Iran Red Crescent Med J. 2018 February; 20(2):e13305. doi: 10.5812/ircmj.13305.

Published online 2018 February 24. **Research Article**

The Effect of Aerobic Training on Tumor Growth, Adiponectin, Leptin and Ghrelin in Mice Models of Breast Cancer

Sadegh Amani Shalamzari,[1,2] Amin Daneshfar,[3] Mozhgan Hassanzadeh Sablouei,[4] Maria A. Fiatarone Singh,[5] and Abdolreza Kazemi[2,6,*]

[1]Faculty of Physical Education and Sport Sciences, Kharazmi University, Karaj, Iran
[2]Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
[3]Department of Humanity, Faculty of Kinesiology, Tarbiat Modares University, Tehran, Iran
[4]Department of Sport Science, Faculty of Kinesiology, Islamic Azad University Guilan Branch, Guilan, Iran
[5]University of Sydney, Faculty of Health Sciences and Sydney Medical School, NSW, Australia; Hebrew Senior Life and Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA
[6]Department of Physical Education and Sport Sciences, School of Humanities, Valie-Asr University, Rafsanjan, Iran

[*]*Corresponding authors*: Abdolreza Kazemi, Assistant Professor, Department of Physical Education and Sport Sciences, School of Humanities, Vali-e-Asr University, Rafsanjan and Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, IR Iran. Tell: +98-3432322307, E-mail: rkazemi22@yahoo.com

Received 2017 July 02; **Revised** 2017 September 25; **Accepted** 2017 December 18.

Abstract

Background: Breast cancer is widespread in Iran and exercise training is an adjuvant strategy for managing this illness.
Objectives: The aim of this study was to investigate the effects of aerobic training on tumor growth and its relationship with changes in adiponectin, leptin, and ghrelin in mice with breast cancer.
Materials and Methods: In this animal experimental study, which was conducted during year 2016 in Iran, 20 female BALB/c mice were randomly divided to two groups: Tumor Control (TC) and Exercise (E). The MC4L2 cancer cells were injected in the mice. The E group then performed progressive aerobic training for six weeks. Tumor volume, food intake, weight, and muscle endurance of all mice were measured weekly. At six weeks, the mice were sacrificed and tumor, gastrocnemius muscle, and heart weights were measured. Level of cytokines/hormones were quantified using the Enzyme Linked Immunosorbent Assay (ELISA) methodology in tumor, serum, muscle, and adipose tissue.
Results: Aerobic exercise training was associated with a significantly decreased growth rate and final weight of the tumor (1.11 versus 2.74 g) compared to the TC group ($P < 0.05$). Exercising mice also had greater food intake, muscle endurance, heart weight (0.12 versus 0.09 g), and muscle weight (0.078 versus 0.045 mg) when compared with the TC group ($P < 0.05$). In addition, the E group had significantly increased adiponectin in all sites except the tumor, decreased leptin in all sites, and increased ghrelin in serum compared to the TC group ($P < 0.05$).
Conclusions: Aerobic exercise training in mice with breast cancer attenuated tumor burden and cachexia, and improved appetite, muscle size and function and fitness relative to non-exercising controls.

Keywords: Adipokine, Breast Cancer, Cachexia, Ghrelin

1. Background

Cancer cachexia is defined by an ongoing loss of skeletal muscle mass with or without loss of fat mass, associated with progressive functional impairment. Loss of muscle mass leads to lower strength, which in turn leads to decreased mobility and quality of life and increases mortality by 25% to 30% in patients with cancer (1). Since cachexia is a multifactorial syndrome, effective treatment strategies must be multifaceted as well; there is some evidence that regular physical activity by itself can be an effective therapeutic strategy for cancer cachexia (2, 3). Cachexia is characterized by a negative protein and energy balance driven by a variable combination of reduced food intake and abnormally high metabolism (4). Food intake and metabolism are regulated by hormones, neuropeptides, and cytokines, including, ghrelin, leptin, and adiponectin (5). These cytokines, which are involved in the regulation of feeding, may play an important role in both general and cancer cachexia (6).

Ghrelin, a 28-amino acid peptide, is produced in the stomach, stimulates food intake and growth hormone secretion, suppresses inflammation, reduces energy expenditure, and attenuates muscle catabolism (7). These func-

tions suggest that ghrelin could improve cachexia. In this regard, it has been shown that ghrelin injection in mice with cancer (8) led to increased food intake and body weight and improved cachexia compared to control mice. Another way to increase endogenous ghrelin levels appears to be via modulation of physical activity levels. For example, ghrelin levels in the circulation increased following moderate-intensity aerobic training in healthy adults (9). However, there are no studies investigating the effect of exercise training on ghrelin levels in animal or human models of cancer (including mice with breast cancer).

