Research Article

PGC1-α mRNA expression cross talk with tumor volume growth and total antioxidant capacity in breast cancer model mice: following discontinuous aerobic exercise and vitamin D intake

Ali Jafari[1], Dariush Sheikholeslami-Vatani[1*], Neda Khaledi[2], Farnoosh Khosrobakhsh[3]

Abstract

The modifications of PGC-1α induce the change of the carcinogenesis and tumor growth and lead to increased antioxidant enzymes. The present study aimed to determine the cross talk between PGC1-α mRNA expression, tumor volume growth, and total antioxidant capacity in breast cancer model mice, followed by discontinuous aerobic exercise and vitamin D. In the present study, 40 female NMRI mice were randomly assigned into five equal groups (n=8): healthy control group (H.C), cancer control group (Ca.C), cancer with the vitamin D group (U.Ca.VD), cancer exercise training group (Ca. Ex), and cancer exercise training with the vitamin D group (Ca.Ex.VD). As the results indicate, the bodyweight of cancer groups (p=0.041, F=3.61) and the tumor growth rate significantly reduced compared to the H.C group. The results indicated that the PGC-1α mRNA expression and TAC (p=0.013, F=5.16) change significantly different between the study groups. Besides, based on the results, a significant positive correlation was observed between PGC1-α and tumor volume growth among the groups, whereas a negative relationship exists between PGC1-α and TAC and among TAC and tumor volume growth only in the Ca. Ex.VD group. The correlation between the variables confirms using vitamin D treatment with the implementation of discontinuous aerobic exercise, as a synergistic effect, improves the total antioxidant capacity and is effective in controlling tumor growth. We recommend that further studies be done on exercise training along with supplementation intake synergistic.

Key Words: **Breast cancer, Discontinuous exercise training, Vitamin D, PGC1-α mRNA, Total antioxidant capacity**

1. Department of Physical Education and Sport Sciences, University of Kurdistan, Sanandaj, Iran. 2. Collage of Physical Education and Sport Sciences, University of Kharazmi, Tehran, Iran. 3. Department of Biological Sciences, University of Kurdistan, Sanandaj, Iran.

*Author for correspondence: d.vatani@uok.ac.ir

A J: 0000-0002-6280-7622; D SH-V: 0000-0002-9771-8806; N KH: 0000-0002-3859-513X; F KH: 0000-0002-7250-4537

Received 28 April 2022; Accepted 20 June 2022

Introduction

Peroxisome proliferator-activated receptor γ (PPARγ) belongs to a nuclear hormone receptor superfamily that regulates the expression of genes involved in cell differentiation, proliferation, the immune/inflammation response, and lipid metabolism (Watkins et al., 2004).Two PPARγ isoforms are known as PPARγ1 and PPARγ2 (Fajas et al., 1997;Tontonoz et al., 1994). The PPARγ coactivator-1 (PGC-1) family is composed of PGC-1α, PGC-1β, and PGC-1-related coactivator (PRC). PGC-1α was initially identified as a transcriptional coactivator involved in mitochondrial function and thermogenesis in brown fat (Puigserver et al., 1998). Also, PGC-1α was described initially as a PPARγ interacting protein. Some investigators have recently studied the expression and clinical significance of PGC-1α in cancer (Jafari et al., 2021; Shiota et al., 2010;Yu et al., 2013). PGC-1α is one of the different types of PPARγ that is expressed in mitochondria and its role has been proven in energy metabolism, apoptosis, and stressful conditions of mammalian cells (Guo, 2021).

PGC-1α is highly expressed in the mitochondria and tissues in response to the high demand for energy-related to energy metabolism, mitochondrial biogenesis, homeostasis, and other biological pathways (Jones et al., 2012; Patti et al., 2003; Puigserver et al., 1998). Recent studies have clarified the expression levels of PGC-1α related to cancer progression, proliferation, invasion, and metabolic pathways via modulation of mitochondrial function in diverse cancers (LeBleu et al., 2014; Weikum et al., 2018; Zhang et al., 2007). Literature works supporting the tumor-promoting functions of PGC-1α have increased (Andrzejewski et al., 2017; Lee et al., 2011). Shiota et al. (2010) showed that PGC-1α promotes cell growth through the activation of androgen receptors in prostate cancer cells by observing cell growth inhibition with PGC-1α knockdown experiments. Besides, PGC-1α increased in tumor samples from arsenic-induced skin cancer patients and was associated

with increased cell proliferation and enhanced mitochondrial biogenesis (Lee et al., 2011). Bhalla et al. (2011) showed that PGC-1α promotes carcinogenesis and tumor growth through the induction of lipogenic enzymes (acetyl-CoA carboxylase and fatty acid synthase) using genetically modified PGC-1α mice. Other study demonstrated that PGC-1α knockout mice had decreased chemically induced liver and colon carcinogenesis, suggesting that PGC-1α stimulates carcinogenesis. Similarly, Shin et al. (2015) first demonstrated that overexpression of PGC-1α enhances cell proliferation and tumorigenesis via the upregulation of Sp1 and acyl-CoA binding protein. It was also reported that PGC-1α overexpression leads to increased antioxidant enzymes (catalase, superoxide dismutase) and decreased ROS-induced apoptosis (Shin et al., 2015). Similarly, PGC-1α knockdown significantly decreased the T cell number and induced apoptosis in PGC-1α-positive melanoma cell lines, suggesting that PGC-1α is crucial in the survival of PGC-1α-positive melanoma cells (Vazquez et al., 2013). Moreover, superoxide dismutase 2 protein levels were decreased in PGC-1α depleted melanoma cells. In addition, ectopic expression of PGC-1α in melanoma cells increased the expression of ROS detoxifying genes. These data support the hypothesis that PGC-1α plays an important role in activating the ROS detoxification gene program to maintain melanoma cell survival (Vazquez et al., 2013). Now, all strategies related to the incidence of cancer, including prevention, treatment of interventions face development challenges. Today, exercise training and dietary supplements are considered prevention and treatment approach.

