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Research paper

Continuous Moderate Exercise Training Can Increase Serum Level of Irisin in Breast Cancer Patients

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Abstract

Background and Aims: Serum Irisin may serve as a novel indicator for breast cancer detection and early diagnosis. Irisin may offer therapeutic benefits for breast cancer prevention and treatment. Exercise training modalities have been recently proposed as a complementary treatment strategy in cancer patients. How exactly exercise results in these health benefits is not known. This randomized control trial compared the effect of high-intensity interval (HIIT) and continuous moderate training (CMT) on serum Irisin levels in breast cancer patients.

Method: Thirty-nine overweight breast cancer patients were randomly assigned to High-Intensity Interval Training (HIIT), Continuous Moderate Training (CMT), and usual care groups. The serum level of Irisin was measured at baseline and twelve weeks of intervention.

Results: The serum level of Irisin in the continuous moderate-intensity training group increased significantly at post-intervention. There was a significant difference in Irisin between CMT and HIIT groups at the post-intervention testing.

Conclusion: Twelve weeks of moderate continuous intensity exercise increased the circulatory levels of Irisin in breast cancer patients. Continuous moderate training is likely to reduce the inflammation in these patients through the anti-inflammatory function of Irisin.

Key words: Breast cancer, Continuous moderate training, HIIT, Irisin

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Introduction

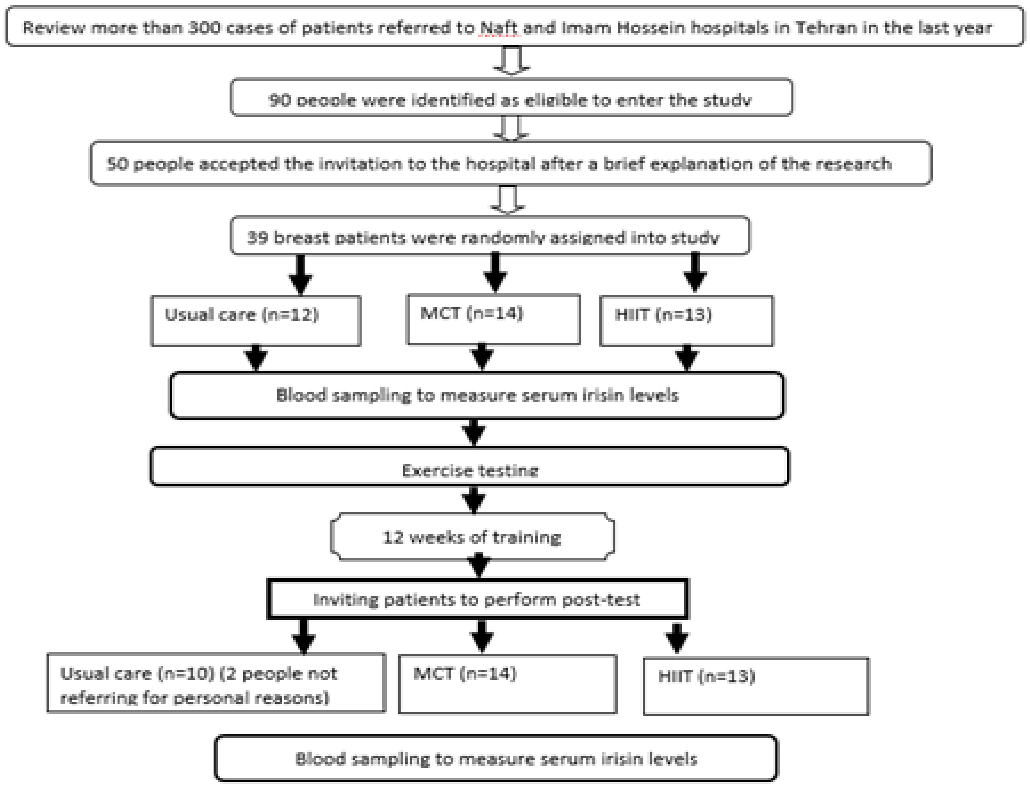
Breast cancer is the most common type of cancer among women (25% of all cancers) and is the leading cause of death among women in terms of cancer (Bray et al., 2018). Regular physical activity is associated with many health benefits and a reduced risk of breast cancer (Fournier et al., 2014; Williams, 2014). In addition, an encouraging number of studies have shown that exercise performed after cancer diagnosis reduced the risk of recurrence and mortality (Betof et al., 2013). There is enough evidence showing that exercise plays an effective role in cancer care and increases the survival of breast cancer patients (Cannioto et al., 2021). However, identifying the optimal exercise protocols for cancer patients is a priority research question in exercise oncology (Morris et al., 2020) and exact mechanisms involved in the anticancer effects of regular physical exercise are not well known..

