We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Role of Heat Shock Proteins in Skeletal Muscle

Thiago Gomes Heck, Sofia Pizzato Scomazzon,
Mirna Stela Ludwig and Paulo Ivo Homem de Bittencourt Jr.

Additional information is available at the end of the chapter

1. Introduction

The ability of the human organism to respond with rapid and appropriate modification during physiological challenges is an essential feature for its own survival. These modifications are inseparable from a satisfactory adjustment of the physiological processes of the whole body, where physiological systems tend to maintain relatively constant composition of the internal environment (milieu intérieur), despite the constant challenges that the body is submitted daily to, which is known as homeostatic control [1, 2].

The homeostatic regulation of any physiological variable depends on cooperative mechanisms which are activated simultaneously or in succession. Thus, intense or critical challenges to life require numerous and complex mechanisms to restore or to maintain the homeostasis. In this way, challenges generate a stress situation to the body and, depending on the severity, can cause harmful effects. Moreover, moderate challenges, which are also caused by stress, results in profitable physiological adjustments [3]. In this context, stress response may be considered a nonspecific response of the body to any demand. A stressor is an agent that produces stress at any time by different ways. The physiological adaptations of the body represent the chronologic development of the response to stressors when their action is prolonged [4].

Since the muscle represents about ¾ of the body mass, a healthy muscular system is associated with the status of the other system of the body. Sick muscle system has harmful effects on human healthy and to its the capacity of interaction in this world. Skeletal muscle is a high plastic tissue that may be modified by use or disuse. Muscle composition, at chemical and structural levels, can be altered and these modification are related to the specific type of exercise that the organism is submitted. For example, the well know effect of strenght training is the hypertrophy of the muscle, which is associated to an increase in force production capacity. Strenght training may induce hypertrophy (muscle fiber enlargement)

and hyperplasia (increase in muscle fiber number) while endurance training promotes elevated muscle oxidative capacity (increase in mitochondrial number). All these adaptations are related to the health status of the body. On the other hand, the absence of exercise stimulus results in atrophy process and loss of functional capacity, marked by both impaired force production and metabolism of the muscle [5].

In this chapter, we focus on cell physiology and molecular biology of the muscle cells from a special point of view concerning to the stress response: the role of heat shock proteins (HSPs) in muscle.

2. Classical roles of heat shock proteins expression

Living organisms respond at cellular level to unfavorable conditions such as heat shock, and other stressful situations of many different origins, by a rapid, vigorous and transient acceleration in the rate of expression of specific genes: the heat shock genes. The products of these genes are commonly referred to as stress proteins or heat shock proteins (HSPs). Besides activation of heat shock genes, the expression of most other genes is inhibit as a result of stress. Thus, the stress intensity and duration leads to a perturbation of normal gene expression, which, if prolonged, can have drastic consequences for cells and system homeostasis [6, 7].

HSPs are highly conserved proteins in both eukaryotic and prokaryotic organisms and are expressed in many cell types including striated skeletal muscle. The first report about HSPs was documented by Ritossa [8, 9], after a serendipitous heat shock in salivary gland cells of *Drosophila buskii*, but heat shock proteins were only characterized later in 1974 [10]. Actually, HSPs are categorized in families according to their molecular sizes and include HSP110, HSP100, HSP90, HSP70, HSP60, HSP30 and HSP10 subclasses. In this chapter, the role of HSPs in muscle will be discussed in terms of the most studied (due to its evident high expression in mammalian cells under stress conditions) and conserved: the 70-kDa family (HSP70), which comprises a number of related proteins whose molecular weights range from 66 to 78 kDa. Many studies in human, rat and mice will be listed throughout the text, thus it is necessary to learn about the HSP70 isoforms that are encoded by a multigene family in each mammalian that will be listed below.