Leptin, an adipocytokine secreted mainly by adipose tissue, acts to suppress food intake and stimulate energy expenditure (5), and plays an important role in breast cancer development through a variety of pathways. Li et al. (2016) showed that leptin significantly increased tumor volume, lung metastases, and tumor-associated macrophage markers in xenograft tumor-bearing mouse models (10). Del mar Blanquer et al. (2016) showed a role for leptin in metabolic reprogramming (i.e. an enhanced use of glucose for biosynthesis and lipids for energy production). Unlike most normal tissues, cancer cells tend to "ferment" glucose into lactate even in the presence of sufficient oxygen to support mitochondrial oxidative phosphorylation (the Warburg effect). The metabolic adaptations induced by leptin may enhance Michigan Cancer Foundation-7 (MCF-7) tumor growth and may underlie the reverse Warburg effect (11). Notably, energy expenditure through exercise, independent of energy intake, may beneficially modulate leptin levels. For example, four weeks of aerobic training that lowers adiposity has been shown to concomitantly reduce circulating leptin concentrations in obese individuals (12). Aerobic exercise has been shown to be an effective adjuvant therapy in females with breast cancer, yet the role of leptin modulation in these benefits is largely unknown, as there is limited evidence to date assessing the effects of exercise on leptin levels within tumor tissue, adipose tissue and/or circulation in either animal models or human patients with cancer.

Adiponectin (also known as ACRP30) is another member of the adipocytokine family, which may be important in cancer prognosis. It is induced during adipocyte differentiation, and its secretion is stimulated by insulin and Insulin-like Growth Factor-1 (IGF-1). Like leptin, adiponectin is involved in metabolism and inflammation yet in a reverse manner (5). Adiponectin serum levels are inversely associated with body weight, adiposity, and inflammation. Thus, low adiponectin levels are found in obesity and metabolic syndrome (13), and high levels are found in anorexia nervosa (14) and during weight loss (15). However, results are heterogeneous in cancer-related adiposity and weight changes in cancer cohorts. For example, reduced levels of serum adiponectin have been reported in patients with breast cancer (16) compared to healthy controls. However, in another study, no correlation was observed between weight loss and adiponectin levels in patients with breast and colon cancer (6). Finally, in another report, in contrast to increased adiponectin, generally observed after weight loss in healthy individuals, adiponectin levels decreased after weight-loss in advanced lung cancer (17). Exercise can also modulate leptin and adiponectin levels in healthy adults and animals (18). Most commonly, higher adiponectin has been associated with higher levels of physical activity or exercise interventions (18), especially when exercise is associated with weight loss. Fewer studies are available in cohorts with cancer, and as with leptin, the results are mixed. Theriau et al. (2016) showed that Conditional Media (CM) created from the adipose tissue of High Fat Diet (HFD)-fed animals caused an increase in the proliferation on MCF7 cells compared to cells exposed to CM prepared from the adipose of lean chow diet-fed counterparts (19). However, physical activity ameliorated these proliferative effects of HFD-CM on MCF7 cells, by increasing adipoR1 and $p27T^{198}$ by AMPK, reducing $pAktT^{308}$ in a manner that depended on the volume of physical activity exposure. High volumes of physical activity (> 3 km/day) completely abolished the adverse effects of HFD feeding on cancer cell proliferation (19).

Thus, evidence to date about ghrelin and adipokines and exercise in healthy cohorts or those with cancer/cachexia is incomplete and not consistent within or across species. No study to date has investigated the levels of adiponectin, leptin, and ghrelin in muscle, tumor, adipose tissue, and serum, simultaneously, in relation to exercise in either human or animal models of cancer cachexia. Therefore, the novelty of this study is assessing these cytokines at four sites and also measuring functional variables and determining the correlation between them. Therefore, the aim of this study was to investigate the effects of exercise training on cachexia and the major cytokines potentially linked to cancer cachexia: ghrelin, leptin, and adiponectin, in mice with breast cancer. It was hypothesized that six weeks of aerobic exercise training would maintain or improve body weight and muscle function, attenuate tumor growth rate, increase ghrelin, decrease leptin, and increase adiponectin in mice with mammary breast cancer, relative to sedentary control mice. It was further hypothesized that the improvement or maintenance in body weight and muscle function after exercise and the attenuation of tumor growth rate would be significantly related to these beneficial adaptations in ghrelin and cytokines.