Irisin is a myokine secreted from muscles during exercise and directly acts on key functional cells involving energy metabolism and homeostasis. Irisin can promote the conversion of white adipose tissue to brown adipose tissue (Boström et al., 2012). It also plays an important regulatory role in reducing insulin resistance (Bastu et al., 2018). There is an association between Irisin and physical activity, for example, Moreno et al. (2015) found higher circulating Irisin levels in physically active individuals compared to sedentary subjects. Interestingly, serum Irisin levels in cancer patients are lower than healthy individuals and it is estimated that increasing one unit of Irisin (μg/ml) reduces the risk of breast cancer in women by almost 90% , Irisin levels could, therefore, be used as a diagnostic key marker in breast cancer (Provatopoulou et al., 2015).

There is a clear pattern between Irisin and exercise where exercise induces a significant increase in Irisin secretion; for instance, people, who are able to reach a higher VO2max and thus work at a higher exercise workload shows the higher levels of Irisin after exercise (Daskalopoulou et al., 2014). However, there is a gap in the literature with regard to the understanding of different types of exercise intensities on levels of Irisin. Therefore, the aim of this study was to compare the effect of 12 weeks of high intensity interval training (HIIT) and continuous moderate training (CMT) comparing to usual care on serum Irisin levels in the breast cancer patients.

Materials and Methods

Participants; The present study was conducted after receiving the code of ethics from the ethics committee of Shahed University with the code of IR.SHAHED.REC.1389.051. All stages of the research and potential benefits and harms were explained to the participants, and they were assured that their information would be confidential at all stages of the research. Participants had the right to leave the project at any stage of the research if they did not wish to continue the collaboration. The research participants were selected among patients referred to Imam Hossein and Naft hospitals in Tehran. After the corresponding oncologist provided consent, the subjects were deemed eligible for the study if they met each of the following conditions: age ≥ 30 years, insufficient physical activity level (less than 150 min/week), hormone-responsive breast cancer; performing no strenuous exercise such as running, cycling, swimming or resistance training; completing chemotherapy and radiotherapy in the last month, taking aromatase inhibitors or tamoxifen (undergoing hormone therapy) and willing to participate voluntarily in this randomized controlled trial. The exclusion criteria were as follows: current smoking; evidence of metastatic breast cancer; planning to receive any additional adjuvant chemotherapy or surgery; pregnancy or breastfeeding; inability to baseline blood sample; cardiac conditions such as myocardial infarction or coroner artery disease; liver conditions; lymphedema; uncontrolled hypertension, defined as systolic blood pressure≥180 mm Hg or diastolic blood pressure≥100 mm Hg; high-risk or uncontrolled heart arrhythmias, clinically significant heart valve disease, decompensated heart failure, or known aortic aneurysm; and any other condition which, from the investigators' viewpoint, may impede testing the research hypothesis or making it unsafe to engage in the exercise program. The eligibility of patients to participate in high-intensity exercise program had been confirmed by a sport medicine specialist. Written informed consent was obtained from all patients prior to participation. Accordingly, 39 qualified participants who had the desire and conditions to participate in the study were randomly divided into 3 groups: High Intensity Interval Training (HIIT) (n=13); Moderate Continuous Training (MCT) (n=14), and usual care (n=12).



