Medicina Sportiva, Vol. 10 (2):36-41, 2006


# EXERCISE-INDUCED HEAT SHOCK PROTEIN (HSP70) RESPONSE IN HUMAN SKELETAL MUSCLE AND LEUKOCYTES

**Ewa Sadowska-Krępa** [A, E, F], **Barbara Kłapcińska** [A, E, F]

Academy of Physical Education, Department of Physiological and Medical Sciences, Biochemistry Unit, Katowice, Poland

## ABSTRACT

Heat shock proteins (HSPs) represent a family of highly conserved stress proteins found in all organisms from bacteria to humans. There is also substantial evidence that induction of HSPs synthesis occurs *in vivo* in response to a variety of physiologically relevant stressors, such as exercise, hyperthermia, oxidative stress, and other metabolic insults. The main aim of this review was to discuss the family of 70kDa heat shock proteins (HSP70), their structure and functions as well as the regulation of their expression in human skeletal muscle and leukocytes in response to exercise.

*Key words: heat shock proteins, HSP70(72), exercise, heat adaptation, oxidative stress, skeletal muscle, leukocytes*

## Introduction

Exposure of body tissues and cells to diverse environmental stresses may lead to a serious disturbance of cellular homeostasis, which mainly results from deleterious modification of proteins such as unfolding, misfolding or aggregation. In response to an acute or chronic stress, cells produce a set of proteins known as heat shock proteins (HSP) or stress proteins (SP) (1), that efficiently protect cells against stress-induced damage by preventing protein misfolding, assisting in their refolding to the native state or targeting them to proteolysis (2, 3). Another mechanism of cellular protein management is through the chaperone function in the assembly, folding and translocation of preexisting proteins across organellular membranes, this is why they are also termed "molecular chaperones" (4). While heat was the first stressor found to induce HSP synthesis (5), several other metabolic insults, such as excessive formation of reactive oxygen and nitrogen species (ROS, RNS), hypoxia, ischemia, reduced glucose availability, increased intracellular $Ca^{2+}$, microbial infections, exposure to heavy met-

**Table1.** *Nomenclature, cellular localization and major functions of heat shock proteins in skeletal muscle*

| Name of heat shock proteins | Localization | Major cellular function |
|---|---|---|
| **Small HSPs (8-32 kDa)** (ubiquitin, αB-crystallin, HSP20, HSP27, heme oxygenase HO-1) | cytosol, nucleus | play a role in chromatin structure, removal of denatured proteins, stabilization of microfilaments, cytokine signal transduction, suppresses aggregation of denatured proteins, facilitate protein refolding, HO-1 provides protection through elimination of heme with concomitant production of the antioxidants bilirubin and biliverdin |
| **60 kDa HSP** (chaperonin) | mitochondria | chaperone activity, facilitates the folding and assembly of proteins as they enter to mitochondria and stabilizes proteins under stress |
| **70 kDa HSPs** (HSC73, HSP72, GRP78, GRP75) | cytosol, nucleus, sarcoplasmic reticulum, mitochondria | translocation of precursor proteins across mitochondrial membranes, protein stabilization and folding within the mitochondria, binding to denaturing or unfolding pre-ribosomes to facilitate their renaturation, role in signal transduction, protein folding, assembly of secretory proteins |
| **90 kDa HSPs** (HSP90α, HSP90α, GRP94) | cytosol, nucleus, endoplasmic reticulum | binding steroid hormone receptors, regulating the activity of hormone receptors, protein folding ATP dependent |
| **110 –100 kDa HSPs** | cytosol, nucleus, nucleolus | role in stress tolerance, protein folding |