2. Materials and Methods

This study was an animal experimental study, which was conducted at Tarbiat Modares University, Tehran, Iran.

2.1. Cell Line

The MC4-L2 cells that are Estrogen Receptor-positive (ER+) breast ductal carcinoma were used. The process of cell culture was done as reported previously (3, 20). The cells were cultured in T75 flask in DMEM/F-12 medium containing 100 μg/mL penicillin,15 mM HEPES, 100 μg/mL streptomycin, glutamine, and 10% Fetal Bovine Serum (FBS). By using 0.025% trypsin, the cells were detached from the bottom of flasks, and after rinsing with Phosphate Buffered Saline (PBS) and enzyme neutralization using 10% FBS, all content of the flask were emptied into Falcon tubes and centrifuged at 1200 rpm for four minutes. The supernatant was decanted and the cell plate dissolved in the medium containing 10% FBS. Hemocytometer and Trypan blue were used to determine cell count and cell viability, respectively.

2.2. Sample

Animals, purchased from the Pasteur Institute of Iran, were kept under 12-hour dark and light cycle, at temperature of 23 $\pm$ 2°C and suitable humidity, with free access to food and water. The sample size was selected on the basis of similar studies and statistician experts. For the Tumor sample, female BALB/c mice (n = 20) were anesthetized using a suitable dose of Ketamine and Xylazine, and then one million MC4-L2 cells were injected subcutaneously into the right upper thigh of each mouse. Healthy control female BALB/c mice (n = 10) were recruited for comparison of muscle endurance. This study was approved by the Animal Ethics Committee of Sport Sciences Research Institute, during year 2016. The registration number was IR.SSRI.REC.1395.129.

2.3. Study Design

The tumor sample (n = 20) was randomly divided to two groups: Tumor Control (TC) and Exercise group (E). The exercise group performed progressive aerobic training for six weeks. Prior to the initiation of the exercise training, the mice were exposed to treadmill familiarization for five days. Familiarization was done with gradually increasing speeds (10, 12, 14, 16, and 18 m/minute) at 0% inclination. Following the acclimation, the progressive aerobic training protocol began at 16 to 18 m/minute, 0% incline, 10 to 14 min/session, five days/week for five weeks (21). Weekly increases in running speed during the training period were used as a guide to assess adaptation to exercise training. No electrical stimulation was performed. The mice were encouraged to run by a gentle tap on the tail or hindquarters. The TC group was placed on a switched-off treadmill during the 5-week training period for the exact same time as the training group. The exercise group animals stopped training 48 hours before being sacrificed. All outcome testing was done by non-blinded assessors.

2.4. Food Intake, Body Weight and Tumor Volume and Weight Measurements

Food intake was estimated by subtracting residual weekly food weight/cage from initial food weight dispensed to each cage every week to the nearest 1.0 gm. All animals were weighed at baseline and weekly with a digital scale. Tumor size was measured in two dimensions, weekly, during six weeks. The larger tumor dimension was considered as length (L), and the other (at 90 degrees) as width (W). After appearance of the tumor, the length and width of the tumor were measured by a digital caliper once a week. Tumor volume was then estimated using the tumor volume formula: [V = π/6 (W $\times$ L2)]. After sacrificing, the entire tumor and gastrocnemius muscle were dissected and the final tumor weight and gastrocnemius muscle were measured. The degree of precision for tumor and muscle weight was 0.0001 g.

2.5. Muscle Endurance Measurement (Kondziela's Inverted Screen Test)

Mice were placed in the center of the wire mesh screen, a stopwatch was started, and the screen was rotated to an inverted position over two seconds, with the mouse's head declining first. The screen was held steadily 40 to 50 cm above a padded surface. The time when the mouse fell off was noted, or the mouse was removed if the criterion time of 120 seconds was reached. The test was scored as paper of Deacon (22).

2.6. Blood and Tissue Sampling

The mice were euthanized 48 hours after the last exercise or sham exercise session. During the euthanasia period, blood (1.5 ml) was withdrawn. Blood samples were then centrifuged for 15 minutes at 4000 rpm, serum was collected and then serum and tissues were stored at -80 °C for further analysis.

2.7. Training Effectiveness

The Heart Weight to Body Weight (HW/BW) ratio was used as an indicator of the effectiveness of training (23). After sacrificing, the heart weight was measured and the ratio was calculated using the final body weight.