In humans, there are at least 13 distinct genes so far studied [11]. For the rationalization of the current nomenclature, human HSP70 genes (rat and mouse, also) have given the locus symbol HSPAx, where A defines members of HSP70 family and X designates the individual loci. In this sense, HSPA8 is the human gene that encodes a 73-kDa constitutive form of HSP70 (HSP73 or HSC70, the cognate form), while HSPA1A gene, located at the major histocompatibility complex (MHC) III region, encodes an inducible form (HSP72 or simply HSP70). In humans, but not in the rat or the mouse, there is an even higher inducible form (HSP70B') encoded by HSPA6 gene. Other representative members, besides mitochondrial (HSP75) and endoplasmic reticulum (HSP78) members of HSP70 family, are found in the intracellular space. While the constitutive form is expressed in a wide variety of cell types at basal levels (being only moderately inducible), the so-called inducible HSP70 forms (which

are barely detectable under non-stressful conditions) could be promptly synthesized under a condition of 'homeostatic stress', this being any 'homeostasis threatening' condition, such as heat, glucose deprivation, lack of growth factors and so forth. Habitually, research groups indistinctly use HSP70 as a unified term for both constitutive and inducible form. However, HSP70 is the preferable form to be used when one refers to the inducible HSP72 protein encoded by HSPA1A gene [12, 13]

In rodents, the Hsp70 family consists of at least nine members that differ from each other by the intracellular localization and expression pattern. Two of them, Grp78 and Grp75, are localized in the lumen of the endoplasmic reticulum and in the mitochondrial matrix, respectively, whereas the remaining seven HSP70s reside mostly in the cytosol. The only cytosolic HSP70 abundantly and constitutively expressed in all cells is Hspa8 (related to Human constitutive form HSP73). The related form for HSP72 in rodents are two proteins encoded by almost identical genes, *Hspa1a* and *Hspa1b*, termed collectively Hsp70i. As in humans, the expression of Hsp70i in rodents is low or undetectable in most "resting" normal cells and tissues, but it increases rapidly in a variety of stress conditions [14-16].

The heat shock response is regulated by high conserved cis-acting regions of the DNA (heat shock elements – HSE) and also by high conserved DNA associated trans-acting proteins named Heat Shock Transcription Factors (HSF) (Morimoto et al., 1992). While more simple organisms as insects (*Drosophila melanogaster)* and yeasts (*Saccharomyces cerevisiae)* have only one HSF, rodents have at least 2 HSF (HSF1 and HSF2) and humans have 3 isoforms of HSF (HSF1, HSF2 e HSF4) [17-20]. Possibly, more complex organisms could have used multiple HSF against different challenges during evolution. Comparing the structure of each isoform of HSF in one single species, the sequence of amino acids may be 40% identical, as HSF1 and HSF2 in mice [21, 22]. Comparing among species, the homology may be as great as 92%, as seen between HSF1 of human and rodents, or in HSF2 (95% homology between humans and rodents). Interestingly, HSF1 and HSF2 may be activated by distinct physiological phenomenon (Sistonen et al., 1992). While HSF1 and HSF2 are found in all kinds of cells, HSF4 is specifically for cells such as neurons or cardiac and skeletal muscle cells. It has also inhibitory function in heat shock response as negative regulator of HSPs expression [23, 24]. Additionally, in experimental models and cell culture procedures, is possible to identify differences in the activation of HSF1 and HSF2. The former is activated seconds after stress and this response is attenuated quickly, while the latter appears to present a latency period between the event and the response, but it remains activated for 72 h. This HSF different latency for activation suggest a cooperative role of HSF isoforms in cell protection [19] and that these genes are high conserved in nature [7].

In unstressed cells, HSP70 may bind to the regulatory protein HSF for prevention of the trimmer formation of HSF that is required for HSPs transcription. Under stressful conditions, the free HSP70 captures the denatured proteins and then dissociates from the HSP70-HSF complex allowing the formation of HSF trimmer, thus triggering a HSP70 production. Once synthesized, new HSP70 molecules may be involved in a variety of cellular processes and exert different functions [25, 26].

HSP70s are known to function as intracellular molecular chaperones that facilitate protein transport, prevent protein aggregation during folding and protect newly synthesized polypeptide chains against misfolding and protein denaturation. The molecular chaperones properties of such a protein allow them to assist the non-covalent assembly/disassembly of other macromolecular structures without being permanent components of such structures. Additionally, molecular chaperones assist the unfolded protein to achieve its single correct three-dimensional configuration (by still unknown mechanism it has evolved to generate this folded state), without becoming a constituent of the final folded protein [25, 26].