als, ethanol, UV radiation or antibiotics, can induce HSP response (6, 7, 8). It has been demonstrated in numerous reports that expression of stress proteins is up-regulated in response to physical exercise. It seems that HSPs can protect cells from some kind of noxious stimuli, which can cause necrosis or apoptosis (9). The HSPs expressed in skeletal muscle contain several inducible and constitutively expressed intracellular proteins with molecular mass from 8 to 110 kD (10) that are classified into families according to their size and function. The most important HSPs in skeletal muscle are: small HSPs, HSP of 70 kDa (HSP70), HSP of 60 and 90 kDa (HSP60, HSP90) and heme oxygenase-1 (HO-1). Their cellular localization and major functions are presented in Table 1 (10-14). The most abundant are studies on the 70 kDa family, which revealed that HSP synthesis is enhanced in skeletal muscle of rodents in response to an acute treadmill running (15-18) and endurance training (19). Less numerous are data from human studies, however enhanced expression of HSPs in human skeletal muscle and blood after an acute bout of exercise or training has been reported by a growing number of researchers (10, 11, 14, 19). As indicators of stress, HSPs proteins may provide information about the cellular changes induced by exercise, therefore they may be of interest for monitoring overtraining (20).

**The 70 kDa heat shock proteins family and function**

Molecular chaperones, such as members of the 70 kDa family in humans represent a highly conserved group of proteins, which is evidenced by an approximately 50 % sequence identity with bacterial stress proteins found in *Escherichia coli* (12). The 70kDa family contains the constitutive HSP70 (HSP70c/HSC73) and the inducible HSP70 (HSP70i/HSP72) (6,12) forms that exhibit an extremely high sequence homology (~95%) and similar biochemical properties (2). However, in contrast to constitutively expressed HSC73, HSP72 is present only in small quantities in the unstressed cells but it can be rapidly synthesized in the cytoplasm in response to different kinds of metabolic stress, the exercise included. There are two other forms of HSP70 referred as the glucose-regulated 78-kDa (GRP-78) and 75 kDa (GRP-75) proteins, as they are specifically induced by glucose deprivation. GRP-78, the form constitutively expressed in the lumen of the endoplasmic/sarcoplasmic reticulum is involved in the assembly of secretory proteins (21), whereas mitochondrial GRP-75 assist in the translocation of the precursor proteins into mitochondria (22). Glucose regulated proteins (GRP), induced by metabolic stressors, provide less or no protection against thermal stress.

Synthesis of HSP70 is controlled by the heat-shock factor (HSF) (6, 23, 24) that binds to the heat shock promoter element (HSE) located upstream from the coding region of HSP70 gene (25). Multiple HSF factors have been identified thus far, among them three (HSF-1, HSF-2 and HSF-4) have been identified in humans (26). It is noteworthy that the exercise –induced regulation of stress proteins is mainly mediated *via* HSF-1 binding to HSE. Activation of HSF-1 in response to stressors such as heat, hypoxia, ischemia/reperfusion, oxidative/nitrosative stress, energy depletion or exercise occurs within minutes (10, 14, 27) and causes migration of HSF trimer complex from the cytoplasm into the nucleus, where is binds to the promoter region of the Hsp70 gene. As Hsp mRNA is transcribed, it leaves the nucleus for the cytosol where new HSP70 protein is synthesized (23, 28*)*. It was demonstrated that the maximal rate of HSP70 production occurs 3 to 5 hours after heat shock and ceases after 8 hour (12), whereas during training in humans there is a time lag of several days until stress protein levels increase substantially (29). Interestingly, it appears from the studies with cell cultures that transcriptional activation of the HSP70 gene is independent on stress protein synthesis (30), which suggests that HSP promoter activity and protein synthesis may be uncoupled (31) and that translation process may be under distinct regulatory mechanism. Due to obvious difficulties in sample acquisition most human studies evaluating HSP70 response to exercise have focused primarily on expression of HSPs in either skeletal muscle or circulating leukocytes.

