Effects of High Intensity Interval Training (HIIT) on Extra Cellular Heat Shock Protein 72 (EC HSP72) in Young Inactive Women

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ABSTRACT

Background: Heat shock protein (HSP) 72 is a unique, ubiquitous molecule. In vitro and in vivo animal models have shown that increased HSP 72 is associated with improved cellular survivability and tolerance to stressors. Objective: The aim of present study was to evaluate the changes in EC HSP72 following 8 week high intensity interval training. Results: We recruited 20 young inactive women with no history of cardio respiratory disses, nonsmokers and don’t receive any meditation as subjects. Subjects were measured for height, weight and BMI one week before the study began. Subjects divided in two groups: experimental and control. A baseline blood samples were taken before and 24 h after the end of training protocol. Data were given as mean ± standard deviation and were analyzed using by t-test. The level of significance was set as 0.05. The results indicated significant increase in serum concentration of EC HSP72 (P=0.000). Conclusion: we found that high intensity interval training has a positive effect on heat shock protein 72 in young inactive women.

INTRODUCTION

Heat shock proteins (HSPs) play an important physiological roles in both the normal and stressed cell. Indeed, it has been thought that HSPs provide the ability of cellular protection against a variety of stresses [1,2]. HSPs can be divided into several groups based upon their molecular masses. Of particular interest is the inducible form of the 70 kilo Dallon family of HSPs (HSP72) [3]. EC HSP 72 has been shown to be released in vivo from the human liver, muscle and the brain during exercise. Intra (IC) and extracellular (EC) HSP 72 have different functions. IC HSP 72 confers cellular protection from subsequent stressors, while EC HSP 72 has a whole-body systemic role in antigen presentation and immunity and may also be of importance for the immune response to exercise [4,5]. Investigators have suggested that EC HSP 72 responses depend upon the training intensities, durations and type of protocol [6].

One of the protocols that is recently considered as interest of researchers is high intensity interval training (HIIT). HIIT Consists of alternating periods of intensive aerobic exercise with periods of passive or active moderate/mild intensity recovery [7]. It seems that in comparison with continuous training, HIIT has better effect on immune system. Because of importance of HIIT, the effect of that protein was studied in most of variables and it has been shown that both short term and long term HIIT has too benefits. One of them is the anti-inflammatory effect of HIIT. Since it is being argued more and more that inflammation is at the root cause of nearly all disease, a whole new consideration comes into play about HIIT.

There are a few studies have measured serum or plasma HSP 72 level in response to training. To date, there has been only one study reporting elevated plasma HSP72 concentrations in elite soccer players vs. sedentary controls [8]. So, systematic investigation of the influence of regular training on plasma concentrations of eHSP72 in inactive subjects is a promising task. Isanezhad et al. [9] investigated EC HSP72 in Vistar rats and reported that EC HSP72 increased after 8 week continuous training. In most studies the effect of training on
HSP72 was studied intracellularly [10,11,12,13] and in the others, the effect of acute exercise was studied [14,15,16,17]. There are, to our knowledge, no studies that examined the effect of HIIT on serum HSP72. So, the aim of our study was to examine the effect of 8 week HIIT as the most important part of most of the sports, on serum HSP72.

**Research Method:**

Twenty healthy physically inactive females were randomly divided into two groups. First group (n=10) performed HIIT, whilst the second group served as resting controls (n=10). The subjects were informed about potential risks and discomfort and written informed consent was obtained.

**HIIT Protocol:**

Subjects were required to run between markers placed 20m apart. The start point was located at the midpoint of the markers (Figure 1). Before commencement of the test subjects were given a familiarization trial of five low-intensity runs following test procedures. The 40-m MST protocol consisted of sprinting from the midpoint to the first marker, turning running 20 m in the opposite direction to the second marker, turning and running again through the mid-point, a total distance of 40 m. This procedure was repeated on each run with subjects completing four sprints in first and second week, 5 sprints in third and fourth weeks, five sprints in fifth and sixth weeks and seven sprint in seventh and eighth weeks. A 30-s recovery period was allowed between each successive sprint. Individual 40-m and split 10-m times (distance covered from the center of the course to the first marker) were recorded manually using a digital stopwatch by the same experimenter. Reliability for timing was established using a test retest method during a pilot study before the experiment itself. Subjects were encouraged to perform maximally on each occasion.

![Fig. 1: Course outline showing distance and direction taken by subjects, during the 40-m maximal intensity shuttle run test. A = 10m, B = 20m, C = 10m.](image)

We used the Heart Rate max (HRmax) to determine the intensity of exercise training. The intensity of training was upper %90 HRmax in all stage of protocol. Polar was used to control the heart rate and intensity of training.

**Measuring HSP 72:**

To obtain serum samples for HSP72, arterial blood samples were spun at 2200g for 15 min at 4°C in tubes containing a clot-inducing plug. A High sensitivity ELISA was used to determine the HSP72 content in serum (EKS-700 Stressgen, Victoria, BC, Canada).

