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Effect of Continuous and Interval Exercise on The Expression of Heat Shock Proteins in Animal Model

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In this study, elderly female animals as a model have been selected to evaluate the level of heat shock protein molecules in the spleen before and after different types of trainings (continuous and interval). It is noticed that the interval training could highly increase the heat shock protein level in both mid and post test, while a significant increase was noticed in the post test continuous training, but not in its mid test. Making generalization about this model it is concluded that in the elderly female animals, in case heat shock protein level is normal, the body is in a healthy condition and training can preserve the normal level of heat shock protein. Being in agreement with the previous results which confirmed an increase in the heat shock protein level, in muscles, healthy heart, liver and brain after exercise, present study indicated an increase also in spleen.

Key Words: Continuous training, Interval training, Humeral immune system, Immunoglobulin, Wistar rat.

INTRODUCTION

Heat shock proteins (HSPs) are highly conserved proteins that are expressed by all prokaryotic and eukaryotic cells. They are described originally as proteins expressed following heat stress. However, it is now established that the expression of heat shock proteins can be induced by various stressful stimuli such as oxidative stress and exposure to heavy metals[1-3].

The medical importance of stress proteins is apparent from numerous studies on infection, inflammation, autoimmune diseases, tumor immunity and organ transplantation immunology. In many experimental models and clinical situations, heat shock protein-responsive lymphocytes have been shown to participate in the immune response[4-6]. The stress response following exercise can be invoked in an effort to maintain or regain cellular homeostasis. As has been detailed in several recent reviews[7,8], exercise results in activation (or inactivation) of numerous cell signaling pathways, which vary according to the training protocol. Moderate changes in intracellular ATP concentration with increase in ADP and AMP[9,10], decrease in carbohydrate storage[11], hypoxia[12], ischemia[13] and intracellular pH level drop[14], could be all responsible for exercise-induced expression of heat shock proteins.

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The first study to document the effect of exercise on the stress response was conducted by Hammond *et al.*[15], in which rats were swam to exhaustion. Hearts were extirpated and analyzed for HSP70, after immediate post exercise. No difference between control hearts and swimmers was observed. Subsequent studies employing treadmill running rats[16,17] and exercising humans have demonstrated that acute exercise is a sufficient stressor to induce the synthesis of numerous heat shock proteins. Changes in exercise intensity, which lead to differences in muscle recruitment patterns and hence muscle loading, also appear to have a significant effect on heat shock protein expression[18]. Elevated levels of several heat shock proteins persist with repeated bouts of exercise, despite adaptations which would tend to reduce the relative stress of exercise. In young rats (10 weeks of age), the myocardium exhibit an increase of 12 fold in HSP70 level, following 8 to 10 weeks of treadmill run training with moderate intensity[19]. Using middle aged animals (10 months old) observed smaller (25 to 45 %) but significant increase in both HSP70 and HSP60.

Fehrenbach *et al.*[20] suggest that exercise training might allow individuals to mount a faster heat shock protein response to external stressors while perhaps attenuating the magnitude of the increase accompanying training. Some factors such as age, gender and strain may play a crucial role in the synthesis of heat shock proteins in response to exercise. The ability of cells to induce heat shock proteins following stress is reduced in aged individuals. Tissues from aged animals and blood cells of elderly humans both show a decrease in the production of stress proteins following thermal stress[21]. In comparison with muscles of young animals, the production of HSP70 was severely blunted in response to a period of mild, non damaging contractile activity, although this dose not appear to be true in skeletal muscles of rats following a period of whole body hyperthermia[22].

The ability of aged rats' muscles to adapt following a 10 week training regimen on a treadmill seems to be fiber type specific. Work by Naito *et al.*[23] demonstrated that exercise training of aged rats on a treadmill for 10 weeks, results in a similar accumulation of HSP70 in predominantly fast skeletal muscles[24]. The lack of adaptation in heat shock protein content in aged animals may be related to a more general failure of adaptation to stress, particularly in predominantly type 2 fiber-containing muscles. It is worth mentioning here that other data indicated a reduction in the ability to adapt following damaging exercise, in aged animals[25].

In this study, we evaluate the different time durations (short and long) and different intensities (continues and interval) of exercise, on the expression of HSP70 in aged rats.

EXPERIMENTAL

Twenty four old female Wister rats were obtained from Pasteur Institute of Iran and housed individually in plastic cages, used for this study. The animals had free access to food (a standard laboratory food) and water. The temperature of the animal room was set between 9-22 °C. The relative humidity was 45 to 65 % and a 12/12

h light/dark cycle was applied. Using and taking care of the rats were in accordance with protocols, approved by Pasteur Institute of Iran.

After 4 weeks of familiarization, rats were randomly assigned to one of three groups namely control, continuous and interval. Six of twenty week old rats were trained by continuous and six others by interval training, 5 days/week (Table-1). The rats in control group were housed in cages without any training facility. Animals were divided into mid test (6 weeks training) and post test (12 weeks training) groups. During the last week before beginning the training protocol, rats were trained at 7 m/min for 5 min/day which increased to 9 m/min for 15 min/day, in order to become familiar with the surroundings. The running speed was initially set at 12 m/min and reached 22 m/min in the12th week. The activity included two 5 min warm-up periods at 7 m/min which increased up to 9 m/Min at the beginning and reached 15 m/min in the last week of training program. There was also a final 5 min considered as warm-down at 15, 12, 9 and 7m/min.

Rat's spleens were aseptically removed and minced. The released cells were washed three times in RPMI-1640 medium. Erythrocytes were lysed in 0.17 M NH_4Cl for 3 Min and the obtained spleeny cells were washed twice in RPMI-1640. The rest of cells were lysed in 1 mL lyses buffer (50 Mm Tris, 5 µM EDTA, 0.5 % Triton-100, 5 µg/mL leupeptin, aprotinin, pepsatine A, antipain, chymostatine) and then centrifuged at 16000 g for15 min. The quantity of sample proteins were measured by the means of spectrophotometer at 280 nm and equalized. The samples were then stored[1] until analysis at -20 °C.

For the detection of HSP70, equal amounts of protein were separated by SDS-electrophoresis on 10 % polyacrylamide gel and transferred to PVDF membrane. Non-specific binding sites were blocked by 2 h incubation of the membrane in BSA 2 %. The membrane was then incubated with antiHSP70 antibody diluted in TBS (1:1000) for 2 h. Bound antibody was detected using peroxides conjugated with anti mouse antibody and bound peroxides were visualized using DAB[2].

Statistics: A 2×4 ANOVA (factor1; continuous *vs.* interval *vs.* control groups, factor 2; pre-test *vs.* middle-test *vs.* post-test) used to test for global effects over the study period. Differences were considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

To evaluate the presence of 73kD constitutive and 72kD inducible forms of HSP70 (HSP/HSC70), the protocol in Table-1 was applied for 24 rats and heat shock protein was determined by western blotting. The results indicated that the heat shock protein concentration in the interval training increased significantly ($p < 0.05$) in mid and post test groups, compared with the control group (Table-3). In continues group, the post test showed a significant ($p < 0.05$) increase in the heat shock protein level while the mid test indicated no significant increase compared with the control group. Also a significant increase was noticed in the level of heat shock protein in continues (post test) compared to interval (post test) group.

TABLE-1
RESULTS OF AGE AND WEIGHT VALUE FOR DIFFERENT TEST OF EXERCISE IN EACH GROUP

Subject	n	Test 1	Test 2	Test 3	Test 4
Age					
Interval	8	20 months	21.5 months	23 months	24 months
Continuous	8	20 months	21.5 months	23 months	24 months
Control (old)	8	20 months	21.5 months	23 months	24 months
Weight					
Interval	8	328.13 ± 2.800	324.13 ± 3.227	321.50 ± 1.309	324.13 ± 3.527
Continuous	8	322.75 ± 8.137	314.00 ± 6.392	318.38 ± 4.173	321.88 ± 4.190
Control (old)	8	322.75 ± 8.137	326.38 ± 3.249	325.63 ± 4.138	323.00 ± 2.828

TABLE-2
TRAINING PROGRAM USED IN CONTINUOUS AND INTERVAL GROUPS

Week	Group 1 (continuous training)	Group 2 (Interval training)
1	10-14º (12m/min)	2 × 5-7º (12m/min)
2	15-19º (12m/min)	2 × 7.5-9.5º (12m/min)
3	20-24º (13m/min)	2 × 10-12º (13m/min)
4	25-29º (14m/min)	2 × 12.5-14.5º (14m/min)
5	30-36º (15m/min)	3 × 10-12º (15m/min)
6	37.5-43.5º (16m/min)	3 × 12.5-14.5º (16m/min)
7	45-51º (17m/min)	3 × 15-17º (17m/min)
8	52.5-58.5º (18m/min)	3 × 17.5-19.5º (18m/min)
9	60-68º (19m/min)	4 × 15-17º (19m/min)
10	70 -78º (20m/min)	4 × 17.5-19.5º (20m/min)
11	80º (21m/min)	4 × 20º (21-22m/min)
12	80º (22m/min)	4 × 20º (22m/min)

TABLE-3
EXPRESSION OF HEAT SHOCK PROTEIN IN AGED WISTER RAT HAVING CONTINUOUS AND INTERVAL TRAINING

Sample	Continues (post) Mean ± SEM	Continues (mid) Mean ± SEM	Interval (post) Mean ± SEM	Interval (mid) Mean ± SEM
Test	4.56 ± 0.2	2.88 ± 0.97	6.61 ± 0.5*	6.23 ± 1.45
Control	–	2.66 ± 1.00	–	–

$p < 0.05$ indicates a significant difference in comparison with pre training.
SEM = Standard error of the mean.

Due to remarkable central role in locomotion, the cellular stress response to exercise has been studied with preferential attention, in skeletal muscles. An induction of the synthesis and accumulation of HSP72, GRP75, GRP78 and HSP60 was reported in rat muscle[26], indicating that exercise activates the expression of heat shock inducible proteins (HSPs) as well as glucose-regulated proteins (GRPs).

The nature of the exercise-activated signal, responsible for heat shock protein induction in skeletal muscle, has been investigated in various reports[27]. Since the accumulation of HSP72 and GRP75 (mtHSP70) in skeletal muscles occurred independently of core body temperature[28] and HSP72 induction was observed following concentric (non-damaging) exercise[29], neither heat nor mechanical stresses alone have been suggested to play a major role in this phenomenon. Oxidative stress[30] and reduced glycogen availability[26,31] have been proposed more recently as main determinants of HSP72 induction following exercise in skeletal muscle.

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