**Exercise and HSP70 expression in skeletal muscle**

One of the first uncovered physiological functions of HSP70 family proteins was acquired thermotolerance (8)*,* which is defined as the ability of a cell or organism to withstand more severe, potentially lethal thermal stress, after a brief sublethal exposure to heat (28). Thermotolerant cells were found to produce less HSPs during a second heat stress than the previously unheated cells, which suggests that regulation of HSP synthesis depends on the basal level of these proteins in the cell. Physical activity is inevitably associated with hyperthermia which may be, at least partly, responsible for exercise-induced HSP response. Core body temperature in humans can reach 40-42$^{o}$C during heavy exertion in the heat (32). However, exercise-induced HSP70 accumulation can be independent of the changes in body temperature (33), which implies that other cellular changes, e.g. enhanced oxidative stress induced by exercise, may contribute to HSP induction in human muscle (10, 11*)*. It has been evidenced the that a single 30 min running bout led to a significant induction of Hsp70 mRNA expression in muscle *vastus lateralis* biopsy samples taken from untrained subjects but this challenge was not sufficient to have an effect on the already high basal level of HSP70 protein (3*)*. It

seems, however, that the Hsp70 mRNA level could be a useful marker of exercise induced stress in specific muscle. On the other hand, significant increases of HSP70 production were evidenced in *vastus lateralis* biopsy samples taken from highly trained rowers during a 4 week training programme performed prior to the World Championships, with a maximum increase observed at the end of the 2nd training week when the athletes were subjected to maximum training loads (29). The view that HSP production protects the vital function and prevents muscle damage was supported by observation of plasma CK, that was elevated at the beginning of conditioning programme and was decreased with training. Interestingly, skeletal muscle HSP70 responses depended upon training intensity. It appeared that HSP70 in skeletal muscle of well trained rowers was increased significantly after 3 weeks high-intensity strength training, then decreased at the end of one week of recovery, but remained unchanged both throughout subsequently applied 3 weeks-long phase of low-intensity endurance training and at the end of one week of recovery (34). In the latter study a discrepancy was observed in HSP70 mRNA and HSP70 protein level, as Hsp70 mRNA reached a maximum increase (257 %) immediately after completion of a high-intensity phase of training, then gradually decreased towards the basal level. This observation seems to support the view of different transcriptional and translational regulation mechanisms in HSP70 expression in response to exercise in human skeletal muscle (3, 30).

Important finding derived from a study with untrained humans subjected to prolonged cycling test to volitional fatigue, at a workload that corresponded to 63% $VO_2$ max, was that HSP72 mRNA level in *vastus lateralis* increased progressively during exercise and that this increase coincided with reduced intramuscular glycogen availability (6). Interestingly, the rise in muscle temperature form 34 to 37.5°C (that did not grow further) and a three-fold increase in muscle lactate in the first 10 min of exercise were without effect. These data suggest that reduced glucose availability can induce a heat shock response. To test this hypothesis the same research group compared HSP70 gene and protein in *vastus lateralis* biopsy samples taken from both limbs, while one limb was reduced by 40 % in resting glycogen content by an exercise bout performed 16 h before the experimental trial (35). While no differences in expression of HSP72 mRNA and HSP72 protein in both glycogen depleted and control legs were observed pre-exercise, a two-fold increase in expression of HSP72 gene and protein was recorded only in glycogen depleted leg immediately after a 4 to 5 h (until exhaustion) exercise on the two-legged knee extensor apparatus It should be stressed that since the amount of work, and thus the amount of heat generated in both limbs were the same, the enhanced HSP72 expression in glycogen depleted leg could not be accounted for any differences in intramuscular temperature. It is noteworthy, that during this non-damaging exercise, as evidenced by a lack of any changes in CK activity in aterial and venous serum of each limb at any time point of the experimental trial, it appeared that HSP72 protein was not released into the circulation during or after this prolonged concentric exercise. The authors speculate that the increased HSP72 expression within muscle cells in response to exercise performed at a reduced carbohydrate availability may be induced by other changes, such as enhanced generation of ROS (36*)* and/or decrease in sarcoplasmic reticulum $Ca^{2+}$ re-uptake (37*).* Indeed, there are numerous studies, mostly from animal models, supporting the view that exercise-induced oxidative stress may stimulate the formation of HSP (18,38).