Observational studies have shown that increased activity levels are associated with a lower risk of different types of cancer (McGuirk et al., 2013; Tennakoon et al., 2014). Both pre-and post-diagnosis physical activity is further associated with reduced cancer-specific and overall mortality in patients suffering from colorectal-, breast-, and prostate cancer (Haq et al., 2013; Lee et al., 2009; Taguchi et al., 2014). Besides maintenance or improvements in physical capacity, exercise interventions have been proven to reduce the frequently observed side effects of cancer and their medical treatments such as fatigue, polyneuropathies, depressions, lymphedema (Fisher et al., 2011; LeBleu et al., 2014; Lee et al., 2009). Malicka et al. (2015) demonstrated that moderate-intensity training reduced the number of induced tumors in rats. Other studies showed that in animals physically active at puberty (rodent treadmill, tunnels, and ladders), the risk of developing the disease decreases, the possible tumor development is delayed and smaller-sized tumors are observed (Alvarado et al., 2016; Faustino-Rocha et al., 2016; Steiner et al., 2013; Sturgeon rezi et al., 2017; Wang et al., 2009; Westerlind et al., 2003). Currently, a longer survival time is observed in individuals after cancer treatment who regularly exercises (Demarzo et al., 2008; Fairey et al., 2005; Hutnick et al., 2005; Jones et al., 2009; Schlotter et al., 2008; Singh et al., 2005). It should also be noted that Demarzo and Garcia (2004) demonstrated an increase in breast tumor incidence in rats after intense exercise compared with untrained rats. Experiments on rats are a recognized model of experimental breast cancer research. Consequently, a vast body of literature suggests that exercise interventions during and after medical treatment improve the quality of life in patients with cancer.

Vitamin D is one of the key controllers of systemic inflammation, oxidative stress, and mitochondrial respiratory function which reduces cell proliferation and enhances cell differentiation-key anti-cancer effects of vitamin D (Watanabe et al., 2015; Wimalawansa, 2019). Studies have demonstrated alterations of gene expression levels linked to tumorigenesis that are influenced by the vitamin D status (Wu et al., 2013). In addition, vitamin D markedly influences the regulation of cell replication (O'Malley et al., 2014; Ramnath et al., 2014). Besides, gene activation following the interaction of 1, 25(OH) 2D with VDR is important for mitochondrial integrity and respiration, and many other physiological activities. Moreover, the vitamin D signaling pathway plays a central role in protecting cells from elevated mitochondrial respiration and associated damage and overproduction of reactive oxygen species (ROS) (Ricca et al., 2018). Peroxisome proliferator-activated receptor-coactivator 1α (PGC-1α) is bound to mitochondrial deacetylase (SIRT3). PGC-1α directly couples to the oxidative stress cycle (Chen et al., 2011) and interacts with Nrf2. This complex regulates the expression of SIRT3; vitamin D metabolites influences this process (Song et al., 2017). Besides, the activation of the mitochondrial PGC−1α-SIRT3/Nrf2 path is dependent on intracellular calcitriol concentration (Wimalawansa, 2019). Along this way, this study analyzed the cross talk between PGC1-α mRNA expression, tumor volume growth, and total antioxidant capacity in breast cancer model mice, followed by discontinuous aerobic exercise and vitamin D.

Materials and Methods

Animals and Experimental Design

In our study, sixty female NMRI mice weighing 24±2 gr, aged 4 to 5 weeks were purchased from Razi Vaccine and Serum Research Institute, Iran. Mice were housed at the Kharazmi University animal laboratory on Polycarbonate cages under controlled environmental conditions (12/12 h light/dark cycle, TM;

23°C, HM; 42°) with free access to food and water. First, cell culture was done as much as the injectable dose. Then, to delay the metastasis stage 4, mice were piloted to determine the best dose to induce the 4T1 mouse breast cancer cell line to tumorigenesis for two weeks which is described below. After two weeks of determining the appropriate dosage and familiarization with the environment and cell culture, 8 mice were randomly isolated as healthy control (H.C) and kept in the same conditions as the other animals until the end of the study period without any treatment. After the pilot stage (n=4) and induction of 4T1cell line (n=48), no clear breast cancer tumors were observed in 12 animals and they were excluded from the study design. Then, 36 animals with breast cancer (along with 8 mice in the untrained healthy control group) were randomly divided into the 5 groups: (I) untrained healthy control (U.H.C), (II) untrained cancer control (U.Ca.C), (III) cancer with vitamin D supplementation (Ca.VD), (IV) Cancer with exercise training (Ca.Ex), and (V) Cancer with exercise training and vitamin D supplementation (Ca.Ex.VD). It should be noted that two mice from the Ca.Ex.VD group (six mice remained) and one of the Ca.Ex group (seven mice remained) died during the treatment. 48 hours after the last exercise training bout, the animals were sacrificed by intraperitoneal administration of 20-30 mg/kg ketamine 10% and 2-3 mg/kg xylazine 2%. Body weight was recorded weekly during the study. The present study was conducted according to the biosafety guidelines of the World Health Organization regarding laboratory animals and approved by the university's biosafety committee (Approval ID: IR.UOK.REC.1397. 026).