Exercise Testing

Before measurements of VO_{2peak}, the participants (in all three study groups) were informed about the test and instructed to exercise to their maximum limit. To measure the aerobic capacity ($VO2_{peak}$), the gas exchange was measured using a breath-by-breath automated exercise metabolic system (ZAN 600 USB, Oberthulba, Germany) until peak oxygen uptake ($VO2_{peak}$) was reached. $VO2_{peak}$ was defined by the average top three consecutive 15-s values in the last 2 min of exercise. Participants were encouraged to give a maximal effort during the test. The protocol involved a slow progression towards a static speed of 3.3 MPH at the start of minute three, with an initial minute at 2.0 MPH and a second minute at 2.7 MPH. At minute three the speed increased to 3.3 MPH and a grade of 1%. The grade increased 1% each minute thereafter. Participants continued walking on the tread mill at increasing incline until exhaustion, unless indications for terminating the maximal test were observed (ACSM 2009). Participants cooled-down at 1.5-2.0 MPH and a level grade for a period of two to five minutes. Expired air was analyzed for O2 and CO2. Heart rate, blood pressure, and rating of perceived exertion score were determined at the end of each two-minute stage. Confirmation of a maximal effort was determined by meeting three out of four of the following criteria; 1) plateau in heart rate and oxygen uptake with increased workload, 2) respiratory exchange ratio > 1.1, 3) rating of perceived exertion > 17, and 4) heart rate > 90% (Burnett et al., 2013).

This data was used to report change in VO_{2peak}. Further, the direct measure of VO_{2peak} was used to personalize the exercise program for optimal benefit for each participant.

Exercise Training

The patients met the study's criteria trained three times per week for 10 weeks under supervision of an exercise physiologist. All training consisted of "uphill" treadmill walking as described previously (Wisløff et al., 2007)(Table1). The high intensity aerobic exercise (HIIT) group carried out a 10-min warm-up period at an intensity corresponding to 50–60% of VO_{2peak} (65–75% of HR_{peak}) before walking four intervals of 4 min at 80–90% of VO_{2peak} (85–95% of HR_{peak}). The training session was terminated by a 3-min cool-down period at 50–60% of VO_{2peak}. . This gave a total exercise time for the high intensity group of 38 min. . The moderate intensity exercise training (MCT) thus consisted of 47 min continuous exercise at an intensity of 50–60% of VO_{2peak} (70% to 75% of maximal heart), representing the same total training load as the high intensity aerobic exercise group. (Rognmo et al., 2004). All participants exercised using a heart rate monitoring device (Polar Electro, Kempele, Finland) during every training session. The Borg 6-to-20 scale was used to assess the rate of perceived exertion during and after each training session. The speed and inclination of the treadmill was adjusted continuously to ensure that every training session was carried out at the assigned heart rate throughout the training period. The control group was asked to follow advice from their family doctor with regard to physical activity; in addition, they met for 47 minutes of continuous treadmill walking at 70% of peak heart rate every 3 weeks (Wisløff et al., 2007). The researchers used the high-intensity interval training protocol that has previously been reported as a safe exercise training regimen for heart failure and coronary artery diseases (Rognmo et al., 2004).

Table 1- Training Protocol

Exercise mood	Group	Time of training		Intensity
uphill treadmill walking	HIIT	38 min	Warm up for10min	65–75%of HRpeak
			4×4 min	85–95% of HRpeak
			3×3 min	50 to 70% of HRpeak
			Cooldown 3 min	65–75% of HRpeak
	MCT	47 min	Warm up for10 min	65–75%of HR_{peak}
			Main training 39 min	70 to 75% MHR
			Cooldown 3 min	65–75% of HR_{peak}

Measurement of Serum Levels of Irisin

To measure serum levels of Irisin, all participants were invited to be in the hospital laboratory in the condition of 12 hours of fasting; and 5 cc of blood was taken from them. Blood samples were collected in a gel tube and transferred to a specialized oncology laboratory with due and thorough laboratory conditions. The samples were centrifuged at 3000 RPM for 10 minutes, then serum was separated and stored at -70 ° C until post-test (Provatopoulou et al., 2015). Final blood sampling was performed 48-72 hours after the last training session, again in the 12-hour fasting state. The human kit from the German company Zellbio by ELISA was used to measure Irisin. The starting and ending time of the measurement for each subject was recorded in the pre-test so that all conditions for the post-test could be repeated exactly. Participants were asked to follow their usual diet during the training period and to refrain from taking any supplements without consulting their doctor. At the beginning of the training, the participants of the training groups were invited to refer to the rehabilitation department of the hospital for training. The training groups performed the following protocols for 12 weeks, three sessions per week. All exercises were performed under the supervision of a physician with accurate monitoring of heart rate and blood pressure in the rehabilitation center of Imam Hossein (AS) and Naft hospitals in Tehran. After the training period, post-test was performed on all participants with pre-test conditions.