A substantial amount of data indicates that increased energy demand during physical exercise requires a multifold rise in oxygen utilization by active tissues (39*),* which is associated with an enhanced generation of reactive oxygen species (ROS), mainly in mitochondria. Skeletal muscle also contains nitric oxide synthases and a number of other potential sources for the formation of reactive nitrogen species (RNS) (40). Both ROS and RNS react non-specifically with cellular components bringing about damages to proteins, lipids and nucleic acids. Evidence from animal studies indicates that muscle cells are endowed with enzymatic defense mechanisms, which involve superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), as well as with non-enzymic antioxidants. It has been evidenced that exercise-induced hyperthermia, oxidative stress and inflammatory reactions following muscular work stimulate the synthesis of the protective heat shock proteins 70 kDa (72 kDa), in striated muscles and heart (3,8,13,16,33,41). The production of HSP70 in cells of skeletal muscles is necessary to maintain myofibrillar integrity and assist in the assembly of cellular proteins. It seems that members of the 70 kDa family, specifically HSP72, may have a complementary protection against oxidative stress. Smolka et al. (16) have suggested that HSP72 is part of a secondary defense system acting to provide fast additional protection when the main antioxidant enzymatic system is compromised by attack of the exercise-induced ROS.

The data presented by Khassaf et al.(11) have revealed that human skeletal muscle (*vastus lateralis*) responds to a single bout of non-damaging one-legged cycling exercise at 70% peak maximum oxygen uptake for 45 min by upregulating expression of superoxide dismutase (SOD) and heat shock proteins (HSP 70 and HSP60). It is noteworthy that a significant increase in

HSP70 (3100 % of the pre-exercise value) was reached by 6 days post-trial, while SOD activity increased to a peak value at 3 days post-exercise and catalase activity remained unchanged. These data indicate that elevated content of HSP70 in human skeletal muscle is maintained for several days post-exercise, which may facilitate protection against potential damage during next bout of exercise. On the other hand, exercise-induced muscle injury varies with the type of contraction, with more severe damage resulting from exercises including high force eccentric contractions. (42, 43). The study of Thompson et al. (44) revealed a highly significant increases of HSC/HSP70 (primarily in HSP70 with a small contribution of HSC70) and HSP27 proteins in response to a single bout of eccentric resistance exercise (two sets of 25 repetitions performed by the lowering portion of a *biceps brachii*) as evaluated 48 h post-exercise. The same research group (45) demonstrated that the response to the second bout of the same damaging eccentric exercise, performed 4 weeks after the first bout, was attenuated when indirect indicators of damage, such as plasma CK activity, were compared, although a basal HSP expression during the 2$^{nd}$ bout was apparently lower. When HSP response to eccentric exercise was evaluated in both upper (*biceps brachii*) and lower limbs (*vastus lateralis*) involved in high-force eccentric contractions (BB) and downhill (-10 degrees) running (VL) it appeared that the post-exercise HSP70 expression increased significantly in the BB but not in the VL. These data suggest that the exercise-induced responses in HSP70 expression are exercise-specific and local, not systemic (41).