Most proteins destined for cell organelles are synthesized in cytosol and must cross one or more organelle membranes to reach their functional destination. For example, in the mitochondria, 95% of the proteins are made as precursor proteins in the cytosol and are mainly post translationally imported into the mitochondrial sub-compartments. In this situation, cytosolic HSP70 play an important role for maintenance of a transport-competent conformation of precursor proteins. The precursor protein is translocated in an unfolded state and are refolded later, sorted to their final destination and assembled into functional complexes [27].

The chaperone function of HSP70 includes the inhibition of the formation of nascent polypeptides. This inhibition is dose-dependent over a range of 0.1-0.4 nmoles of HSP70 and this effect is greater for the larger polypeptides. All these data suggest that high concentration of HSP70 can perturb the normal folding of nascent proteins, block cell growth and impair the cell viability. Then, these are reasons that may explain why the cells of human body have to carefully auto-regulate the levels of HSP70. Many characteristics and functions of HSFP70 are listed in table 1 and are discussed in this text.

Since the skeletal muscle is one of the most adaptable tissues of the body, every structural aspect of the muscle that can change in response to the stimulus challenge (or to its lack) may require the chaperon action of HSP70 at the molecular level. For example, major adaptations to (dis)use muscle, such as fiber type distribution, fiber diameter, myosin heavy chain profile and mitochondrial distribution, are factors that are associated to the up- or down-regulation of HSP70.

3. HSP70 function in the muscle

The ability of muscle cells to express HSPs (mainly HSP70s) represents a cytoprotection mechanism because HSP70 proteins share the same overall structure. They are composed of an actin-like N-terminal nucleotide binding/ATPase domain of 45 kDa, a substrate-binding domain (SBD) of approximately 15 kDa and a C-terminal domain of approximately 10 kDa that is involved in co-chaperone binding (HU et al, 2006). It is of note that N- and C-terminal domains have expressive relevance to antigen presentation, an important way by which HSP70s participate in immune responses. With this structure, HSP70 may act as a molecular chaperone inside the muscle cell: they facilitate protein transport; prevent protein aggregation during folding; protect newly synthesized polypeptide chains against misfolding and protein denaturation [28, 29].

Protein	Gene name	Human gene ID	Basal levels	Synthesis under stress condition	Cellular location	General functions and process	Specific functions
HSP72 (HSPA1A, HSPA1B)	HSPA1A, HSPA1B	3303, 3304	Low	Fast	Nucleus, nucleolus, citoplasm, cytoskeleton (Mainly in nuclear locus)	Protection against stress Participation in protein metabolism (protein degradation, folding and synthesis) Adaptation to stress Cellular development Cellular energy metabolism	Passes the newly synthesized, unfold protein to leading to folded protein Carries proteins for translocation into different cellular compartments Serve as cohort proteins to other proteins as a drug delivery vehicle Prevent aggregation of non native proteins Facilitate the functional restoration of denatured proteins or the degradation of irreversible damage proteins. Augmented glycolitic activity.
HSP73 (HSPA8)	HSPA8	3312	High	Slow	Distributed throughout the cell, but concentrated over mitochondria or in the nucleolus		

Table 1. Characteristics and functions of HSP70

Historically, studies about the effects of exercise on heat shock protein expression have been dedicated to HSP70 analysis in cardiac or skeletal muscle after exhaustive animal protocols (for review see Noble *et al.*, 2008) [30]. It has been demonstrated that 30 minutes after an exercise bout there is an increase in mRNA expression of muscle inducible form of HSP70 (HSP72) and that is a later heat shock response related to mRNA of constitutive form of HSP70 (HSP73) [31]. Similar response may be observed in humans, and are related to glycogen depletion and the muscle heating [32]. This acute heat shock response (intracellular HSP70 content) remained increased 24 hours after an acute exercise session, according to exercise load [33].

