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The comparison study of effect of endurance and interval training on Hsp70 levels in mice with breast cancer tumor

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Abstract

The perpose of this study was to compare effect od endurance and interval traning on levels 30 female balb/c mice were utilize randomly divided in 3 groups as fallow:1:tumor-control, 2:endurance training-tumor, 3: interval trianing-tumor.endurance training protocle was done for 6 weeks at 25% to 75% vo2max and training protocle was done for 6 weeks at 20% to 55% vo2max between 1 until 10 interval rep*1 min.Blood samples were collected after protocle and ELIZA method used for measuring The result of this study showed rate of Hsp70 in interval training groups decaesd and in endurance training increased and there were significant difference between groups.also data analyze showed tumor volume in interval training group decaeed significant and in endurance griup increased significant..based on the result of this research;doing exercise with change the rate of Hsp70 can is effective in improving cancer treatment as a supplement in addition to other treatments

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Introduction

Over 1.38 million women are diagnosed with breast cancer annually and more than 458 000 will die from the disease worldwide. Early detection and advancements in treatment options have led to a steady improvement in 5-year relative survival rate in developed countries, such as Canada, where the 5-year relative survival rate is currently 87% (Holmes *et al.*, 2005). This trend has resulted in a growing population of breast cancer survivors who are potentially faced with a number of long-term side effects of cancer and its treatment. Some of these include decreased aerobic capacity and strength, weight gain, fatigue and reduced quality of life. The use of conventional and novel therapies is associated with a diverse range of debilitating physiologic (e.g., physical deconditioning, weight gain, cardiac and pulmonary dysfunction, etc.) and psychosocial (e.g., fatigue, nausea, depression, anxiety, etc.) symptoms that can have profound implications.

On quality of life (Vogel *et al.*, 2008). The negative impact of surgery is dependent on the tumor location (site) and extent of resection. Today physical exercises are seriously considered as one of the main components of rehabilitation in most chronic diseases which has been shown to increase the factors influencing the quality of life (Bonay M *et al.*, 1994). For instance, breast cancer had a 30 percent reduction by running. The interval-training exercise intervention was effective in improving the functional capacity of stage II breast cancer patients on adjuvant chemotherapy (Brunelli *et al.*, 2007). A factor which is effective on tumor growth is heat shock proteins (HSPs). They play an important role as intracellular chaperones in the immune system and may protect cells from the harmful effects of environmental stress factors (Fehrenbach *et al.*, 1999). Hsps are highly conserved proteins in nearly all organisms and are induced by various environmental and pathophysiological stresses. Hsp70 interacts with abnormal proteins including mutated or altered oncogene and tumor suppressor gene products (Osuch *et al.*, 2002). Which are closely associated

with the development or progression of various tumors. Studies have implicated that enduring training up regulates hsp expression and may provide an extra protection against oxidative stress. On the other hand, hsp70 is over-expressed in various tumors such as those of the lung, colon, breast and pancreas (Lanng *et al.*, 2007). Recent studies have identified that hsps have important extracellular function. Have demonstrated that hsp72 binds with high affinity to the cell surface receptors of human monocytes and enters the cell via a specific signal transduction pathway (Doyle *et al.*, 2006).

Hsp70, the most highly conserved and abundantly induced of the stress proteins, appears to play dual and opposing roles in cancer. On the one hand, Hsp70 promotes growth and survival of tumor cells by engaging misfolded or aggregated proteins and proteins involved in cell proliferation. As such, it endows tumor cells with stress resistance. However, Hsp70 can also promote tumor immunity by stimulating innate immune mechanisms and enhancing cross-presentation of tumor antigens to lymphocytes (Taylor *et al.*, 2004).

Following exercise, the stress response can be invoked in an effort to maintain or regain cellular homeostasis. As has been detailed in several recent and addressed elsewhere in this text, exercise results in activation (and inactivation) of a number of cell signaling pathways, which vary in a muscle- and exercise-specific fashion (Ciocca *et al.*, 1993). Moderate changes in intracellular ATP concentrations with increases in ADP and AMP, decreased carbohydrate stores, hypoxia, ischemia, and reduced intracellular pH could all be responsible for exercise-induced expression of HSPs. Increases in intracellular calcium and exercise-dependent may also release of hormones such as epinephrine and norepinephrine result in HSP accumulation. Finally, direct exercise-induced muscle stretch or damage resulting in cytokine release and initiation of the inflammatory response can alter HSP production (Ocke *et al.*, 1995). Furthermore, it has been shown that exercise is a powerful stimulus of intracellular Hsp70 expression

in immune cells. In muscle and other tissues like myocardium, liver, spleen, and brain. However, studies about effect of continuous and interval training on plasma levels of hsp70 in tumor cells are rare (Asea *et al.*, 2000).