**Exercise and HSP70 expression in leukocytes**

Strenuous physical exercise is known to induce an acute response of the immune system, such as leukocytosis, activation of blood neutrophils and increases in inflammatory cytokines (IL-1, IL-6, IL-8, TNFα) (46, 47). Neutrophils that infiltrate damaged muscle fibers have been recognized as containing NADPH oxidase, the enzyme that may substantially contribute to the generation of ROS, the phenomenon known as "respiratory burst" (48). It has been postulated by Fehrenbach et al. (7) that protection and tolerance against exercise-induced stress may be, at least partially, provided by HSPs that play an important role in the activation of the immune response. These authors revealed that subjection of well-trained male athletes to strenuous endurance exercise such as a half-marathon resulted in up-regulation of HSP70 mRNA expression in leukocytes and production of proinflammatory plasma cytokines TNFα and IL-8. The basal values of HSP70 mRNA expression were significantly higher in trained compared with untrained subjects. It was also reported that the HSP70 mRNA expression after heat shock (2h, 42°C) applied *in vitro* to blood samples withdrawn at rest was significantly more pronounced in leukocytes of trained than the untrained men. This report on parallel post-exercise increases in HSP expression and in levels of TNFα, and especially IL-8 as marker of enhanced generation of reactive oxygen/nitrogen species, may imply that other exercise-associated stress agents, such as oxidants and proinflammatory cytokines, may be involved in HSP regulation. Supportive to this hypothesis are findings that the response of leukocyte expression of HSP72 to exhaustive treadmill exercise is attenuated in subjects receiving α-tocopherol (49). Previously, the same group of researchers (50) reported that endurance exercise completed at higher ambient temperature (28°C) provides thermotolerance. This was evidenced by a higher HSP72 protein level in leukocytes compared with that recorded after run at 18°C and a blunted HSP expression found after second run at 28°C or after a heat shock (42°C, 2 h), applied *in vitro* to leukocytes derived from those individuals who completed both runs at higher temperature. These findings are consistent with those reported previously by Ryan et al. (32) who found that leukocytes obtained from humans following 2 h of exercise in a hot environment (46°C) and subsequently incubated *in vitro* at 41°C, synthesized less 70K stress proteins compared with those obtained from individuals who exercised in more moderate (30°C) ambient conditions. Their findings suggest that cells such as leukocytes may regulate the amount of these stress proteins in response to repeated exposures to elevated temperatures. The marathon runners represent a special population subset able to tolerate core temperatures up to nearly 42$^0$C without signs of heat illness (51).

Another interesting aspect of the HSP70 response to endurance exercise has been revealed in a study on the effect of a half-marathon run on HSP70 expression on the surface of leukocytes (52). It appeared that exercise-induced stress affected mainly monocytes and granulocytes while only a few lymphocytes expressed HSP70 in their cytoplasm. It was found that HSP70 protein on the surface was nearly undetectable on a majority of white cells withdrawn from blood of untrained individuals as well as of the athletes at rest. However, after the run an increased HSP expression on the surface of monocytes and neutrophils was observed. A greater increase of cell surface HSP positive leukocytes following heavy training was also evidenced by Whitham et al. (53) in male endurance cyclists. Another important finding of the Fehrenbach (52) study was a significantly lower percentage of HSP70 positive (expressed in the cytoplasm) monocytes and granulocytes obtained at rest from half-marathon runners compared to untrained individuals. These observations are consistent with those reported

later by Shastry et al. (54) who also found that baseline HSP70 protein levels in leukocytes of moderately trained humans were significantly lower than levels in untrained subjects matched for height, weight, sex, per cent body fat and age. It is noteworthy that age may be especially important because there is an age-related attenuation in HSP70 expression, as it was evidenced by a significantly lower basal percentages of HSP72-positive monocytes and granulocytes in the elderly subjects (sexagenarians and physically active octogenarians), compared to young (25-y-old) adults (55).

More recent studies (56, 57) focused on another previously unknown function of HSP70 as an extracellular protein with regulatory effects on human monocytes, acting both as chaperone and cytokine. Binder et al. (58) shed light on important obligatory role for HSP70(72) proteins in chaperoning antigenic peptides in the cytosol and their presentation by major histocompatibility complex I (MHC I) molecules. It was found that HSP72 binding to the cell surface of human monocytes activated production of pro-inflammatpory cytokines (IL-6, IL-1$\alpha$, TNF$\alpha$) and stimulated inducible nitric oxide synthase (iNOS), NADPH oxidase and phagocytosis (56). It was shown that acute exercise led to an increase of HSP72 in the blood (19), although it is not clear from which tissue circulating HSP72 originates. As HSP72 release into circulation from non damaged muscle has been excluded (59), it seems however likely that it may originate from damaged muscle, leukocytes, liver or heart (7, 32, 59). It was evidenced quite recently (14) that both duration as well as intensity of exercise have a major influence on the concentration of soluble HSP72 (sHSP72) in the blood. The most pronounced sHSP72 response was induced by the long lasting competitive endurance exercise such as marathon run. Exhaustive exercise (about 30 min treadmill running at 80 % $\dot{V}O_2$max) resulted in a significantly greater increase in sHSP72 compared to a moderate exercise test of the same duration (about 30 min treadmill running at 60 % $\dot{V}O_2$max).