In humans, repeated bouts of eccentric exercise showed an impressive result in terms of HSP70 expression. Sets of Eccentric contractions contractions of the elbow flexors promote ~2-fold increase in HSP70 expression in biceps brachii. Four weeks later, the basal levels of HSP70 was reduced but the muscle still presents the heat shock response to exercise in the same magnitude but in less HSP70 content [34, 35]. Accompanying this effect, higher levels of both serum creatine kinase, soreness, lower levels of both relaxed arm angle and measured torque (indicators of muscle damage) occurs after the first bout, but the levels of these parameters are lower in the second bout. This may represents an association between muscular adaptations and the phenomenon called "acquired thermo tolerance" in terms of HSP70 expression. This study suggests that equivalent HSP70 response imply equivalent levels of stress in each bout and that may be an indicative that the heat shock response is a marker of muscle adaptation. Moreover, basal modification of HSP70 levels remains four weeks after the first bout of exercise and suggests that a single exercise challenge can promote deeper molecular adaptations in muscle cells [34].

Since the heat shock response is a prompt molecular adaptation to the stress condition, to localize the elements that contemplates this phenomenon in muscle is a interesting point of research. The sarcoplasmic reticulum contains microdomains that are involved in translation and processing of transcripts which encode proteins requiring compartmentalization to specific organelles within the myofiber [5]. In skeletal myofibers, ribosomes are localized to both the subsarcolemmal and intermyofibrillar cytoplasm. HSP70 has been shown to be concentrated in a subsarcolemmal fashion and it localizes to the nucleolus and myofibrils in response to stress condition. Although various modes of exercise can induce HSP70 expression, it is clear that it has a different pattern of heat shock response between slow and fast fibers. Slow and more oxidative fibers express greater HSP70 content in response to exercise possibly by preferential recruitment or a more sensitivity to temperature challenges. In this way, the muscle that have different localization of HSP70 mRNA in according to the type of the stimulus: exercise is different to heat treatment [36]. Exercise promotes a concentrated and punctuated perinuclear localization of HSP70 mRNA near the periphery of skeletal myofibers after exercise session (1 to 24h). This result represents that HSP70 proteins are prompt to response because HSP70 mRNA appears rapidly close to the nuclei that transcribes this gene. Diffuse HSP70 mRNA was also observed any time after exercise bout that represents a quickly cytoplasmic distribution of heat shock response proteins [36, 37]. Corroborating this discussion, Paulsen et al [38] showed that maximal eccentric exercise induces a 20 fold increase in HSP70 mRNA 24h after exercise and an increase about 200% in cytosolic HSP70 content.

The chaperone function of HSP70 is more than microscopic laboratory measurements research field. Muscle disuse results in muscular atrophy that is represented by decrease in muscle mass, fiber cross sectional area and total myofibrillar protein content. In this situation contractile protein breakdown exceeds protein synthesis. Moreover, in atrophied muscle there occurs an increase in the proportion of fibers containing the fast myosin heavy chain by transformation from the slow myosin heavy chain (MyHC-I/β) to the fast myosin heavy chain (MyHC-IId/x). As early as 18 h after muscle disuse and persisting for as long as for 18 days, it is possible it is possible to measure a decrease in HSP72 in soleus muscle [39]. Interestingly, previous heat treatment is a strategy to induce HSP70 expression in muscle and this molecular adaptation results in maintenance of muscle mass during 7 days period of immobilization [40]. In this way, HSP70 expression appears to have, not a full protective effect on muscle mass, fiber cross sectional area and total myofibrillar protein content, but a preventive effect on the decrease in MyHC-I/β and the increase in MyHC-IId/x induced during the atrophy process [41]. These evidences suggest that HSP70 can inhibit a key signaling pathway for atrophy in muscle cell preventing the muscular atrophy.

Heat treatment has also been tested in humans. Short wave diathermy therapy is a clinical strategy that means to increase deep heating of tissues with higher water content. This strategy may promote a 58%-increase in HSP70 expression in *vastus lateralis* [42]. It is possible that the previous heat treatment cannot reduce markers of muscle damage but it is able to reduce muscular pain, to preserve strength and to improve range of motion following eccentric contractions. Curiously, there is a gender difference in heat shock response in both basal and exercise-induced HSP70 levels, with men showing lower pre-exercise levels and an attenuated HSP70 response as compared to women's values. The gender difference may be explained by the effects of estrogen modulation on heat shock response [42].