Cell Culture and Induction of Mouse Model Breast Cancer

The mouse breast cancer cell (4T1; NCBI No: C604) was obtained from the cell bank of Pasteur Institute of Iran. Cell lines were initially cultured according to the instructions. Mice in cancer groups were subcutaneously injected with 1×105 4T1 cells into the right flanks, and sterilized distilled water with the same volume was injected subcutaneously into a healthy control group (Yu et al., 2013).

Discontinuous aerobic exercise (DAE) was performed for 6 weeks, three sessions per week, on a rodent treadmill (Danesh Salar of Iranian Co, IRI) in the evening (from 5:00 PM to 7:00 PM). Training intensity (running speed on the treadmill) and duration gradually increased every two weeks for the training groups. Due to the running speed and treadmill slope (zero degree), the intensity of training in the present protocol was equal to 50-75 percent of maximal oxygen consumption (VO2max) in mice (Atoum et al., 2017). The rest period between exercise bouts was considered 1:4 (Wolin et al., 2012).

Previous studies have confirmed the anti-cancer effects of vitamin D, (Jones et al., 2010). In the current study, vitamin D (Sun Vit®, Iran Hormone) was purchased in the form of ampoules and mixed with sesame oil (5 µg vitamin D+150 µl sesame oil) to be injected subcutaneously into the target groups (Ca.VD and Ca.Ex.VD) once every two days. Other groups (Ca.C and Ca.Ex) except the healthy control group (H.C) were treated with the same volume of sesame oil as a placebo.

Tumor Volume Measurement and Tissue and Blood Sampling

Tumor volume was measured in two dimensions. The largest dimension of the tumor was considered as the length of the tumor (L), and the other dimension (at 90°) was considered as the width of the tumor (W). At the end of each week, the length and width of the cancerous tumor were measured by a digital caliper (EKO; TURBO, NO: EDC-20), and the tumor volume was calculated according to the following formula [V=1/2 (L2 × W)] (Atoum et al., 2017).

To blood sampling and tissue procurement, the animals were first anesthetized by subcutaneous injection of ketamine-xylazine. Blood samples were then taken directly from the animal's heart (1.5 ml). The blood was then placed into tubes containing EDTA and centrifuged at 3000 g for 10 minutes, and after serum separation, it was kept at -20°C until the final assay. Immediately following blood sampling, the heart was completely removed and after weighing it was fixed in buffered formalin (10%) for 24 hours. Cardiac tissue was eventually stored at -80°C for further evaluation.

Total RNA Extraction and Real-time PCR Analysis

Total RNA was extracted from cardiac tissue using the RNX-PLUS reagent method (SINACLON, Cat. No: EX6101). In brief, after the myocardial tissue has reached 0°C from a frozen state, about 30 to 50 mg of the cardiac tissue is homogenized using a hand mixer (NS-16010) by adding liquid nitrogen. The homogeneous tissue was then incubated for 5 minutes at room temperature. Then, all stages, extraction RNA, a PCR thermal cycler (BIORAD T100), the real-time thermal cycling system (Corbett 5Plex Rotor-gene 6000; Qiagen), were done according to the instructions. Primer pairs for target genes were obtained from metabion (© 184 metabion international AG, Germany).

Table 1. Primer sequence (5'⟶3')

		(5'⟶3')
PGC-1α	Forward	TGAACTAAGGGATGGCGACT
	Revers	AAGAAGGCGACACATCGAAC
Gapdh	Forward	AAATGGTGAAGGTCGGTGTG
	Revers	GAATTTGCCGTGAGTGGAGT

All primers were checked for specificity to the genes of 185 interests by Blast analysis (Table 1).

Statistical analysis

After determining data normality, the Pearson correlation was used to determine the correlation between PGC-1α gene expression, tumor volume growth and total antioxidant capacity (TAC). Eventually, the obtained data were analyzed by the SPSS software, version 22. All data were presented as means ±SD and p≤ 0.05 was considered statistically significant in all tests.

Results

As the results indicate, the bodyweight of cancer groups significantly reduced compared to the U.H.C group after six weeks (p=0.041, F=3.61) (Table 2).

Tumor volume measurement was performed in six weeks of implementation (Figure 1).

The findings demonstrated that the tumor growth rate of the Ca. Ex.VD group was less than the other groups. The results of fold changes in the expression level of the PGC-1α mRNA indicated that these changes were significantly different between the study groups (Figure 2).

Furthermore, the results of the TAC assay revealed that there was a significant elevate in serum total antioxidant capacity in the cancer groups under treatment: U.Ca.VD, Ca. Ex, and Ca. Ex.VD (p=0.013, F=5.16) (Figure 3).

Additionally, the results reveal a significant positive correlation between PGC1-α and tumor volume growth and TAC among the groups, whereas a negative relationship between PGC1-α and TAC in the Ca. Ex.VD group. Also, the results presented a negative correlation between TAC and tumor volume growth in the Ca. Ex.VD group. Results demonstrated a decrease of 11.68% of tumor growth in this group. The results of the correlation are represented in Table 3.