In conclusion, multifaceted metabolic changes caused by strenuous physical activity are involved in stimulation of synthesis of HSPs in human skeletal muscle and leukocytes. The observation of the HSP70 response to exercise seems to be useful for monitoring beneficial or unfavorable effects of intensive exercise or extensive training.

## References

1. Locke M, Noble EG. Stress proteins: the exercise response. *Can J Appl Physiol* 1995; 20: 155-67.
2. Welch WJ. Mammalian stress response: Cell physiology, structure/function of stress proteins, and implications for medicine and disease. *Physiol Rev* 1992; 72: 1063-81.
3. Puntschart A, Vogt M, Widmer HR. et al. HSP70 expression in human skeletal muscle after exercise. *Acta Physiol Scand* 1996; 157: 411-7.
4. Moseley PL. Heat shock proteins and heat adaptation of the whole organism. *J Appl Physiol* 1997; 83: 1413-7.
5. Ritossa F. A new puffing pattern induced by temperature shock and DNP in Drosophila. *Experientia* 1962; 18: 571-3.
6. Febbraio MA, Koukoulas I. HSP72 gene expression progressively increases in human skeletal muscle during prolonged, exhaustive exercise. *J Appl Physiol* 2000; 89: 1055-60.
7. Fehrenbach E, Niess AM, Scholtz E, et al. Transcriptional and translocational regulation of heat shock proteins in leukocytes of endurance runners. *J Appl Physiol* 2000; 89: 704-10.
8. Kilgore JL, Musch TI, Ross CR. Physical Activity, muscle, and the HSP70 response. *J Appl Physiol* 1998; 23: 245-60.
9. Latchman DS. Heat shock proteins: protective effect and potential therapeutic use (Review). *Inter J Molec Med* 1998; 2: 375-81.
10. Liu Y and Steinacker JM. Changes in skeletal muscle heat shock proteins: pathological significance. *Frontiers in Bioscience* 2001; 6: D12-D25.
11. Khassaf M, Child RB, McArdle A. et al. Time course of responses of human skeletal musle to oxidative stress induced by nondamaging exercise. 2001; 90: 1031-5.
12. Kiang JG and Tsokos GC. Heat shock protein 70kDa: molecular biology, biochemistry, and physiology. *Pharmacol. Ther* 1998; 80: 183-201.
13. Power SK, Locke M, Demirel H. Exercise, heat shock proteins, and myocardial protection from I-R injury. *Med Sci Sports Exerc* 2001; 3: 386-2.
14. Fehrenbach E. Cellular responses to environmental stress. In: Moore FC, Vőlker K. eds. Molecular and cellular exercise physiology. Human Kinetics, Champaign, Il. 2005: 199-218, ISBN :0-8360-4518-X.
15. Milne KJ, Noble EG. Exercise-induced elevation of HSP70 is intensity dependent. *J Appl Physiol* 2001; 93: 561-8.
16. Smolka MB, Zoppi CC, Alves AA. et al. HSP72 as a complementary protection against oxidative stress induced by exercise in the soleus muscle of rats. *Am J Physiol Intergrative Com Physiol* 2000; 279: 1539-45.
17. McArdle, Pattwell D, Vasilaki A, et al. Contractile activity-induced oxidative stress: cellular origin and adaptive responses. *Am J Physiol Cell Physiol* 2001; 280: C621-C7.
18. 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 Radic Biol Med* 1991; 11: 239-46.
19. Walsh RC, Koukoulas I, Garnham A. et al. Exercise increases serum Hsp72 in humans. *Cell Stress Chaperones* 2001; 6: 386-93.
20. Lehmann M, Foster C, Dickhutt HH. et al. Autonomic imbalance hypothesis and overtraining syndrome. *Med Sci Sports Exerc* 1998; 30: 1140-5.
21. Munro S and Pelham RB. An HSP70-like protein in the ER: identity with the 78 kDa glucose-regulated protein and immunoglobulin heavy chain binding protein. *Cell* 1986; 46: 291-300.
22. Mizzen LA, Chang C, Garrels JI. et al. Identification, characterization, and purification of two mammalian stress proteins present in mitochondria, GRP75, a member of the HSP70 family and HSP58, a homolog of the bacterial GRoEl protein. *J Biol Chem* 1989; 264: 20664-75.
23. Morimoto RI. Regulation of heat shock transcriptional response; cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes & Dev* 1998; 12: 3788-96.
24. Knowlton AA, Sun L. Heat-shock factor-1, steroid hormones, and regulation of heat-shock protein expression in the heart. *Am J Physiol Heart Circ Physiol* 2001; 280: 455-64.
25. Amin J, Ananthan J, Voellmym R. et al. Key features of heat shocj regulatory elements. *Moll Cell Biol* 1988; 8: 3761-9.
26. Schuetze TJ, Gallo GJ, Tempst P. et al. Isolation of a cDNA for HSF2; evidence for two heat shock factor genes in humans. *Proc Natl Acad Sci USA* 1991; 88: 6911-5.
27. Locke M, Noble EG, Tanguay RM. et al. Activation of hetshock transcription factor in rat heat after heat shock and exercise. 1995; 268: C1387-C94.