If muscle disuse is a trouble, the reuse of the musculature may represent many stages of soreness. After immobilization, the reload process to the muscle implies in newest molecular adaptations. If a less-required muscle is submitted to a challenge, the HSP70 expression increases greatly (~200%) in the first two weeks of reload process and return to basal levels (above disuse levels) as early as in 8 weeks [39]. This effect is accompanied by increase in percentage of slow type I MyHC fibers (MyHC-I/β). Although many factors appear to be related to the down- and up-regulation of HSP70 function, the expression of this protein is closely related with the morphological and functional changes of muscle cells.

Although initially the HSP70s have been described essentially in studies that addressed molecular chaperone action of such proteins, HSP70s have also been studied as limiting of protein aggregation, facilitating protein refolding and maintaining structural function of proteins [43]. Intracellular HSP70s have further been demonstrated to be anti-inflammatory [44, 45], providing cytoprotection through anti-apoptotic mechanisms, inhibiting gene expression and regulating cell cycle progression [46].

Besides the now classical molecular chaperone action, the most remarkable intracellular effect of HSP70 is the inhibition of nuclear factor κB (NF-κB) activation, which has profound implications for immunity, inflammation, cell survival and apoptosis. Indeed, HSP70 blocks

NF-κB activation at different levels. For instance, HSP70 inhibits the phosphorylation of inhibitor of κB (IκBs), while heat-induced HSP70 protein molecules are able to directly bind to IκB kinase gamma (IKKγ) thus inhibiting tumour necrosis factor-α (TNFα)-induced apoptosis [47, 48] . In fact, the supposition that HSP70 might act intracellularly as a suppressor of NF-κB pathways has been raised after a number of discoveries in which HSP70 was intentionally induced, such as the inhibition of TNFα-induced activation of phospholipase A2, the suppression of inducible nitric oxide (NO) synthase (iNOS, encoded by NOS-2 gene) expression paralleled by decreased NF-κB activation. Hence, HSP70 is anti-inflammatory per se, when intracellularly located, which also explains why cyclopentenone prostaglandins (cp-PGs) are powerful anti-inflammatory autacoids [49, 50].

Another striking intracellular effect of HSP70 is the inhibition of apoptosis. Caspases form an apoptotic cascade by the intrinsic pathway, characterized by the release of mitochondrial pro-apoptotic factors into the cytosol, while stimulation of cell surface receptors triggers the extrinsic pathway by external signaling factors that may induce the apoptotic process. The inhibitory potential of HSP70 over apoptosis occurs via many intracellular downstream pathways (e.g. JNK, NF-κB and Akt), which are both directly and indirectly blocked by HSP70 either, besides the inhibition of Bcl-2 release from mitochondria. Together, these mechanisms are responsible for HSP70 anti-apoptotic function in cells under stress conditions [51-53].

These intracellular effects of HSP70 are closely related to aging and disuse (or both, in a synergic way) effects on muscle wasting, because there is comprehensive evidence that NF-κB activity is increased during disuse and is required for muscle atrophy. NF-κB activation is actually decrease in the first week of immobilization but it is increased in longer atrophy process (by 3-fold) and aged disused muscle (5-fold increase), both reversible effects with HSP70 overexpression that inhibit NF-κB activity owing to increasing the levels of IκBα that are available to bind and to retain NF-κB proteins in cytosol [40].

In terms of metabolic function, increased HSP70 protein expression (~50%) by heat treatment, muscle-specific transgenic over expression, or pharmacological means can protect against diet- or obesity-induced hyperglycemia, hyperinsulinemia, glucose intolerance, and insulin resistance. This protection was tightly associated with the prevention of JNK phosphorylation, another role for HSP70 in the blocking of inflammation [54].