Discussion

The results of the current study indicated that discontinuous aerobic exercise and vitamin D induce changes among variables

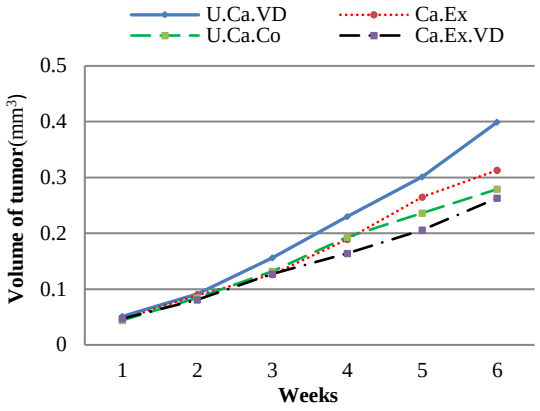

Figure 1. Average tumor volume is plotted relative to the number of weeks during six weeks of discontinuous exercise training (DET) and vitamin D supplement intervention in four cancer groups. U.Ca.C= Untrained Cancer Control Group, U.Ca.VD= Untrained Cancer with Injection of Vitamin D Group, Ca.Ex= Cancer Exercise Training Group, Ca.Ex.VD= Cancer Exercise Training with Injection of Vitamin D Group. Data are presented as Mean ± SD. *Significance level (P≤0.05).

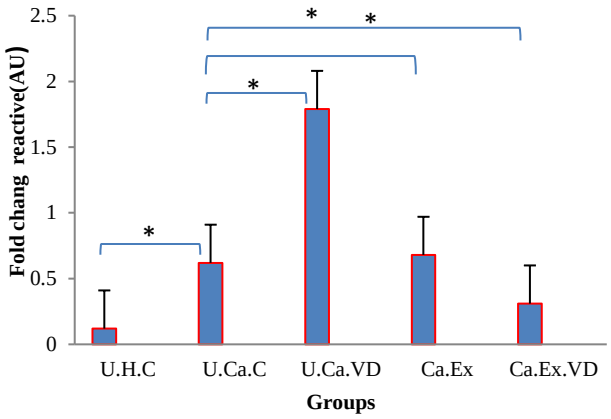

Figure 2. The effect of 6 weeks of discontinuous exercise training (DET) and vitamin D supplement on PGC-1α mRNA expression in five experimental groups. U.H.C= Untrained Healthy Control Group, U.Ca.C= Untrained Cancer Control Group, U.Ca.VD= Untrained Cancer with Injection of Vitamin D Group, Ca.Ex= Cancer Exercise Training Group, Ca.Ex.VD= Cancer Exercise Training with Injection of Vitamin D Group. Data are presented as Mean ± SD. *Significance level (P≤0.05).

Table 2. Weight of animals in different groups (means ± SE).

		U.H.C (n=8)	U.Ca.C (n=8)	U.Ca.VD (n=8)	Ca.Ex (n=7)	Ca.Ex.VD (n=6)
Weight (gram)	First week	31.25±1.58	29.13±2.64	30.88±1.36	31.00±2.00	30.67±3.08
	Last week	35.75±2.12	28.38±7.9*	31.63±1.6*	31.29±1.98*	31.87±2.40*

* P≤0.05

so that a significant positive correlation existed between PGC1-α and tumor volume growth and TAC between groups, except in the Ca. Ex.VD group; thus, there is a significant positive relationship between PGC1-α mRNA and tumor volume growth, whereas a negative correlation was observed between PGC1-α mRNA and TAC in this group. According to previous studies, long-term exercise training implementation leads to PGC-1α overexpression and increased antioxidant enzymes subsequently (catalase, superoxide dismutase) and decreased ROS-induced apoptosis (Faustino-Rocha et al., 2016). The role of PGC-1α has been proven in energy metabolism, apoptosis, and stressful conditions (Guo, 2021) and biological pathway (Jones et al., 2012; Patti et al., 2003; Puigserver et al., 1998). Alternatively, Bhalla et al. (2011) showed that PGC-1α promotes carcinogenesis and tumor growth through the induction of lipogenic enzymes.

In the current study, exercise training along with vitamin D interventions not only diminished PGC-1α mRNA and tumorigenesis but also promoted the total antioxidant capacity. While there was a completely reverse results in other groups. Studies showed that exercise training in mice modeling of breast cancer, the possible tumor development is delayed and smaller-sized tumors are observed (Alvarado et al., 2016; Faustino-Rocha et al., 2016, 2017; Steiner et al., 2013; Sturgeon et al., 2017; Wang et al., 2009; Westerlind et al., 2003). Although, the intensity and duration of exercise are important variables, which can interfere with oxidative stress and antioxidant status (Jeon et al., 2018; Moysés-Oliveira et al., 2019; Ramez et al., 2020). The

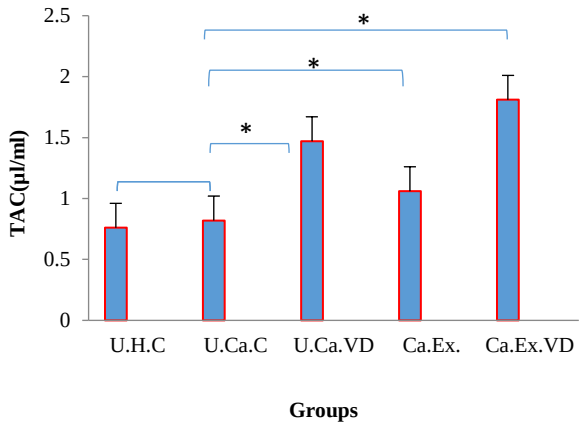

Figure 3. Average total antioxidant capacity is plotted relative to the number of weeks during six weeks of discontinuous exercise training (DET) and vitamin D supplement intervention in five groups. U.H.C= Untrained Health Control Group, U.Ca.C= Untrained Cancer Control Group, U. Ca.VD= Untrained Cancer with Injection of Vitamin D Group, Ca.Ex= Cancer Exercise Training Group, Ca.Ex.VD= Cancer Exercise Training with Injection of Vitamin D Group.* P≤0.05

Table 3. Correlation between PGC1-α, tumor volume and TAC.