2.8. Cytokine Measurements

Serum and muscle, fat and tumor tissue were removed and frozen (-80°C) immediately after the mice were sacrificed. Fresh-frozen tissues (100 mg) were homogenized. The homogenates were immediately centrifuged at 12000 g for 20 minutes at 4°C, and the supernatant was removed as the detergent-soluble fraction. Protein concentrations were determined using the Bio-Rad Protein Assay with BSA for the standard curve. The samples were stored immediately in aliquots at -80°C for subsequent ELISA analysis. Assays of ghrelin (Code number RAB0207, Sigma Aldrich USA, Intra-Assay: CV < 10%, Inter-Assay: CV < 15%, Detection Range: 0.1 - 1,000 ng/mL), leptin (SEA084mu, cloud-clone corp. wuhan USA, Intra-Assay: CV < 10% Inter-Assay: CV < 12%, Detection Range: 0.156 to 10 ng/mL), and adiponectin (ab108785 Abcam USA, Intra-Assay: CV < 4.8% Inter-Assay: CV < 7.1%, Detection Range: 0.78 ng/mL to 50 ng/mL) were performed by ELISA kits. All assays were measured in duplicates and the mean was reported.

2.9. Statistical Analysis

All data are preented as mean ± Standard Deviation (SD) after ascertaining normality of distribution visually and statistically. Independent t-tests were used to assess the main effects of exercise training for outcomes measured only once at the conclusion of the study (tumor weight, heart weight, and heart weight/body weight ratio). Repeated measures Analysis of Variance (ANOVA) was used to assess the main effect of Time and Group x Time interaction for outcomes measured repeatedly across the experiment (body weight, food intake, muscle endurance, and tumor volume during six weeks, as well as cytokine levels). It should be noted that all repeated measurement assumptions were checked. When the Group x Time interaction was significant in the ANOVA model, post-hoc t tests of Bonferroni were used to ascertain the specific time points, at which the change over time differed between groups. Relationships between changes in variables of interest (e.g. change in adipokines and change in tumor size) were analyzed via linear regression models and Pearson correlation coefficient. All analyses were performed using the SPSS statistical software Ver. 19(IBM, Chicago, IL, USA) with the significance level set at P < 0.05.

3. Results

3.1. Body Weight and Food Intake

The mean body weight and food intake between the 2 tumor groups is shown in Table 1. Body weight did not differ significantly between TC and E groups across six weeks (P = 0.189). However, the E group had a significantly higher food intake than TC (P = 0.042). Unexpectedly, there was a negative correlation between food intake and body weight during the six weeks in TC (r = -0.93, P = 0.008), which was not seen in the E group (r = 0.68, P = 0.134).

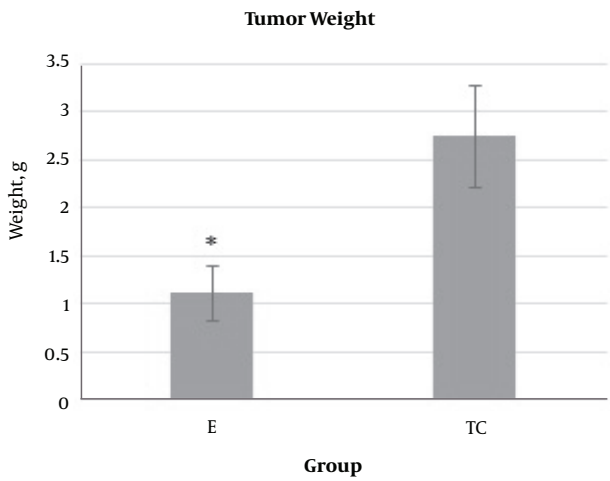
3.2. Muscle Weight

As expected, gastrocnemius muscle weight was higher in E (0.0787 ± 0.012 mg) compared to the TC group (0.045 ± 0.009 mg; P = 0.001) after sacrificing at six weeks. The mean difference between the two groups for muscle weight was 0.033 mg (CI: 0.016, 0.045).

3.3. Tumor Size and Tumor Weight

As hypothesized, the final tumor weight after sacrifice was significantly lower in the E group compared to the TC group (P = 0.001), Figure 1. The mean difference between the 2 groups for tumor size was 1.625 g (CI: 0.064, 2.33). Consistent with this finding, the rate of tumor growth was attenuated in the E group compared to the TC group over six weeks (P = 0.001, ES = 0.78). This E effect was not observed at week 1 (P = 0.63; mean diff 2.55g CI: -8.77, 25.25) or week 2 (P = 0.24; mean diff 27.92g CI: -30.99, 144.82), yet the 2 groups were significantly different from week 3 to 6 (week 3: P = 0.001; mean diff 166.99g, CI:73.35, 345.49; week 4: P = 0.001; mean diff 326.33g, CI:14.45, 576.58; week 5: P = 0.001; mean diff 777g, CI:141.5, 1317.51; week 6: P = 0.001; mean diff 1327g, CI:302.22, 1945.22).