28. Kregel KC. Molecular biology of thermoregulation. Invited review: Heat shock proteins: modifying factors in physiological stress response and acquired thermotolerance. *J Appl Physiol* 2002; 92: 2177-86.
29. Liu Y, Lormes W, Wang L. et al. Different skeletal; muscle HSP70 responses to high-intensity strength traioning and low-intensity endurance training. *Eur J Appl Physiol* 2004; 91: 330-5.
30. Bruce JL, Price BD, Colemen N. et al. Oxidant injury rapidly activates the heat shock transcription factor but fails to increase levels of heat shock proteins. *Cancer Res* 1993; 53: 12-5.
31. Wu BJ, Kingston RE, Morimoto RI. Human HSP70 promoter contains at least two distinct regulatory domains. *Proc Natl Acad Sci USA* 1986; 83: 629-33.
32. Ryan AJ, Gisolfi CV, Moseley PI. Synthesis of 70 K stress protein by human leukocytes: effect of exercise in the heat. *J Appl Physiol* 1991; 70: 466-471.
33. Skidmore R, Gutierrez JA, Guerriero V, Kregel KC. HSP70 induction during exercise and heat stress in rats: role of internal temperature. *Am J Physiol* 1995; 268: 92-7.
34. Liu Y, Lormes W, Wang L. et al. Different skeletal; muscle HSP70 responses to high-intensity strength traioning and low-intensity endurance training. *Eur J Appl Physiol* 2004; 91: 330-5.
35. Febbraio MA, Steensberg A, Walsh R. et al. Reduced glycogen availability is associated with an elevation in HSP72 in contracting human skeletal muscle. *J Physiol* 2002; 538: 911-7.
36. Reid MB. Plasticity of skeletal, cardiac, and smooth muscle: invited review: Redox modulation of skeletal muscle contraction: what we know and what we don't. *J Appl Physiol* 2001a; 90: 724-31.
37. Stephenson GD, Nguyen LT, Stephenson GM. Glycogen content and excitation-contraction coupling in mechanically skinned muscle fibres of the cane toad. *J Physiol* 1999; 519: 177-87.
38. Kelly DA, Tiidus PM, Houston ME. et al. Effect of vitamin E deprivation and exercise training on induction of HSP70. *J Appl Physiol* 1996; 81: 2379-2385.
39. Sen CK. Oxidants and antioxidants in exercise. *J Appl Physiol* 1995; 79: 675-86.
40. Reid MB. Nitric oxide, reactive oxygen species, and skeletal muscle contraction. *Med Sci Sports Exerc* 2001b; 33: 371-6.
41. Thompson HS, Maynard EB, Morales ER. et al. Exercise-induced HSP27, HSP70 and MAPK responses in human skeletal muscle. *Acta Physiol Scand* 2003; 178: 61-72.
42. Clarkson PM, Tremblay I. Exercise-induced muscle damage, repair, and adaptation in humans. *J Appl Physiol* 1988; 65: 1-6.
43. Clarkson PM and Sayers SP. Etiology of exercise-induced muscle damage. *Can J Appl Physiol* 1999; 24: 234-48.
44. Thompson HS, Scordilis SP, Clarkson PM. et al. A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. *Acta Physiol Scand* 2001; 171: 187-93.
