6/9 rats, 12 tumors in MIT in all animals, and 21 tumors in HIT in 4/6 rats (Table II).

Immunohistochemistry. The expression of Ki-67 was observed in nuclei of tumor cells (Figure 1). In the secondary

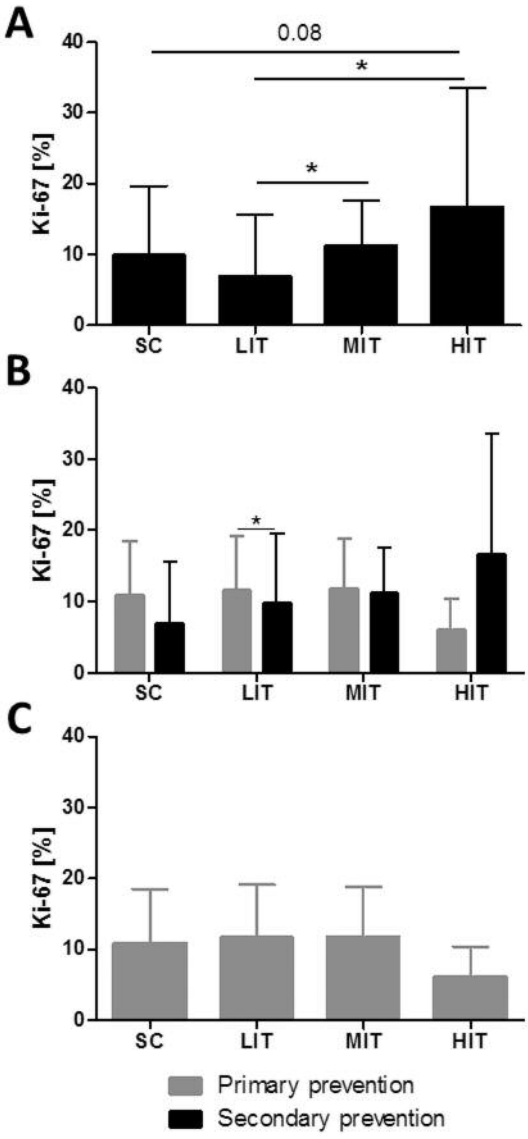

Figure 2. *The expression of Ki-67 antigen in low- (LIT), moderate- (MIT), and high-intensity training (HIT) and untrained (SC) study groups. The secondary prevention model (A), the comparison between the secondary and primary prevention models (B) and the primary prevention model (C) (subject of our previous study; 4).*

prevention model, we observed an evident trend to increasing Ki-67 antigen expression together with physical exercise (Figure 2A). The weakest Ki-67 expression was observed in LIT and in relation to MIT and HIT the differences were statistically significant. In addition, the strongest expression was found in the HIT group. In relation to the SC the difference between groups approached the level of statistical significance (p=0.08). The comparison of Ki-67 expression between the groups in the secondary and primary prevention models showed a statistically significant difference only between the animal groups undergoing low intensity training (Figure 2B).

Discussion

Low physical activity (<4 Metabolic Equivalent of Task [MET]/week) can result in overweight and obesity. These conditions, in turn, are associated with an increased risk of developing type 2 diabetes mellitus, arterial hypertension, atherosclerosis, ischemic heart disease and cancer, including breast cancer. Nielson *et al*. in their analysis demonstrated that overweight and obesity resulted in an increase in sex hormone levels, insulin resistance, and an increase in the levels of inflammatory factors, thereby increasing the risk for the development of breast cancer (4, 29).

Many authors report in their studies that high physical activity in individuals after breast cancer treatment can slow the disease progression and increase the cure rates. Irwin *et al*. demonstrated that high physical activity (≥9 MET/week) in women treated due to breast cancer and its increase from low to high after treatment reduced the risk of cancer-related death by 39% (21, 30). Studies of other teams confirmed these observations (18, 31-34). Holik *et al*. (19) in their studies demonstrated that in women an increment of 5 MET-hours per week was associated with 15% lower risk of breast cancer related death. McTiernan *et al*. (35) found that regular moderate physical activity for 12 months reduced the risk for developing breast cancer.

Goh *et al*. demonstrated that physical exercise could reduce the risk of breast cancer and increase survival rates. Modulation of the immune system by aerobic physical training or beneficial effects of myokines can be the mechanism of this phenomenon (36, 37). Fairey *et al*. examined the increase in NK cell activity due to physical training. Despite its growth, the authors did not find statistically significant differences between trained and untrained groups (13). Hutnick *et al*., Demarzo *et al*., as well Mathur and Pedersen showed an increase in lymphocytes count and their activity in the peripheral blood together with increased physical activity (12, 15, 38). Lima *et al*. compared the tumor mass and cell proliferation in trained and untrained rats. In the group of trained rats, tumor mass and proliferation were statistically significantly lower than in the group of untrained rats (39). Reports about the impact of the varying intensity of exercise on carcinogenesis are inconclusive. Westerlind *et al*. indicated an increase in both proliferation and apoptosis in animals which trained with moderate intensity (2). Other authors showed that low-intensity physical training is carcinogenic, whereas moderate and high intensity training (over 35% and 70% of maximum intensity) inhibited the growth of tumor cells (40-42). Cohen *et al*. in their study stressed the

protective activity of moderate intensity training (43). According to those authors, low and high intensity of training resulted in an increase in the prevalence of tumor changes. Similarly, Saez *et al*. indicated the carcinogenic effect of intense exercise training (44). This thesis was confirmed by the results of our study. As the exercise intensity increased, the increase in Ki-67 antigen expression was observed (Figure 2A). The inverse relationship was noted in the primary prevention model (Figure 2C). Statistically significant differences were also observed between the groups of rats which trained with different intensity. The lowest Ki-67 antigen expression was observed in the LIT group, the results of which were statistically significantly different from the MIT and HIT results (Figure 2A). In addition, differences in Ki-67 antigen expression were observed between the primary and secondary prevention models. In the secondary prevention model, it was statistically significantly lower in the LIT group (Figure 2B). Most attention is paid to the imbalance between the processes of proliferation and apoptosis, which is affected by the frequency, duration and intensity of physical exercise. Research is continued to identify specific repetitive relationships that could be applied in the prophylaxis and in supportive therapy in cancer patients (44-46).

Conclusion

Based on the obtained results, it can be assumed that low-intensity physical training is safer for breast cancer subjects. Moderate and high-intensity training, however, can increase the proliferation of tumor cells, thus, being a risk factor for cancer severity. Further studies are needed to confirm the obtained results.

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