Figure 1. Comparison of Tumor Weight (g) Between Exercise (E) and Tomur Control (TC) Groups



*P < 0.001.

Table 1. Body Weight and Food Intake Throughout the Study Period Between the Groups[a,b]

	Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Body weight, g	TC	17.83 ± 0.98	18.03 ± 0.91	18.60 ± 1.12	19.05 ± 1.03	19.66 ± 1.07	20.81 ± 1.22
	E	17.75 ± 0.69	17.98 ± 0.77	18.26 ± 0.83	18.61 ± 0.78	19.72 ± 1.03	19.93 ± 1.23
Food intake, g	TC	165.50	166.05	162.82	160.23	155.04	140.62
	E	162.90	169.5	166.84	167.33	170.55	172.04

Abbreviations: TC, Tumor Control; E, Exercise; g, grams
[a]Data are presented as Means ± Standard deviation
[b]Food intake was measured for the whole cage of each group and therefore has only a single mean value for each group.

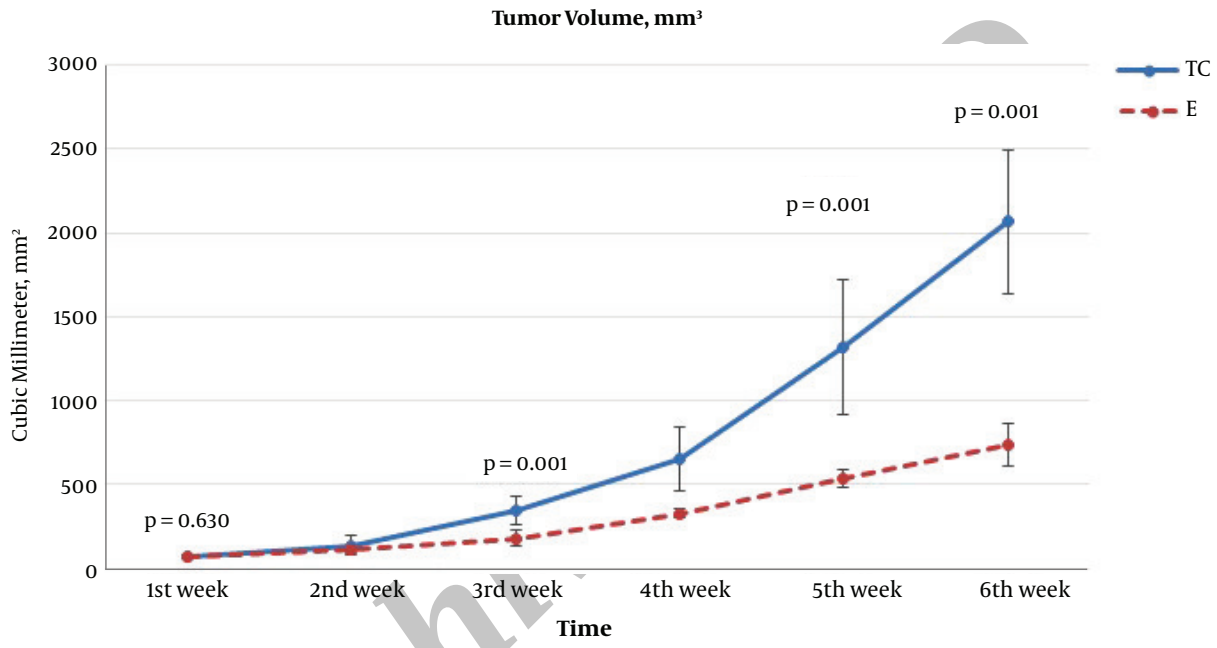


Figure 2. Tumor Volume (mm^3) Between Exercise (E) and Control (TC) Groups

3.4. Muscle Endurance Test

Kondziela's inverted screen test was used to determine muscle endurance of the mice in the healthy control, TC, and E groups. Healthy mice held on for more than 120 seconds on the device. There was a significant difference among the three groups (P = 0.001) overall. There were significant differences among all three groups from week 3 to 6. The healthy group was the strongest group and E was stronger than TC, Figure 3.

3.5. Fitness Adaptations

The heart weight to body weight (HW/BW) ratio is an index of efficiency of training (23), with higher ratios indicating better training adaptation. The HW/BW ratio was higher in E (0.006 ± 0.001) than in the TC group (0.004 ± 0.001; P = 0.001), as expected. The mean difference between the 2 groups for HW/BW ratio was 0.002 (95% CI: 0.001, 0.003). The correlation between HW/BW ratio and muscle weight and tumor weight was r = 0.736 (P = 0.001) and r = -0.663 (P = 0.005), respectively. The relationship between HW/BW ratio and the endurance test at the sixth week was r = 0.621 (P = 0.010).

3.6. Adipocytokine Changes

Adiponectin: As hypothesized, serum adiponectin was higher in E than TC at the end of the trial (P = 0.001). Similarly, fat adiponectin was higher in E than TC (P = 0.002), as was muscle adiponectin (P = 0.014). However, tumor adiponectin was similar between the two groups (P = 0.47), Table 2.

Leptin: As hypothesized, leptin was significantly lower in E compared to TC in serum, fat, muscle, and tumor, Table

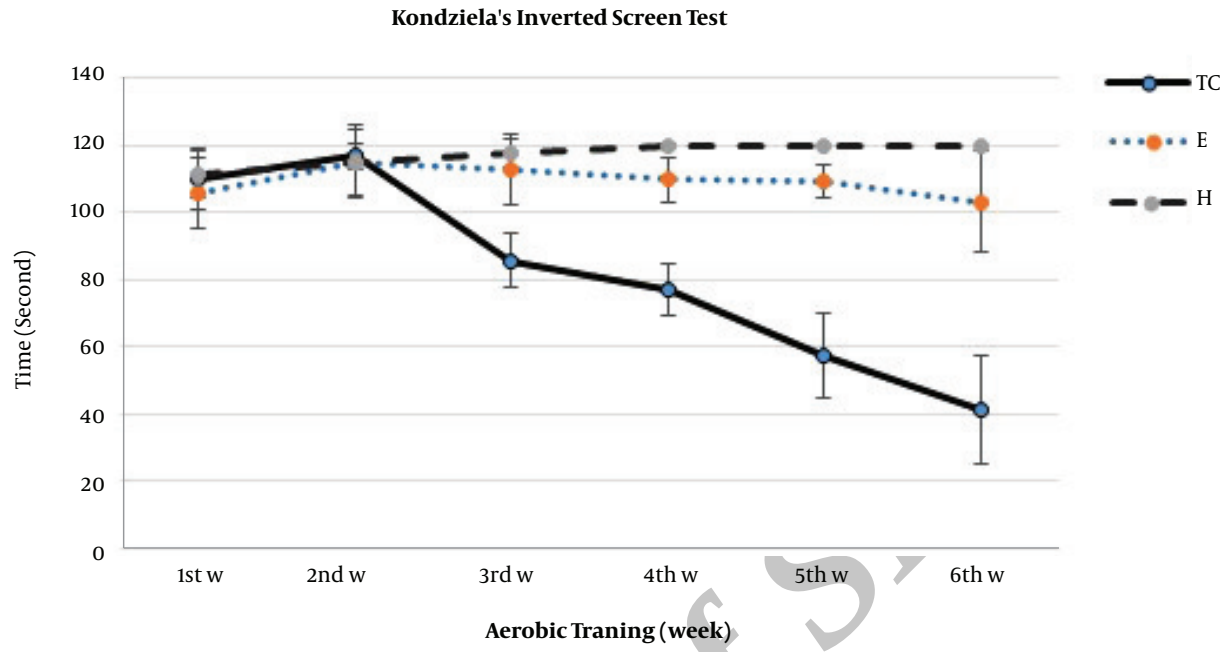


Figure 3. The Result of Kondziela's Inverted Screen Test (sec) Between the Groups (TC: Tumor Control, E exercise, H Healthy)

2.

Ghrelin: Ghrelin was only measured in serum and tumor, and results were mixed, Table 2. It was significantly higher in serum in the E compared to the TC group, as hypothesized, yet lower in E than TC in presence of tumor.

Table 2. Levels of Adiponectin, Leptin and Ghrelin in Tissues Between the Groups[a]

Adipocytokine	TC	E	P Value
Adiponectin, pg/ml			
Serum	7.91 ± 1.46	12.16 ± 2.21	0.001
Tumor	35.91 ± 2.41	36.92 ± 3.09	0.471
Fat	23.16 ± 2.38	30.56 ± 4.86	0.002
Muscle	25.21 ± 2.71	29.57 ± 3.47	0.014
Leptin, ng/ml			
Serum	3.42 ± 0.38	2.15 ± 0.11	0.001
Tumor	3.67 ± 0.67	2.95 ± 0.32	0.016
Fat	10.41 ± 1.11	5.02 ± 1.14	0.001
Muscle	6.20 ± 2.33	3.91 ± 1.21	0.027
Ghrelin, pg/ml			
Serum	1454.40 ± 18.12	1526.41 ± 32.11	0.001
Tumor	1122.20 ± 100.80	1028.11 ± 50.31	0.033

Abbreviation: TC, Tumor Control; pg, picogram; ng, nanogram; ml, milliliter; E, Exercise
[a] Data are presented as Means ± Standard deviation
[b] ANOVA test, P < 0.05 was considered significant.

3.7. Associations Between Adipokines and Other Variables

Data from E and TC groups were pooled to analyze these relationships. There were inverse associations between leptin and adiponectin in tumor (r = -0.846 P = 0.008), serum (r = -0.754 P = 0.031), muscle (r = -0.607 P = 0.110), and adipose tissue (r = -0.892 P = 0.003), as expected. This study also investigated the relationship between these factors and the outcomes related to cancer progression and cachexia.

3.8. Tumor Size

As hypothesized, tumor size was directly related to leptin in serum (r = 0.94, P = 0.001), adipose tissue (r = 0.83, P = 0.001), and tumor (r = 0.59, P = 0.016). Similarly, tumor weight was directly related to leptin level in serum (r = 0.78, P = 0.001) and adipose tissue (r = 0.87, P = 0.001), as expected. Also, as hypothesized, tumor size (r = -0.67, P = 0.004) and tumor weight (r = -0.87, P = 0.001) were both inversely related to serum ghrelin. By contrast, tumor size was directly related to tumor ghrelin (r = 0.72, P = 0.002).

3.9. Body Composition

There was a significant positive relationship between leptin and body weight in adipose tissue (r = 0.557, P = 0.025) and serum (r = 0.512, P = 0.043), yet not in muscle (r = 0.468, P = 0.068) or tumor (r = 0.173, P = 0.523). As expected, muscle weight was inversely related to tumor (r =

-0.56, P = 0.025), serum (r = -0.84, P = 0.001) and adipose tissue (r = -0.72, P = 0.002) leptin levels, and positively related to serum ghrelin (r = 0.81, P = 0.001).

3.10. Fitness

As expected, the HW/BW ratio (aerobic fitness index) was inversely related to leptin in tumor (r = -0.42, P = 0.109), serum (-0.63, P = 0.008), muscle (r = -0.62, P = 0.010), and adipose tissue (r = -0.61, P = 0.013). Also, as anticipated, muscle endurance was inversely related to leptin in tumor (r = -0.62, P = 0.009), serum (-0.82, P = 0.001), and adipose tissue (r = -0.87, P = 0.001), and directly related to ghrelin in serum (r = 0.78, P = 0.001).

There were no significant relationships between adiponectin and any of the above variables.

4. Discussion

As hypothesized, aerobic training significantly decreased both the growth rate and final weight of the tumor compared to non-exercising controls. Also, as hypothesized, exercising mice had greater food intake, muscle endurance, heart weight/body weight (fitness), and muscle weight than controls. In addition, many of the hypothesized potentially beneficial adaptations to exercise in cytokines were observed, including significantly increased adiponectin at all sites except the tumor, decreased leptin at all sites, and increased ghrelin in serum. Finally, as anticipated, the levels of cytokines after training predicted some of the beneficial physiological outcomes related to cancer cachexia noted above. Thus, the current data provides evidence for the beneficial effect of aerobic training in the modulation of cytokines involved in tumor cachexia and growth, resulting in attenuation of tumor burden, greater food intake, increased muscle size and endurance and better cardiovascular and musculoskeletal fitness in mice with breast cancer compared to non-exercising control mice with breast cancer.

4.1. Effect of Exercise on Cachexia and Cytokines

Cachexia is associated with loss of muscle mass as well as muscle function. On the 3rd week, muscle endurance declined as tumor volume increased, and both of these changes were attenuated by concomitant aerobic exercise compared to sedentary mice. For determine cachexia, advanced devices must be used to measure the composition of the mouse body, which was a limitation of the current study. Other positive adaptations to the exercise intervention included a higher food intake, HW/BW ratio, and gastrocnemius muscle size. Therefore, several common features of cancer cachexia in mice were mitigated by the aerobic training intervention.