		PGC1-α	Tumor volume	TAC
	PGC1-α	-	-	r= 0.72
		-	-	p=0.031
U.H.C	Tumor volume	-	-	-
		-	-	-
	TAC	r= 0.72	-	-
		p=0.031	-	-
U.Ca.C	*PGC1-α*	-	r= 0.68	r= 0.71
		-	p=0.021	p=0.032
	Tumor volume	r= 0.68	-	r= 0.74
		p=0.021	-	p=0.043
	TAC	r= 0.71	r= 0.74	-
		p=0.032	p=0.043	-
U.Ca.VD	*PGC1-α*	-	r= 0.87	r= 0.83
		-	p=0.018	p=0.035
	Tumor volume	r= 0.87	-	r= 0.89
		p=0.018	-	p=0.014
	TAC	r= 0.83	r= 0.89	-
		p=0.035	p=0.014	-
Ca.Ex	*PGC1-α*	-	r= 0.70	r= 0.75
		-	p=0.036	p=0.017
	Tumor volume	r= 0.70	-	r= 0.76
		p=0.036	-	p=0.045
	TAC	r= 0.75	r= 0.76	-
		p=0.017	p=0.045	-
Ca.Ex.VD	*PGC1-α*	-	r= 0.87	r= - 0.89
		-	p=0.028	p=0.011
	Tumor volume	r= 0.87	-	r= - 0.82
		p=0.028	-	p=0.038
	TAC	r= - 0.89	r= - 0.82	-
		p=0.011	p=0.038	-

research revealed that exercise, especially long and intense exercise can increase ROS, oxidative stress, and lipid peroxides enhancing the activity of catecholamines, prostanoids, NADP (H) oxidase, the activity of macrophages (Clarkson et al., 2000). Moreover, the increase in PGC-1α and antioxidant defense following exercise training can remove active oxygen species produced by the exercise training and confer resistance against oxidative stress that is a therapeutic strategy for treating some tumors and cancer.

Accumulating studies suggest that the protective role of exercise training in cardiac diseases involves the activation of PGC-1α expression. It has been demonstrated that aerobic interval training suppresses pathological remodeling via the promotion of nuclear PGC-1α expression, thus inhibiting mitochondrial dysfunction following MI in rats (Watkins et al., 2004; Yu et al., 2013). These results confirm that exercise training reduces mitochondrial dysfunction in the heart via upregulation of PGC-1α signaling pathways, which provides an emerging approach for treating cardiac diseases. In contrast, silencing of PGC-1α in cancer cells suspended their invasive potential and attenuated metastasis without affecting proliferation, primary tumor growth, or epithelial-to-mesenchymal (EMT) program (Vazquez et al., 2013).

Furthermore, research has reported that hormone, 1, 25(OH)2D modulates cell proliferation through direct and indirect pathways. For example, vitamin D inhibits pathways related to transcription factor NF-κB (Wimalawansa, 2019). People with chronic non-communicable diseases, such as cardiovascular disease, type 2 diabetes, autoimmune diseases, arthritis, and osteoporosis have chronically elevated NF-κB (Ramnath et al., 2014; Watanabe et al., 2015). NF- κB enhances oxidative stress and cellular responses to inflammation and injury, including the following head injury (Wimalawansa, 2019). Whereas, 1,25(OH)2D (calcitriol) suppresses NF-κB and thereby reducing chronic diffuse somatic inflammation (Watanabe et al., 2015; Wimalawansa, 2019). In addition, the activation of the mitochondrial Nrf2/PGC−1α-SIRT3 path depends on intracellular calcitriol concentration (Wimalawansa, 2019). This research did not find the synergistic effects of exercise training and vitamin D implementation. Therefore, different training protocols and possible mechanisms should be investigated to clarify this effect and related mechanisms.

Conclusion

Considering the cross talk between the variables, these data suggest that taking vitamin D supplementation along with the implementation of discontinuous aerobic exercise, as a synergistic effect, improves the total antioxidant capacity and is effective in controlling tumor growth. It is recommended that further studies be conducted on exercise training along with supplementation intake synergistic.

What is already known on this subject?

PGC-1α promotes breast cancer metastasis and confers bioenergetics flexibility against metabolic drugs but with the intervention of exercise training and vitamin D, a different issue is raised for which a definite answer has not been given yet.

What this study adds?

We know that taking vitamin D supplementation along with implementation of discontinuous aerobic exercise as a synergistic effect improves the total antioxidant capacity and is effective in controlling tumor growth.

Acknowledgements

We would like to thank Z. Mohsenzadeh, Kh. Shojaei, M. Mohabbat and Kh. Rahnama for their assistance in data collection.

Funding

This project was financially supported by a Ph.D. grant provided by the University of Kurdistan, and funding from the Iran National Science Foundation (No. 97011583).

Compliance with ethical standards

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

Ethical approval The project was found to be in agreement with the ethical platform and the national norms and standards for conducting medical research in Iran (Approval ID: IR.UOK.REC.1397. 026).

Informed consent Animal study.

Author contributions

Conceptualization: A.J., D.Sh-V.; Methodology: N.Kh., F.Kh.; Software: A.J., D.Sh-V.; Validation: N.Kh., F.Kh.; Formal analysis: A.J., D.Sh-V.; Investigation: N.Kh., F.Kh.; Resources: D.Sh-V.; Data curation: A.J.; Writing - original draft: N.Kh., F.Kh.; Writing - review & editing: A.J.; Visualization: N.Kh.; Supervision: D.Sh-V.; Project administration: F.Kh.; Funding acquisition: D.Sh-V.