**Statistics:**

To evaluate the effect of HIIT on serum HSP72 in both groups (experimental and control) t-test was used. P < .05 was set as the significance level.

**Results:**

The characteristics of subjects have been summarized in table 1. The results of the paired t-test illustrated in table 2. Results show that the amount of HSP 72 have a significant increase after 8 week HIIT (p=0.000).

<table>
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<tr>
<th>Table 1: Characteristics of experimental and control group.</th>
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<td>Subjects (N)</td>
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<td>Control group</td>
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**Discussion:**

The results of present study showed that 8 week HIIT can increase EC HSP72 in young inactive females. This finding is similar to [9,14]. Walsh showed that continuous training in elite athletes cause the increase of EC
HSP72 level. Isaneghad et al. [9] showed that 8 week continues training in rats cause the increase of serum HSP72. The result of present study is in opposite with [6], where they reported that HSP72 don’t increase with exercise. The differences in HSP 72 values between the two studies could be explained by the different mode, intensity and nature of exercise. It seems that exercise intensity is an important factor in determining of HSP72 [6]. However, it was also demonstrated that exhaustive exercise at 80% VO2max induces greater levels of eHSP72 compared with exercise at 60% VO2max of the same duration. Thus, the exercise-induced increase in eHSP72 concentration was described as intensity dependent [10]. Accordingly, the increase of expression in eHSP72 noted in this study, may relate to intensity of exercise. High intensity causes rise in core temperature and metabolic demand and these enhances EC HSP72 [18].

<table>
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<th>Table 2: Pre and Post test changes in serum HSP72.</th>
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<tr>
<td>control</td>
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<tr>
<td>Pre-test</td>
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<td>EC HSP72</td>
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During exercise, the release of HSP72 from damaged cells contributes to circulating eHSP72 levels [6]. It is thus believed that intracellular HSP72 is the potential source of extracellular HSP72, as the release may not depend on the translation of new intracellular protein [19]. In relation to HIIT, the key point is that the amount of HSP72 released from muscle is directly proportional to the amount of muscle being contracted. In addition to the amount of muscle mass involved, an increase in glycolysis metabolism (as one would see with HIIT) causes a further increase in HSP72. Consequently, whole body exercise play a major role in determining the amount of HSP72 released from muscle and, in turn, obtaining the numerous health and performance benefits.

The release of EC HSP 72 during stress may be an adaptive feature of the stress response [19]. The mechanism of release is unclear. The experiments of Johnson et al. [20] suggest that EC HSP 72 is released as a result of sympathetic nervous system activation, where norepinephrine binds to a 1 adrenoreceptors, causing an increase in intracellular calcium, thus stimulating the release exosomes containing HSP 72. Although in present study we didn’t evaluate norepinephrine levels.

EC HSP 72 is thought to stimulate innate immunity [21], act as a danger signal resulting in improved immune responses and facilitated host defense to pathogenic challenges [19]. For example, EC HSP 72 may bind to toll-like receptor (TLR)-2 and/or TLR-4, initiating an immune response through dendritic cells, macrophages and neutrophils [22,23], examples of APC. [58-611 Peptide fragments of HSP 72 can be presented on MHC I on the APC, and provide stimulation to the cytotoxic T lymphocyte (CTL). If the CTL receives a 2nd co-stimulation from the APC, the CTL becomes activated. This is a potent immune response because the HSP-peptide complexes drive the CTL response at a lower concentration (2-3 logs less) than that required for antigen alone [24]. However, if a pathogenic challenge does not occur, then HSP 72 will have little impact on innate immune cell production of pro-inflammatory cytokines and nitric oxide. Therefore, EC HSP 72 may exist as a precautionary measure and decrease the response time to a severe stress or challenge in case a pathogenic challenge occurs. If a pathogenic challenge ensues, then HSP 72 in conjunction with CD14 molecules and TLR could eliminate bacteria, or in chronic disease states, exacerbate the disease state (via pro-inflammatory influences). So, with regard to the role of HSP72 in protection of cell from injury and activate in inflammatory response, EC HSP72 may have a positive role in improvement of systematic inflammatory.

Recent research reveals that, HSP72 has a protective function against cell stress, ischemia, hypoxia, atrophy, cell injury, necrosis, elevation of body temperature, decrease of body temperature, decrease of blood glycogen and glucose, acidosis and other cases that occurs during exercise. So, this increase in HSP72 increases tolerance of humans to exercise induced stress [25]. Increase of HSP72 production, result in increase of the cell resistance to pro inflammatory markers and decrease of HSP72 production, increase tendency of cells to inflammatory markers [26]. What is more, the 8 week HIIT resulted in an increase in the EC HSP72 concentration and these shows the anti inflammatory role of HIIT. So, it can be used as an important strategy to prevention of illness.

Conclusion:

To sum up, basing on the data collected and analysis conducted, we concluded that maintaining an immunological response balance is vital to humans. HIIT stimulated the anti inflammatory response, which was supported by increase of HSP72. The greater understanding of the role of HSP 72 in humans may decrease the risk of heat illnesses.

REFERENCES