4. Muscle activity and HSP70, eHSP70 and cytokines

Cytokines are intracellular signaling molecules, typically proteins or glycoproteins, that mediate various aspects of cell function, including proliferative and adaptive responses. Cytokine signaling is essential for a coordinated inflammatory response. Diseases related to inflammatory processes as cancer, congestive heart failure, AIDS, sepsis and arthritis often lead to muscle catabolism and loss of muscle function, and this effects are attributed to circulating cytokines. On the other hand, exercise is known to alter immunological function in health individuals and this adaptation also is related to altered cytokine levels [55].

Some cytokines are more closely related to exercise challenge. Circulating TNF-α (tumor necrosis factor – α) may promote cellular responses mediated by two receptors located on cell surface, the 55 kDa TNF-receptor 1 and the TNF-receptor 2. The chronic interaction of this cytokine with its receptors resulted in catabolic response, as loss of muscle mass and contractile dysfunction. TNF-α promotes loss of muscle protein associated to oxidative stress signaling that culminates in muscle wasting mediated by the NF-κB activation, a redox sensitive transcription factor. The impaired muscle function induced by TNF-α also may occur without changes in muscle mass [56]

Exercise-associated muscle damage initiates the inflammatory cytokine cascade. Strenuous exercise increases plasma levels of TNFα, IL-1, IL-6, IL-1 receptor antagonist (IL-1ra), TNF receptors (TNFR), IL-10, IL-8, and macrophage inflammatory protein-1. Exercise induces immune changes and also alters neuroendocrinological factors including catecholamines, growth hormone, cortisol, β-endorphin, and sex steroids. It is generally assumed that the "brain–immune" axis also exists during stress. Release and/or expression of enkephalins can be regulated by different factors such as stress, exercise and cytokines [55, 57].

IL-6 is generally considered a pro-inflammatory cytokine released from immune cells and reaching higher levels in the circulation and inside the muscle. However, muscle contraction during exercise is a signal for IL-6 release from the muscle. IL-6 increases ~100-fold after a marathon race and the increase was tightly related to the duration and intensity of the exercise. IL-6 is produced in the skeletal muscles in response to exercise and it has growth factor abilities and contributes to the anti-inflammatory effect of exercise. Interestingly, exercise, IL-6 and HSP70 have particular relationship: exercise training increases IL-6 response to immune related challenge (LPS treatment) and IL-10 plasma concentration; IL-6 can induce HSP70 expression but the absence of IL-6 during exercise do not attenuate the increase of HSP70 expression by exercise; in sedentary, the absence of IL-6 blunted HSP70 response in skeletal muscle after a immune challenge (LPS treatment); and the absence of IL-10 (an anti-inflammatory cytokine produced during exercise) increased the levels of IL-6 after the same immune challenge These data suggest that there are different pathways that leads to IL-6 and HSP70 up-regulation, with and without exercise stimulus [44, 45, 58-61]. These cytokine signaling and HSP70 expression effects on muscle are summarizing in Figure 1.

Physical exercise has many effects on the Central Nervous System (CNS), much more than mood influence. Peripheral signals generated during and after an exercise session, such as IL-6 and IL-10, decrease endoplasmic reticulum stress markers at hypothalamic level, an effect related to the decrease in NF-κB activation. The processes of building certain behaviors and control of them can be analyzed under the optics of neuroimmunomodulation. The expression of 'sickness behavior' can be induced by immune modifications and immune capacities that are associated with distinct behavior in mammals. In this sense, it is clear the participation of mediators including TNF-α, interleukin-1β (IL-1 β), and IL-6 in the CNS. For instance, the release of skeletal muscle-derived IL-6 into the blood is the most remarkable alteration in cytokine pattern observed