References

Alvarado, A., Faustino-Rocha, A. I., Ferreira, R., Mendes, R., Duarte, J. A., Pires, M. J., et al. (2016). Prognostic factors in an exercised model of chemically-induced mammary cancer. Anticancer Res, 36(5), 2181-2188. PMID: 27127121

Andrzejewski, S., Klimcakova, E., Johnson, R. M., Tabaries, S., Annis, M. G., Mcguirk, S., et al. (2017). PGC-1α promotes breast cancer metastasis and confers bioenergetic flexibility against metabolic drugs. Cell Metab, 26(5), 778-787.e775. doi: https://doi.org/10.1016/j.cmet.2017.09.006

Atoum, M., & Alzoughool, F. (2017). Vitamin D and breast cancer: Latest evidence and future steps. Breast Cancer (Auckl), 11, 1178223417749816. doi: https://doi.org/10.1177/1178223417749816

Bhalla, K., Hwang, B. J., Dewi, R. E., Ou, L., Twaddel, W., Fang, H. B., et al. (2011). PGC1α promotes tumor growth by inducing gene expression programs supporting lipogenesis. Cancer Res, 71(21), 6888-6898. doi: https://doi.org/10.1158/0008-5472.can-11-1011

Chen, Y., Zhang, J., Lin, Y., Lei, Q., Guan, K.-L., Zhao, S., et al. (2011). Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS. EMBO Reports, 12(6), 534-541. doi: https://doi.org/10.1038/embor.2011.65

Clarkson, P. M., & Thompson, H. S. (2000). Antioxidants: What role do they play in physical activity and health? Am J Clin Nutr, 72(2 Suppl), 637s-646s. doi: https://doi.org/10.1093/ajcn/72.2.637S

Demarzo, M. M., & Garcia, S. B. (2004). Exhaustive physical exercise increases the number of colonic preneoplastic lesions in untrained rats treated with a chemical carcinogen. Cancer Lett, 216(1), 31-34. doi: https://doi.org/10.1016/j.canlet.2004.06.002

Demarzo, M. M., Martins, L. V., Fernandes, C. R., Herrero, F. A., Perez, S. E., Turatti, A., et al. (2008). Exercise reduces inflammation and cell proliferation in rat colon carcinogenesis. Med Sci Sports Exerc, 40(4), 618-621. doi: https://doi.org/10.1249/

Fairey, A. S., Courneya, K. S., Field, C. J., Bell, G. J., Jones, L. W., & Mackey, J. R. (2005). Randomized controlled trial of exercise and blood immune function in postmenopausal breast cancer survivors. J Appl Physiol (1985), 98(4), 1534-1540. doi: https://doi.org/10.1152/japplphysiol.00566.2004

Fajas, L., Auboeuf, D., Raspe, E., Schoonjans, K., Lefebvre, A. M., Saladin, R., et al. (1997). The organization, promoter analysis, and expression of the human PPARgamma gene. J Biol Chem, 272(30), 18779-18789. doi: https://doi.org/10.1074/jbc.272.30.18779

Faustino-Rocha, A. I., Gama, A., Oliveira, P. A., Alvarado, A., Neuparth, M. J., Ferreira, R., et al. (2017). Effects of lifelong exercise training on mammary tumorigenesis induced by MNU in female Sprague-Dawley rats. Clin Exp Med, 17(2), 151-160. doi: https://doi.org/10.1007/s10238-016-0419-0

Faustino-Rocha, A. I., Silva, A., Gabriel, J., Gil Da Costa, R. M., Moutinho, M., Oliveira, P. A., et al. (2016). Long-term exercise training as a modulator of mammary cancer vascularization. Biomed Pharmacother, 81, 273-280. doi: https://doi.org/10.1016/j.biopha.2016.04.030

Fisher, K. W., Das, B., Kortum, R. L., Chaika, O. V., & Lewis, R. E. (2011). Kinase suppressor of ras 1 (KSR1) regulates PGC1α and estrogen-related receptor α to promote oncogenic Ras-dependent anchorage-independent growth. Mol Cell Biol, 31(12), 2453-2461. doi: https://doi.org/10.1128/mcb.05255-11

Guo, L. (2021). Mitochondria and the permeability transition pore in cancer metabolic reprogramming. Biochem Pharmacol, 188, 114537. doi: https://doi.org/10.1016/j.bcp. 2021.114537

Haq, R., Shoag, J., Andreu-Perez, P., Yokoyama, S., Edelman, H., Rowe, G. C., et al. (2013). Oncogenic BRAF regulates oxidative metabolism via PGC1α and MITF. Cancer Cell, 23(3), 302-315. doi: https://doi.org/10.1016/j.ccr.2013.02.003

Hutnick, N. A., Williams, N. I., Kraemer, W. J., Orsega-Smith, E., Dixon, R. H., Bleznak, A. D., et al. (2005). Exercise and lymphocyte activation following chemotherapy for breast cancer. Med Sci Sports Exerc, 37(11), 1827-1835. doi: https://doi.org/10.1249/01.mss.0000175857.84936.1a

Jafari, A., Sheikholeslami-Vatani, D., Khosrobakhsh, F., & Khaledi, N. (2021). Synergistic effects of exercise training and vitamin D supplementation on mitochondrial function of cardiac tissue, antioxidant capacity, and tumor growth in breast cancer in bearing-4T1 mice. Frontiers in Physiology, 12(465). doi: https://doi.org/10.3389/fphys.2021.640237