More studies in human and animals have been shown that prolonged and intensity training induced lead to increase of Hsp70 levels. So increase levels of Hsp70 inhibit cell death in tumor. But there is not any studies that show exercise training lead to reduce of hsp70 levels.

Therefore, we questioned whether there are different in the effects continuous and interval training on Hsp70 levels in balb/c mice with carcinoma tumor. We also examined which exercise has role in reduce of tumor size.

Materials and methods

Mice

30 Inbred female Balb/c mice, aged six to eight weeks were obtained from Institute of animal. Given free access to food and water, mice were housed for one week and maintained under standard conditions prior to experimentation. All the experiments were performed under supervision of expert veterinarian and according to the ethical standards of the international journal of sports medicine (Galvao DA *et al.*, 2005).

Exercise protocol

Treadmill running was the exercise method used. Tumor bearing Mice were randomly divided into 3 groups as follows: sedentary control group, interval training group, continuous training group. Continuous training protocol was done for 6 weeks at 25% to 75% VO_{2max} . Interval Training protocol was given exercise on a treadmill at 20% to 75% VO_{2max} between 1 until 10 interval rep *1 min for 5 days in 6 weeks at 5% degree. Control group was housed near the treadmill without food and water during the time of exercise and did not exercise.

Hsp70 ELISA assay

to detect and quantify the inducible HSP70 levels in the plasma/serum samples (2 × 100 µl duplicates) of mice, an enzyme immune assay (EIA) (stress gene, Victoria, Canada) was utilized. The sensitivity was described as 0.2 ng/ml. Plasma/serum samples were stored immediately after collection at -80°C until further analysis. Assay procedures were performed according to the manufacturer's instructions. Post-exercise values were corrected for plasma volume change according to Dill and Costill (Bentzen SM *et al.*, 2006).

Statistical Analysis

The results were depicted as the mean ± standard deviations of triplicate determinations. Statistical analysis was performed using one-way ANOVA and two-tailed Student's t test. A value of $p < 0.05$ was considered to be statistically significant.

Results

Effect of Interval and continuous Training on the Level of Heat Shock Protein of the Spleen Cells.

The results indicated that significantly increased were noticed in the level of HSP 70 among the animals trained with continuous training, and significantly decreased in the levels of HSP70 among the animals trained with interval training compared to the control group (group 1). Fig. 1. Hsp70 levels in training and control groups.

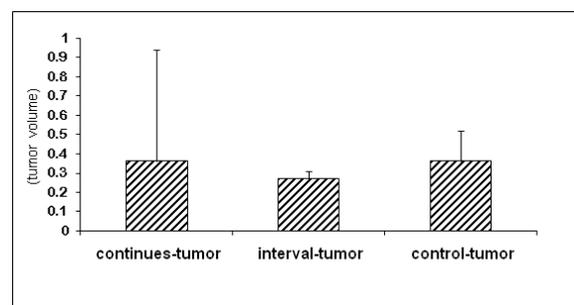


Fig. 1.

Effect of Interval and continuous Training on size of tumor

Also, we found a significant decrease in the tumor size in interval training group in comparison with group 1 and a significant increase in the tumor size in continuous training group in comparison with group 1.

Fig2:Tumor volume in Training and tumor groups

Conclusion

The primary aim of this case study was to investigate the Alterations in serum Hsp70 after interval and continues training in balb/c mice with breast cancer tumor. We found an increase in basal levels of serum Hsp70 after continues training and a decrease in basal levels of serum Hsp70 after interval training .

Physical exercise has consistently been identified as a central element of rehabilitation for many chronic diseases (Martino *et al* .,2004) and has been successful in improving the quality of life and reducing all causes of mortality (Vogel *et al* .,2008). Previous evidence suggests that moderate levels of physical activity may even reduce the risk of death from breast cancer (Galvao *et al* ., 2005). Therefore, exercise may prove to be a valuable intervention to improve not only the quality of life but also the overall survival. Human breast carcinomas are frequently infiltrated by inflammatory cells secreting several cytokines which may regulate the activity of both immune cells and neoplastic cells. American Cancer Association issued guidelines recommend that all patients with cancer are encouraged to exercise during chemotherapy. The recommendations are based on promising initial evidence of the effect of exercise on maintaining or improving the quality of patient status(Osuch *et al*., 2002).

Previous studies revealed that over-expression of hsp70 in breast cancer, as well as in colorectal cancer, were associated with unfavorable prognosis. Furthermore, it has been shown that exercise is a powerful stimulus of intracellular HSP expression in immune cells, muscle and other tissues like myocardium, liver, spleen, and brain (Smolka *et al*.,2000). HSP70 acts as a molecular chaperone, and is involved in folding of nascent polypeptide chains and translocations of precursor proteins across the membranes of cytoplasmic organelles. Hsp70 has also been demonstrated in other malignancies such as endometrial, breast, lung, colonic, and pancreatic

cancers. Such enhanced hsp70 expression may be due to the stressful microenvironment of neoplastic cells inducing aggressive growth, local tissue hypoxia, and glucose starvation. In conclusion, exercise may improve cell survival by protecting proteins from degradation and facilitating their folding (Hendrick *et al*.,1993).

HSPs have been detected in body fluids of cancer patients and in supernatants of tissue cultures recent observations imply a crucial role of extracellular localized and membrane-bound HSPs in inducing an efficient cellular immune response against cancer. It is very well known that endurance exercise results in an up-regulated Hsp70expression in healthy humans(Thompson *et al*.,2002). Apart from the intracellular up-regulation, soluble Hsp70 was significantly increased in the plasma/serum and in the brain of endurance athletes. Soluble Hsp70 proteins are involved in the activation of an anti-tumor immune response mediated by NK cells. These findings might have implications to broaden the understanding of an exercise-induced Hsp70 release in athletes. Furthermore,the increase in serum Hsp70 during Exercise was inversely correlated to the resting values of Serum Hsp70.Days with high pre-exercising serum Hsp70 Levels generally had a smaller increase after exercise.results of this study is agree with some of studies and disagree with other investigates. (Radons *et al*.,2005).

It is Interesting to note that showed The same correlation between pre-exercis evalues and post-Exercise increase of Hsp70 in human muscles biopsies Measured over5–8 weeks of exercise. It is also suggested that basal Hsp70 Is up-regulated after exercise–heat acclimation to protect Against delayed thermal injury by providing cytoprotection With out the need for de novo protein synthesis.Furthermore,Hsp70 when released in to the circulation acts as a cytokine to activate an immune response and ,therefore, plays an important role after exercise(Fairey *et al*.,2003) .Hence Several studies and the present have shown increased Levels of Hsp70 with exercise. The concentration of serum

Hsp70 is a result of the release in to and the uptake from the circulation. The origin of the released Hsp70 to the Circulation is, however, still unknown. A variety of human cells and tissues like the brain, liver and leucocytes have been shown to increase their expression of Hsp70 in response to exercise and could, therefore, be responsible for the increase in serum Hsp70 levels. Further work is required to assess the full biological significance of the changes in extracellular Hsp70 after heat exercise (Kraus *et al.*, 2004).

Hsp also have an anti-apoptotic role, preventing or at least inhibiting programmed cell death. The magnitude of the heat shock response is dependent upon the intensity of the exercise bout, with higher exercise intensities yielding greater Hsp induction. It has also been shown in human studies, for example, that prolonged exercise induced a more pronounced response of serum Hsp70 than shorter more intensive exercise. So increase levels of Hsp70 inhibit cell death in tumor. This finding indicates that continuing with increase levels of hsp70 lead to growth tumor. There are not any studies that show exercise training lead to reduce of hsp70 levels (van Baak *et al.* 1999).

In the present study we demonstrated, for the first time, that interval training results in a decrease of HSP70 levels. So we can conclude that may be reduce size of tumor in interval training group is in response to decrease hsp70 levels but we must investigate other effective factors in tumor.

In conclusion, The present study demonstrated that program and continues training induced a more pronounced response of HSP70 Levels compared with interval training.

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