Statistical Analysis

To compare the research variables and find the significant effect of different types of training protocols on these variables, considering the assumption of homogeneity of data, the data were analyzed using analysis of covariance (ANCOVA) after checking and confirming all the assumptions of this test ($P \leq 0.05$). The baseline values were considered as a covariate variable. Data was statistically analyzed by SPSS software version 18.

Findings

Two patients in the usual care group did not attend the post-test due to personal problems; therefore, the study in the usual care group was completed with 10 patients. The characteristics of the participants such as age, height, weight and body mass index are reported in Table 2 as mean and standard deviation.

Table 2- Physical Characteristic of Research Groups

Characteristic	Group HIIT(n=13) (Mean[١]±SD)	CMT (n=14) (Mean±SD)	CON (n=10) (Mean±SD)
Age (years)	44.54±9.06	49.36±8.05	44.90±5.02
Height (cm)	160.85±4.22	157.93±6.05	159.00±5.33
Weight (kg)	68.72±10.01	76.32±13.48	68.94±11.18
Body mass index (kg . m2)	26.62±4.28	30.37±6.49	27.37±4.88

As shown in Table 3, the results of post-test analysis of covariance showed that there was a statistically significant difference in serum levels of Irisin between the groups. It was observed a significant different in Irisin levels in pretest by ANCOVA test. The results of post hoc test showed that there is a significant difference between HIIT and MCT groups as well as between MCT and usual care groups (Table 4). The serum level of Irisin in the MCT group increased significantly after 12 weeks of exercise training (Table5).

Table 3- Analysis of Covariance of Changes in Serum Irisin Level

Variable	Source	Total Squares	Freedom Level	F	Significance	Eta Coefficient
Irisin Serum Level (ng.mL)	Interactive Effect	1.089	2	2.445	0.103	
	Pre-test Factor	1.064	1	4.777	0.037	
	Group Factor	1.537	2	3.449	*0.044	0.180

* Significant Difference

Table 4- LSD Post hoc Test to Determine Differences between Groups

Groups	Mean Difference	Standard Deviation	Significance	95% confidence Interval	
				Lower Bound	Upper Bound
HIIT- MCT	-0.409	0.190	0.039*	-0.796	-0.023
HIIT-Usual care	0.082	0.207	0.696	-0.340	0.503
MCT - Usual care	0.491	0.204	0.022*	0.076	0.906

*Significant Difference

Table 5. Irisin serum level differences within groups

Variable	Phase	Group		
		HIIT	MCT	Usual care
Irisin Serum Level (ng/mL)	Pre (Mean±SD) test	2.73±0.54	2.77±0.55	2.73±0.59
	Post (Mean±SD) test	2.52±0.36	2.95±0.55	2.44±0.60
P value		0.263	0.460	0.066

Discussion

The results of this study showed that 12 weeks of MCT significantly increased the serum level of Irisin in breast cancer patients. Serum levels of Irisin in the HIIT and usual care group showed a decrease, although it was not significant, but there was a significant difference between the MCT and HIIT groups and between the continuous and usual care group.

Based on recent studies, Irisin may be used as a therapeutic for the treatment of a wide variety of cancers by regulating lipid metabolism, suppressing tumor-promoting inflammation, increasing apoptosis, and inhibiting metastasis (Sumsuzzman et al., 2019). Many studies have reported the anticancer effect of

Irisin against different types of cancer. In-vitro studies indicate that Irisin in combination with doxorubicin, a chemotherapeutic drug widely used in the treatment of breast cancer, inhibits the proliferation and induces the apoptosis of breast cancer cells, resulting in enhanced effects of chemotherapy (Gannon et al., 2015). Irisin can inhibit cell proliferation, migration and invasion in prostate (Tekin et al., 2015) and lung (Nowinska et al., 2019; Shao et al., 2017) cancer cell lines. These data indicated the strong anticancer effects of Irisin. Irisin may reduce cancer risk by lowering the basal systemic levels of risk factors such as proinflammatory cytokines and adipokines (Hanahan & Weinberg, 2011; Spyrou et al., 2018).