Jeon, S. M., & Shin, E. A. (2018). Exploring vitamin D metabolism and function in cancer. Exp Mol Med, 50(4), 1-14. doi: https://doi.org/10.1038/s12276-018-0038-9

Jones, A. W., Yao, Z., Vicencio, J. M., Karkucinska-Wieckowska, A., & Szabadkai, G. (2012). PGC-1 family coactivators and cell fate: Roles in cancer, neurodegeneration, cardiovascular disease and retrograde mitochondria-nucleus signalling. Mitochondrion, 12(1), 86-99. doi: https://doi.org/10.1016/j.mito.2011.09.009

Jones, L. W., Eves, N. D., Peddle, C. J., Courneya, K. S., Haykowsky, M., Kumar, V., et al. (2009). Effects of presurgical exercise training on systemic inflammatory markers among patients with malignant lung lesions. Appl Physiol Nutr Metab, 34(2), 197-202. doi: https://doi.org/10.1139/h08-104

Jones, L. W., Viglianti, B. L., Tashjian, J. A., Kothadia, S. M., Keir, S. T., Freedland, S. J., et al. (2010). Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer. J Appl Physiol (1985), 108(2), 343-348. doi: https://doi.org/10.1152/japplphysiol.00424.2009

Lebleu, V. S., O'connell, J. T., Gonzalez Herrera, K. N., Wikman, H., Pantel, K., Haigis, M. C., et al. (2014). PGC-1α mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. Nat Cell Biol, 16(10), 992-1003, 1001-1015. doi: https://doi.org/10.1038/ncb3039

Lee, H. J., Su, Y., Yin, P. H., Lee, H. C., & Chi, C. W. (2009). PPAR(gamma)/PGC-1(alpha) pathway in E-cadherin expression and motility of HepG2 cells. Anticancer Res, 29(12), 5057-5063. PMID: 20044617

Lee, C. H., Wu, S. B., Hong, C. H., Liao, W. T., Wu, C. Y., Chen, G. S., et al. (2011). Aberrant cell proliferation by enhanced mitochondrial biogenesis via mtTFA in arsenical skin cancers. Am J Pathol, 178(5), 2066-2076. doi: https://doi.org/10.1016/j.ajpath. 2011.01.056

Malicka, I., Siewierska, K., Pula, B., Kobierzycki, C., Haus, D., Paslawska, U., et al. (2015). The effect of physical training on the N-methyl-N-nitrosourea-induced mammary carcinogenesis of Sprague-Dawley rats. Exp Biol Med (Maywood), 240(11), 1408-1415. doi: https://doi.org/10.1177/1535370215587532

Mcguirk, S., Gravel, S.-P., Deblois, G., Papadopoli, D. J., Faubert, B., Wegner, A., et al. (2013). PGC-1α supports glutamine metabolism in breast cancer. Cancer & Metabolism, 1(1), 1-11. doi: https://doi.org/10.1186/2049-3002-1-22.

Moyses-Oliveira, M., Cabral, V., Gigek, C. O., Correa, D. C. C., Di-Battista, A., Stumpp, T., et al. (2019). Search for appropriate reference genes for quantitative reverse transcription PCR studies in somite, prosencephalon and heart of early mouse embryo. Gene, 710, 148-155. doi: https://doi.org/10.1016/j.gene.2019.05.042

O'malley, M., King, A. N., Conte, M., Ellingrod, V. L., & Ramnath, N. (2014). Effects of cigarette smoking on metabolism and effectiveness of systemic therapy for lung cancer. J Thorac Oncol, 9(7), 917-926. doi: https://doi.org/10.1097/jto. 0000000000000191

Patti, M. E., Butte, A. J., Crunkhorn, S., Cusi, K., Berria, R., Kashyap, S., et al. (2003). Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential

Role of PGC1 and NRF1. Proc Natl Acad Sci U S A, 100(14), 8466-8471. doi: https://doi.org/10.1073/pnas.1032913100

Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., & Spiegelman, B. M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell, 92(6), 829-839. doi: https://doi.org/10.1016/s0092-8674(00)81410-5

Ramez, M., Ramezani, F., Nasirinezhad, F., & Rajabi, H. (2020). High-intensity interval training increases myocardial levels of Klotho and protects the heart against ischaemia-reperfusion injury. Exp Physiol, 105(4), 652-665. doi: https://doi.org/10.1113/ep087994

Ramnath, N., Nadal, E., Jeon, C. K., Sandoval, J., Colacino, J., Rozek, L. S., et al. (2014). Epigenetic regulation of vitamin D metabolism in human lung adenocarcinoma. J Thorac Oncol, 9(4), 473-482. doi: https://doi.org/10.1097/jto.0000000000000114

Ricca, C., Aillon, A., Bergandi, L., Alotto, D., Castagnoli, C., & Silvagno, F. (2018). Vitamin D receptor is necessary for mitochondrial function and cell health. International Journal of Molecular Sciences, 19(6). doi: https://doi.org/10.3390/ijms19061672

Schlotter, C. M., Vogt, U., Allgayer, H., & Brandt, B. (2008). Molecular targeted therapies for breast cancer treatment. Breast Cancer Res, 10(4), 211. doi: https://doi.org/10.1186/bcr2112

Shin, S. W., Yun, S. H., Park, E. S., Jeong, J. S., Kwak, J. Y., & Park, J. I. (2015). Overexpression of PGC-1α enhances cell proliferation and tumorigenesis of HEK293 cells through the upregulation of Sp1 and Acyl-CoA binding protein. Int J Oncol, 46(3), 1328-1342. doi: https://doi.org/10.3892/ijo.2015.2834

Shiota, M., Yokomizo, A., Tada, Y., Inokuchi, J., Tatsugami, K., Kuroiwa, K., et al. (2010). Peroxisome proliferator-activated receptor gamma coactivator-1alpha interacts with the androgen receptor (AR) and promotes prostate cancer cell growth by activating the AR. Mol Endocrinol, 24(1), 114-127. doi: https://doi.org/10.1210/me.2009-0302

Singh, M. P., Singh, G., & Singh, S. M. (2005). Role of host's antitumor immunity in exercise-dependent regression of murine T-cell lymphoma. Comp Immunol Microbiol Infect Dis, 28(3), 231-248. doi: https://doi.org/10.1016/j.cimid.2005.02.001

Song, C., Fu, B., Zhang, J., Zhao, J., Yuan, M., Peng, W., et al. (2017). Sodium fluoride induces nephrotoxicity via oxidative stress-regulated mitochondrial SIRT3 signaling pathway. Sci Rep, 7(1), 672. doi: https://doi.org/10.1038/s41598-017-00796-3

Steiner, J. L., Davis, J. M., Mcclellan, J. L., Enos, R. T., & Murphy, E. A. (2013). Effects of voluntary exercise on tumorigenesis in the C3(1)/SV40Tag transgenic mouse model of breast cancer. Int J Oncol, 42(4), 1466-1472. doi: https://doi.org/10.3892/ijo.2013.1827

Sturgeon, K. M., Schweitzer, A., Leonard, J. J., Tobias, D. K., Liu, Y., Cespedes Feliciano, E., et al. (2017). Physical activity induced protection against breast cancer risk associated with delayed parity. Physiol Behav, 169, 52-58. doi: https://doi.org/10.1016/j.physbeh.2016.11.026

Taguchi, A., Delgado, O., Celiktaş, M., Katayama, H., Wang, H., Gazdar, A. F., et al. (2014). Proteomic signatures associated with p53 mutational status in lung adenocarcinoma. Proteomics, 14(23-24), doi: 2750-2759. https://doi.org/10.1002/pmic. 201400378

Tennakoon, J. B., Shi, Y., Han, J. J., Tsouko, E., White, M. A., Burns, A. R., et al. (2014). Androgens regulate prostate cancer cell growth via an AMPK-PGC-1α-mediated metabolic switch. Oncogene, 33(45), 5251-5261. doi: https://doi.org/10.1038/onc.2013.463

Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., & Spiegelman, B. M. (1994). mPPAR gamma 2: Tissue-specific regulator of an adipocyte enhancer. Genes Dev, 8(10), 1224-1234. doi: https://doi.org/10.1101/gad.8.10.1224

Vazquez, F., Lim, J. H., Chim, H., Bhalla, K., Girnun, G., Pierce, K., et al. (2013). PGC1α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. Cancer Cell, 23(3), 287-301. doi: https://doi.org/10.1016/j.ccr.2012.11.020

Wang, M., Yu, B., Westerlind, K., Strange, R., Khan, G., Patil, D., et al. (2009). Prepubertal physical activity up-regulates estrogen receptor beta, BRCA1 and p53 mRNA expression in the rat mammary gland. Breast Cancer Res Treat, 115(1), 213-220. doi: https://doi.org/10.1007/s10549-008-0062-x

Watanabe, R., & Inoue, D. (2015). Current topics on vitamin D. Anti-cancer effects of vitamin D. Clin Calcium, 25(3), 373-380.

Watkins, G., Douglas-Jones, A., Mansel, R. E., & Jiang, W. G. (2004). The localisation and reduction of nuclear staining of PPARgamma and PGC-1 in human breast cancer. Oncol Rep, 12(2), 483-488.

Weikum, E. R., Liu, X., & Ortlund, E. A. (2018). The nuclear receptor superfamily: A structural perspective. Protein Sci, 27(11), 1876-1892. doi: https://doi.org/10.1002/pro.3496

Westerlind, K. C., Mccarty, H. L., Schultheiss, P. C., Story, R., Reed, A. H., Baier, M. L., et al. (2003). Moderate exercise training slows mammary tumour growth in adolescent rats. Eur J Cancer Prev, 12(4), 281-287. doi: https://doi.org/10.1097/00008469-200308000-00007

Wimalawansa, S. J. (2019). Vitamin D deficiency: Effects on oxidative stress, epigenetics, gene regulation, and aging. Biology (Basel), 8(2). doi: https://doi.org/10.3390/biology8020030

Wolin, K. Y., Schwartz, A. L., Matthews, C. E., Courneya, K. S., & Schmitz, K. H. (2012). Implementing the exercise guidelines for cancer survivors. J Support Oncol, 10(5), 171-177. doi: https://doi.org/10.1016/j.suponc.2012.02.001

Wu, W., & Zhao, S. (2013). Metabolic changes in cancer: beyond the Warburg effect. Acta Biochim Biophys Sin (Shanghai), 45(1), 18-26. doi: https://doi.org/10.1093/abbs/gms104

Yu, H., & Xin, Y. (2013). Down-regulated expressions of PPARγ and its coactivator PGC-1 are related to gastric carcinogenesis and Lauren's classification in gastric carcinoma. Chin J Cancer Res, 25(6), 704-714. doi: https://doi.org/10.3978/j.issn.1000-9604.2013.11.11