45. Thompson HS, Clarkson PM, Scordilis SP. The repeaed bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans. *Acta Physiol Scand* 2002; 174: 47-56.
46. Severs Y, Brenner I, Shek PN. et al. Effects of heat and intermittent exercise on leukocyte and sub-population cell counts. *Eur J Appl Physiol* 1996; 74: 234-45.
47. Weinstock C, Konig D, Harnischmacher R. et al. Effect of exhaustive exercise stress on the cytokine response. *Med Sci Sports Exerc* 1997; 29: 345-54.
48. Clark RA. Activation of the neutrophil respiratory burst oxidase. *J Infect Dis* 1999; 179: S309-S17.
49. Niess AM, Fehrenbach E, Schlotz E. et al. Effects of RRR-alpha-tocopherol on leukocyte expression of HSP72 in response to exhaustive treadmill exercise. *Int J Sports Med* 2002; 23: 445-52.
50. Fehrenbach E, Niess AM, Veith R. et al. Changes in HSP72 expression in leukocytes are associated with adaptation to exercise under conditions of high environmental temperature. *J Leukoc Biol* 2001; 69: 747-54.
51. Maron MB, Wagner JA, Horvath SM. Thermoregulatory responses during competitive marathon running. *J Appl Physiol* 1977; 42: 909-14.
52. Fehrenbach E, Passek F, Niess AM. et al. HSP expression in human leukocytes is modulated by endurance exercise. *Med Sci Sports Exerc*. 2000b; 32: 592-600.
53. Whitham M, Halson SL, Lancaster GI. et al. Leukocyte heat shock protein expression before and after intensified training. *Int J Sports Med* 2004; 25: 522-7.
54. Shastry S, Toft DO, Joyner MJ. HSP70 and HSP90 expression in leucocytes after exercise in moderately trained humans. *Acta Physiol Scand* 2002; 175: 139-46.
55. Simar D, Malatesta D, Koechlin C. m et al. Effect of age on Hsp72 expression in leukocytes of healthy active people. *Exp Gerontol* 2004; 39: 1467-74.
56. Asea A, Kraeft SK, Kurt-Jones EA. et al. HSP70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med* 2000; 6: 435-42.
57. Asea A. Stress proteins and initiation of immune response: chaperokine activity of hsp72. *Exerc Immunol Rev* 2005; 11:34-45.
58. Binder RJ, Blachere NA, Srivastava PK. Heat Shock Protein-chaperoned peptides but not free peptides introduced into the cytosol are presented efficiently by major histocompability complex I molecules. *J Biol Chem* 2001; 276: 17163-71.
59. Febbraio MA, Ott P, Nielsen HB. et al. Exercise induces hepatosplachnic release of heat shock protein 72 in humans. *J Physiol* 2002; 544: 957-62.

Received: May 04, 2006
Accepted: July 12, 2006
Published: July 14, 2006

Address for correspondence:
Ewa Sadowska-Krępa
Katedra Nauk Fizjologiczno-Medycznych
Akademia Wychowania Fizycznego
40-065 Katowice, ul. Mikołowska 72A
Poland
tel.(+48-32) 2075154
fax. (+48-32) 251 68 68
e-mail: e.sadowska-krepa@awf.katowice.pl

**Author's contribution**
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection