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Effects of Exercise Therapy on Heat Shock Protein70 Expression in Leukocytes

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Abstract. [Purpose] The purpose of this study was to identify effects of exercise therapy on Heat Shock Protein (HSP70) in obese individuals. [Subjects] This study compared pre- and post-intervention levels of HSP70 between two obesity groups (obese exercise therapy n=7, obese control n=7). [Methods] Exercise therapy subjects were treated on a treadmill for 50 minutes at 55–80% of maximal heart rate for eight weeks. The HSP levels were measured prior to exercise at rest, exhaustion and 30 minutes after exercise therapy. Baseline HSP levels were also measured in seven controls, at rest, exhaustion and 30 minutes after exercise therapy. [Results] The findings show HSP levels in the long-term exercise therapy subjects were significantly lower than those of the control group at exhaustion. In this study, HSP70 levels may have increased significantly after exercise. [Conclusion] We conclude that the lower levels of baseline HSP70 expression in exercise therapy subjects might be the results of chronic adaptation to training.
Key words: Heat Shock Protein70, Exercise Therapy, Leukocytes

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INTRODUCTION

The main role of heat shock proteins is to act as molecular chaperones, minimizing protein aggregation and ensuring proper protein folding and transport during and after physiological stress[1)]. HSP expression is also altered during glucose depletion and oxidative stress[2)]. The metabolic changes caused by exercise are similar to those which induce stress protein synthesis[3)]. Physical exercise can elevate core temperature to 44 °C and muscle temperatures up to 45 °C[4)]. Exercise also causes oxidative stress via an increased generation of reactive oxygen intermediates (ROI)[5)].

In studies evaluating the HSP response to exercise in leukocytes, results are not conclusive, HSP70 transcripts increased significantly in leukocytes immediately after a half-marathon[6)]. Only small amounts of HSP70 were produced in young men performing 2 hours of treadmill exercise in the heat[7)]. For young women, moderate treadmill exercise for 20 minutes at 60% of maximal oxygen consumption and estrogen supplementation did not cause HSP70 levels in blood cells to change 6 or 24 hours after the study[8)]. This study is important in order to understand further the different protective roles that HSPs might play, and the importance of their in leukocytes. With the results of these previous studies as the background, the main purpose of this study was to determine whether HSP70 is synthesized in leukocytes after moderate exercise. We reasoned that studying HSP expression in leukocytes of trained subjects was important, if HSPs are indeed involved in adaptation mechanisms to exercise through alteration of the immune function. Also, the pattern

of HSP expression may be different in trained individuals; if exercise does induce HSP synthesis in these subjects, it may take several hours to reach the peak levels that would result in a detectable difference.

We hypothesized that as a result of regular exposure to exercise-related effects such as increased HSPs, may exist as an adaptation to training and basal levels might be higher in trained individuals than in untrained individuals. This study compared pre- and post-intervention levels of HSP70 in obesity groups after long-term aerobic exercise therapy.

SUBJECTS AND METHODS

Subjects

The study was approved by the Institutional Review Board, and written informed consent was obtained from the 14 male subjects. All members of the moderate obese exercise therapy group (n=7) that participated in the study were between the ages of 22 and 30 years old. The obese exercise therapy group were selected for the study if they ran at least 16 km per week, had been doing so for at least 3 months, and could run for at least 1 hour continuously. The obese control group (n=7) were healthy with no history of musculoskeletal or metabolic diseases. These individuals normally exercised 0–1 hour per week. and were excluded from the exercise group because they did not participate in the prescribed exercise. The baseline level of obesity was determined as 25% of the fat and body mass index (BMI) 25[9,10]. The exercise group performed aerobic exercise therapy from low to moderate intensity for 8 weeks. The exercise intensity was 35–54% of maximal heart rate in the first two weeks and 55–80% of VO_2 max for eight weeks, five days a week. Exercise protocols were 10 minutes for warm up, 30 minutes for main exercise, and 10 minutes for cool-down[11]. None of the subjects had performed the treadmill exercise before, and none used drugs or took vitamin or mineral supplements of significance to the study. Blood samples were taken 5 minutes before exercise, at exhaustion, and after 30 minutes of recovery.

Methods

The heart rate of the obese exercise therapy group was measured with a 5-lead electrocardiograph (ECG). Maximal oxygen consumption (VO_2max) was determined by using a continuous, graded treadmill test to exhaustion. The treadmill grade was increased every 2 minutes while speed was kept constant. The speed was individualized so that exhaustion occurred in approximately 8–12 minutes. Standard criteria for an acceptable VO_2max test included a leveling off of VO_2 with increasing workload, a respiratory exchange ratio (RER) above 1.15 and a peak HR (Telemeter heart checker, Polar, Finland) similar to age-predicted maximal values. Both groups also underwent a dual bioelectrical impedance analysis and BMI test to determine body composition[11,12]. Forty-eight hours after the bioelectrical impedance analysis (Inbody Biospace 3.0, Korea), BMI and VO_2max test (Minato, a baseline blood sample of 10 mL was taken. Both groups then ran for 50 minutes on a treadmill at 70% of their VO_2max. Room temperature was kept at approximately 21°C, and relative humidity was set at 60%.

Blood was collected in a stable state. Both groups stopped eating, smoking, drinking and exercising 21 hours before the test concerning circadian rhythm. After 50 minutes, 10 ml of blood was collected at three different times: at rest, at exhaustion, and after 30 minutes of recovery. In order to measure the HSP 70, we mixed 1.67 ml of PBS (phosphate buffered saline) with 1 ml of blood at room temperature. The mixture was transferred to a tube containing 1 ml of Histopaque-1077 (Sigma, USA) and centrifuge at 500 g at 18°C for 30 minutes.

After centrifugation, the leukocytes were separated and the plasma layer was removed. The leukocytes were transferred to a new tube and mixed with 30 ml of PBS. The mixed solution was washed after centrifuging at 500 g at 18°C for 10 minutes. After repeating the same process as above twice, the number of the cells was measured after mixing with 1 ml of PBS again.

After obtaining cells and adding 250 μl of Cytofix/Cytoperm(Pharmingen, USA) to the cells, the cells were fixed by reacting them on ice for 20 min. The fixed cells were rinsed twice for 15 minutes in 125 μl of 1 x perm/wash solution. The cells were fixed (1×250), first antibody solution was added, and the cells were reacted on ice for 30 minutes. The cells were centrifuged at 500 g, then the precipitate was washed twice for 15 minutes. The rinsed cells and antibody (SPA-812, Stressgen

Table 1. Means and standard deviations on HSP at rest, exhaustion, and 30 minutes recovery before and after long-term aerobic exercise therapy (unit : %)

Group	Rest		Exhaustion		30 minutes Recovery	
	pre	post	pre	post	pre	post
Exercise	47.39 ± 2.79	46.35 ± 2.70	58.78 ± 8.53	43.51** ± 6.24	48.05 ± 6.80	47.17 ± 4.43
Control	48.34 ± 4.84	47.83 ± 2.39	57.51 ± 13.3	59.86 ± 8.32	48.26 ± 10.0	50.07 ± 9.79
Total	47.93 ± 3.98	46.48 ± 2.55	57.85 ± 11.5	51.76 ± 14.6	48.46 ± 8.74	48.83 ± 7.83

Co.) for anti-rabbit immunoglobulin were mixed in a solution and put on ice for 30 minutes. After washing the cells 3 times in the same way as above, the cells were analyzed using flow cytometry (FACS) after addition of 20% of paraformaldehyde solution.

Data are shown as mean ± SD, the t-test and analysis of covariance (ANCOVA) were used for statistical analyses of the data. Statistical significance was accepted for values of p less than 0.05.

RESULTS

Age (Exercise 21.62 ± 1.06, Contro1 21.65 ± 1.07), weight (Exercise 81.60 ± 4.76, Contro1 80.82 ± 6.92), and body fat (Exercise 27.88 ± 2.48%, Contro1 27.17 ± 2.95%), BMI(Exercise 26.37 ± 0.85, Contro1 26.10 ± 1.25) did not differ between Exercise and Control subjects ($p>0.05$). As expected, heart rate increased significantly in response to 50 minutes of exercise ($p<0.01$). The average speed for the 50 minutes exercise therapy was 9.7 ± 1.34 km h^{-1}, with speeds ranging from 8.3 to 13.1 km h^{-1}. The values of HRrest were Exercise 71.75 ± 4.59, Contro1 71.50 ± 4.50 beats/min, and those of HRmax were Exercise 194.62 ± 5.28, and Control 93.62 ± 5.09 beats/min.

The means and standard deviations of HSP of at rest, exhaustion, and 30 minutes recovery before and after the long-term aerobic exercise therapy are shown in Table 1.

HSP70 levels from exercise and control subjects on HSP of pre-post tests at rest following long term aerobic exercise therapy. Exercise and control subjects were not significantly higher than those of pre-post tests [$F(1, 11)=1.376$, $p>0.05$].

There indicates HSP 70 levels from exercise and control subjects on HSP of pre-post tests at the exhaustion following long term aerobic exercise therapy. Exercise and control subjects were significantly higher than levels in pre-post tests [$F(1, 11)=17.279$, $p<0.01$].

There indicates HSP 70 levels from exercise and control subjects on HSP of pre-post tests at 30 minutes recovery following long term aerobic exercise therapy. Exercise and control subjects were significantly higher than levels in pre-post tests [$F(1, 11)=0.473$, $p>0.05$].

DISCUSSION

The primary finding of this study is that a single moderate-to-heavy bout of exercise at 70% of VO_2max does not induce marked HSP70 synthesis in the leukocytes of Exercise group subjects. However, it appears that moderate Exercise group subjects had significantly lower basal levels of HSP70 than did control group subjects of comparable age, height, weight, body fat (%), BMI, HRrest (beats/min), and HRmax (beats/min). These results suggest that HSP70 expression in leucocytes may be affected by regular endurance exercise therapy.

All of the previous studies examining HSP production in human leukocytes after exercise used a different approach. HSP70 levels in young women did not change 6 and 24 hours after 20 minutes of exercise and 3 days of estrogen supplementation[8]. HSP 70 levels increased little in young men after treadmill exercise in the heat[7]. It is possible that these exercise protocols were not sufficient to generate the stress that is needed to stimulate greater HSP70 expression, suggesting that exercise at or below the normal level of training experienced by subjects is not a sufficient stimulus to induce

HSP70 expression in leukocytes. HSP synthesis in lymphocytes was only noted when animals were at or near exhaustion[3]. HSP70 expression was determined in the vastus lateralis in exercise group individuals in response to rowing training. These results show that individuals demonstrated increased HSP70 expression during a high intensity phase of training, but not during a high-volume phase of training[13]. Another study examined HSP70, HSP90 expression in leukocytes in highly trained men after a half-marathon. These subjects exercised for 1.5–2 hours at an average speed of about 14 km/h[14]. The percentage of leukocytes that expressed HSP70 increased significantly immediately after the exercise and remained elevated for up to 24 hours. These increased may have occurred as a result of the longer bout of higher-intensity exercise.

An interesting finding was the baseline HSP70 levels in the leukocytes of the exercise therapy subjects. In order to control for differences that may not have been related to physical activity, controls were used that were matched to the exercise therapy subjects in age, height, weight, body fat (%), BMI, HRrest (beats/min), and HRmax (beats/min). The results of the present study indicate that HSP70 expression in leukocytes after a single bout of moderate-to-heavy exercise is not markedly increased. This could be due to several different factors. There was some variation in the amount of HSP70 that the subjects expressed. The variations among subjects could be due to differences in environmental stress and genetic factors. Subjects with lower basal levels of HSP70 expressed greater synthesis of HSP70 after heat shock[15]. Most studies have indicated that there could be possible gender-based differences in basal HSP expression[1]. In this study, HSP70 levels may have increased significantly after the exercise. The subjects might have been experiencing a chronic adaptation to training. This adaptation might help to minimize the risk of cellular damage. The interesting finding was that HSP levels in long term exercise therapy subjects were significantly lower than those of the control group.

REFERENCES

1) Morimoto RI, Tissieres A, Georgopoulos C: The biology of heat shock proteins & molecular chaperones. Cold Spring, New York: Laboratory Press, 1994, pp1–25.
2) Storz G, Tartaglia LA, Ames BN: Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. Science, 1990, 248: 189–194.
3) Locke M, Noble EG, Alkinson BG: Exercising mammals synthesize stress proteins. Am J Phys, 1990, 258: 723–729.
4) Salo DC, Donovan CM, Davies KJ: HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise, Free radical. Bio Med, 1991, 11: 239–249.
5) Davies KJ, Quintanilha AT, Brooks GA, et al.: Free radicals & tissue damage produced by exercise. Biochem Biophys Res Comman, 1982, 107: 1198–1205.
6) Fehrenbach E, Passek F, Niess AM, et al.: HSP expression in human leukocytes in modulated by endurance exercise. Medic Sci Sports, 2000, 32: 592–600.
7) Ryan AJ, Gisolfi CV, Moseley PL: Synthesis of 70K stress protein by human leukocytes; effect of exercise in the heat. J App Phys, 1991, 70: 466–471.
8) Chang RT, Lambett G P, Moseley PL, et al.: Effect of estrogen supplementation on exercise thermoregulation I premenopausal women. J App Phys, 1998, 85: 2082–2088.
9) KNHE: Korea National Health Examination Survey. 2000, pp 20–59.
10) The Asia-pacific perspective: Redefining obesity & its treatment. World Health Organization Western Pacific International Association for the Study of Obesity. International Obesity Task Force 2000, 2000, pp30–90.
11) ACSM: ACSM's Guidelines for Exercise Testing & Prescription. 7th edition. Lippincott Williams & Wilkins, 2006, 20–155.
12) Walsh RC, Koukoulas I, Garnham A, et al.: Exercise increases serum HSP 72 in Humans. Cell Stress Chaperones, 2001, 45: 386–393.
13) Liu Y, Lormes W, Baur C, et al.: Human skeletal muscle to highly trained rowers. J App Phys, 2000, 186: 101–104.
14) Fehrenbach A, Niess MA, Schlotz E, et al.: Transcription & translational regulation of heat shock proteins in leukocytes of endurance runners. J App Phys, 2000 89: 704–710.
15) Boshoff T, Lombard F, Eiselen R, et al.: Differential basal synthesis of hsp70/hsc70 contributes to inter individual variation in hsp70/hsc70. Cell Mol Life Sci 2000, 57: 1317–1325.