The current results are in line with the results of several animal studies (3, 24), which have shown a reduction in tumor volume after aerobic training. Isanejad et al. (2016) and Khori et al. (2015) reported a reduction in tumor volume after aerobic training associated with changing levels of several miRNAs (miR-21, Let-7a and miR-206) linked to cachexia and their target genes (3, 25). Reduced tumor volume after exercise training in animals has also been associated with reduction in levels of angiogenic cytokines and inflammation products (20) within tumor tissue. In this study, for the first time, reduced tumor volume in the aerobic training group as well as modification of 3 cytokines influencing appetite and energy metabolism (leptin, ghrelin and adiponectin) were indicated. Notably, in the cases of ghrelin and leptin, these changes were associated with some of the features of cancer cachexia measured.

Ghrelin plays an autocrine/paracrine role in a number of processes associated with cancer development, including cell proliferation, cell migration, and apoptosis. A small number of studies have focused on ghrelin, specifically in the tumor tissue of cancer patients. Jeffrey et al. (2005) showed that ghrelin levels in breast cancer cells are higher than in normal cells, and ghrelin stimulates proliferation of cancer cells (26). In the present study, it was shown that aerobic training reduced the levels of ghrelin in tumor tissue, correlated with a reduction in tumor volume compared to sedentary tumor-bearing mice. Therefore, it is possible that reduced tumor growth with exercise may be related in part to reduced levels of ghrelin in tumor tissue.

By contrast, consistent with other studies, increased serum ghrelin levels were observed in the mice after aerobic training. Thus, it may be that serum ghrelin acts in a different manner to ghrelin in tumor tissue, preventing cachexia, and inhibiting tumor growth. Circulating ghrelin has a strong orexigenic effect (27). In tumor-bearing states, cachectic factors, such as cytokines, can elicit effects on energy homeostasis that mimic leptin and suppress orexigenic ghrelin. As shown in Table 1, food intake in the aerobic training group was higher than the control group. Although individual levels of food intake and serum ghrelin could not be correlated as food intake was measured for the group as a whole, the findings are consistent with the possibility that aerobic exercise stimulates food intake via increased circulating ghrelin. Ghrelin is known to inhibit leptin and pro-inflammatory cytokine expression by human monocytes and T cells (28), which could be one mechanism by which ghrelin increases appetite. Thus, a beneficial adaptation to exercise training in cancer cachexia may be modulation of ghrelin and other appetite-regulating neuropeptides.

Adiponectin and leptin are altered with adiposity and

exert antagonistic effects on cancer cell proliferation.

Adipokines are bioactive particles that mediate metabolism, inflammation, angiogenesis, and proliferation. Leptin and adiponectin represent two adipokines that elicit generally opposing molecular effects. There are associations between increased serum leptin levels and increased tumor growth, while adiponectin exhibits an inverse, relatively strong correlation with cancer development. Leptin has been shown to increase proliferation, migration, and invasion of cancer cells (29), while lower plasma adiponectin levels are associated with larger tumor size and metastasis in clear-cell carcinoma of the kidney (30). Adiponectin can also antagonize the actions of leptin. For example, leptin phosphorylates and activates the signal transducer and activator of transcription (STAT)3 and STAT5 and Janus Kinase (JAK)2, which are involved in cancer development. By contrast, adiponectin can increase protein tyrosine phosphatase 1B (PTP1B), which then de-phosphorylates STAT3 and JAK2, thus antagonizing leptin signaling.

In this study, levels of serum adiponectin were higher and leptin levels were lower after exercise compared to non-exercising control mice, consistent with studies of physical activity in humans. Although normally secreted from adipose tissue, leptin can be secreted from breast cancer cells. Levels of leptin in tumor tissue and adipose tissue were significantly lower with exercise, yet levels of adiponectin were not different in tumor tissue and significantly higher in adipose tissue after exercise. It is possible that the exercise training suppressed leptin signaling and improved cachexia in this cancer model by increasing levels of adiponectin. However, as these regulatory factors were only measured at the end of training, it is not possible to ascertain the time course and causal relationships among them. These adaptations to training require further study with sequential sampling over the full time course of tumor growth and cachexia development.

In summary, ghrelin, leptin, and adiponectin can modify energy metabolism and appetite and may have important roles in cancer cachexia and tumor progression in both animals and humans. Aerobic training for six weeks in a murine model of breast cancer successfully attenuated tumor growth and cachexia, including food intake, body composition and muscle function/fitness compared to sedentary mice, and some of these benefits were related to beneficial cytokine/hormone adaptations in these animals. Future investigations of the time course and molecular mechanisms underlying these adaptations, optimal dose-response patterns, and relevance to human cohorts with breast and other cancers are warranted.

Acknowledgments

This research was funded by the Physiology Research Center of the Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.

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