Irisin may be the missing link between breast cancer and the protective effect of physical activity. It was found that Irisin reduces the number of malignant breast cancer cells and cancer cell motility and increases the sensitivity of breast cancer cells to chemical treatments such as doxorubicin. Thus, it can be used as an adjunct therapy in some cancers, especially breast one. Irisin can also prevent apoptosis by inhibiting the kappa-light chain-enhancing factor (NF-kB). This may indicate the anti-inflammatory effect of Irisin against pro-inflammatory cytokines (such as tumor necrosis factor alpha) (Gannon et al., 2015). Provatplo et al. (2015) reported that Irisin levels in women with breast cancer were significantly lower in comparison to healthy women and that serum levels were positively correlated with tumor stage (Provatopoulou et al., 2015). In a study by Adriana et al. (2021), assuming that high-intensity interval training (HIIT) may increase immune protection compared to continuous moderate intensity training (MCT). The effect of these two exercises on some cytokines, NK and iris cells were evaluated in postmenopausal women over 60 years old who were at high risk for breast cancer. The researchers stated that exercise intensity had no significant effect on circulating Irisin, and it did not change in either of the two exercise groups (Coletta et al., 2021). The difference in the characteristics of the subjects, including age and health status, is obvious. Adriana et al.'s subjects were women at high risk for cancer, with an average age of 60 years. However, in the present study, the subjects were women treated from breast cancer who were under 50 years old in both training groups. It seems that in women who have experienced breast cancer and received various treatments, continuous intensity exercise may have more effect on the Irisin levels. Another point that cannot be ignored is that in the study of Adriana et al., the final blood sampling started 3 days after the last training session and lasted up to 5 days after the training session; Irisin in study of Adriana appeared to have lost the golden time for evaluation, whereas in our study the final blood draw was performed 48 to 72 hours after last session of exercises.

According to study by Hu et al. (2015), it may be possible to compare the results of the study on healthy individuals with those with metabolic syndromes. Hu studied the acute response of Irisin to three types of exercise (high-intensity interval exercise, moderate-intensity continuous exercise, and resistance exercise) in both healthy individuals and those with metabolic syndrome (at least three cases of obesity, high triglycerides, low HDL, high blood pressure and high blood sugar). He concluded that the response of Irisin in the two groups with healthy and metabolic syndrome to exercise is indistinguishable; this response was observed as an increase in Irisin immediately after three types of exercise. However, the increase in Irisin after resistance training was slightly higher than the other two types of training. A further increase in Irisin in response to strength training may be due to muscle injury followed by an increase in creatine kinase (Huh et al., 2015). The results of the present study indicate the priority of MCT over HIIT in affecting the serum level of Irisin. Investigating the effect of different types of exercise on serum Irisin level can be useful in selecting the type and intensity of exercise for patients after breast cancer treatment to help them further improve. In this regard, Inanloo et al. (2017) examined the effect of four weeks of aerobic exercise on serum levels of Irisin in type 2 diabetic women and concluded that continuous intensity exercise can significantly increase serum levels of Irisin in these patients (Balaghi Inaloo et al., 2017). Regardless of the subjects participating in the two studies, the results of the present study can be considered in line with those of Inanloo's study; both studies emphasize the positive effect of continuous intensity exercise on serum Irisin.

Chen et al. suggested that, under physiological stresses, irisin transfer from circulation into tissue was increased (Chen et al., 2017). As we know, exercise is one of the conditions under physiological stresses. Concerning exercise intervention, HIIT seems to be more effective in transfer from circulation into tissue compared to CMT in obese group, because HIIT might induce greater stress responses than CMT. Thus, these might contribute to an increase in physiological stress, which consequently resulted in increased irisin uptake into adipose tissue. Therefore, lower serum levels of Irisin in HIIT group compared to CMT intervention may be due to Irisin uptake in tissues like adipose in HIIT groups. This study didn’t measure the Irisin in adipose tissue, therefore, further researches are still needed to investigate the role of exercise in affecting irisin uptake (i.e., exocytosis mechanism) in adipose tissue in breast cancer patients. In addition, some evidence demonstrated that post-exercise serum irisin level maintained higher in the continues exercise than interval training the in the obese female subjects (Rejeki et al., 2021).

Conclusion

The 12 weeks of continuous moderate intensity exercise training significantly increased serum levels of Irisin in breast cancer patients, while high intensity interval exercise was not able to provide a significant effect.

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