during exercise so that IL-6 is now considered as an exercise factor, a 'myokine' [60], not just an inflammatory mediator. Additionally, as previously hypothesized, [61] the exercise-evoked IL-6 may also act on the CNS to induce the fatigue sensation. In other words, the skeletal muscle must be considered as an auxiliary endocrine organ that interacts with the immune system and CNS, so that IL-6 is a robust exercise marker. Myokine signals are correlated with sensation of fatigue, and may be inducer of sleep or illness response and pyrogenic behavior.[62-67].
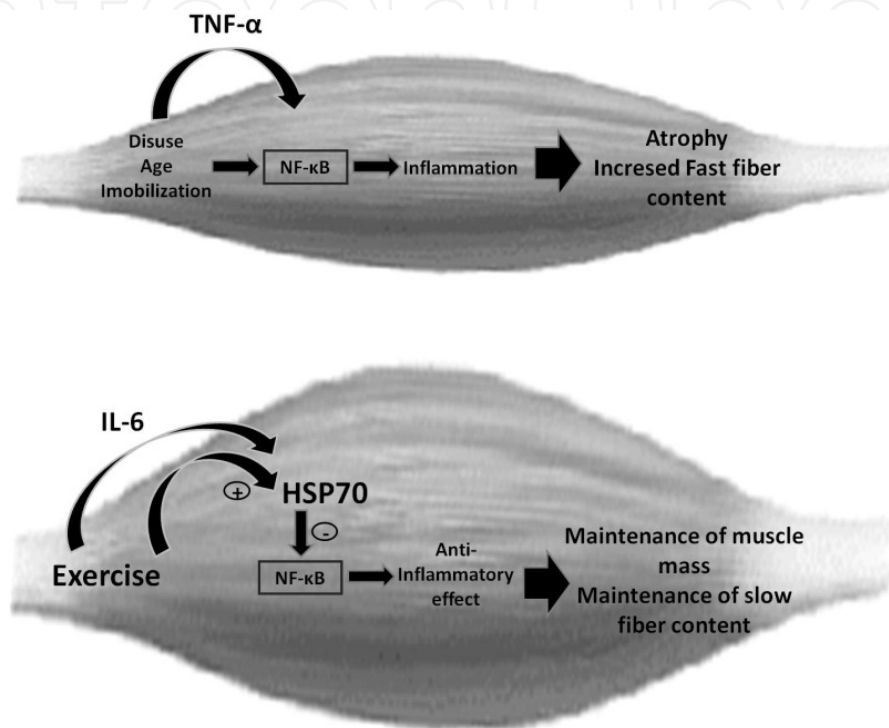

Figure 1. The HSP70 role in inhibition of NF-κB induced atrophy

More recently, however, it has been demonstrated that the presence of HSP70s in the circulation (extracellular HSP70, eHSP70) in response to exercise [32, 44, 68-70]. Since exercise is able to induce high concentrations of HSP70s in both muscle and plasma, the most obvious hypothesis was, primarily, that skeletal muscle should be the releaser of HSP70 during exercise. However, further studies have revealed that this is not the case, at all. The lack of evidence supporting the proposition that the muscle could be the major source of circulatory eHSP70 precluded the 'muscle hypothesis' and suggested that other tissues/cells should be responsible for the increase of eHSP70 in the circulation. In the early phase after high-intensity exercise, eHSP70 is elevated in peripheral blood.

Once HSP70 protein release from muscle to extracellular fluid could eventually happen by lysis process, and considering that the lysis of muscle fiber occurs only under severe cellular stress condition, the presence of eHSP70 during moderate exercise was found to be unfeasible. Though it had been shown that both the intensity and duration of exercise have effects in plasma[71] and muscle[33] HSP70 concentration, this rise in circulating levels of HSP70 precedes, however, any gene or protein expression of HSP70 in skeletal muscle,[32, 72] which

is another strong argument against the 'muscle hypothesis'. Afterwards, eHSP70 blood concentration returns to the lower basal levels as soon as 2 h after the end of the physical effort, remaining practically undetectable for 24h. Similarly to the cytokines released by immune cells during exercise, serum eHSP70 concentration does rise after exercise sessions, mainly because of the contribution of lymphocytes [73]. As a corollary, lymphocyte-derived HSP70s may interplay with CNS to induce the state of 'fatigue behavior' activation [45]. Then, the equilibrium of immune signals during exercise is required to maintenance of the homeostasis and this equilibrium may be observed by several markers, listed in the Figure 2 in relation to the degree of exercise or disease challenge.
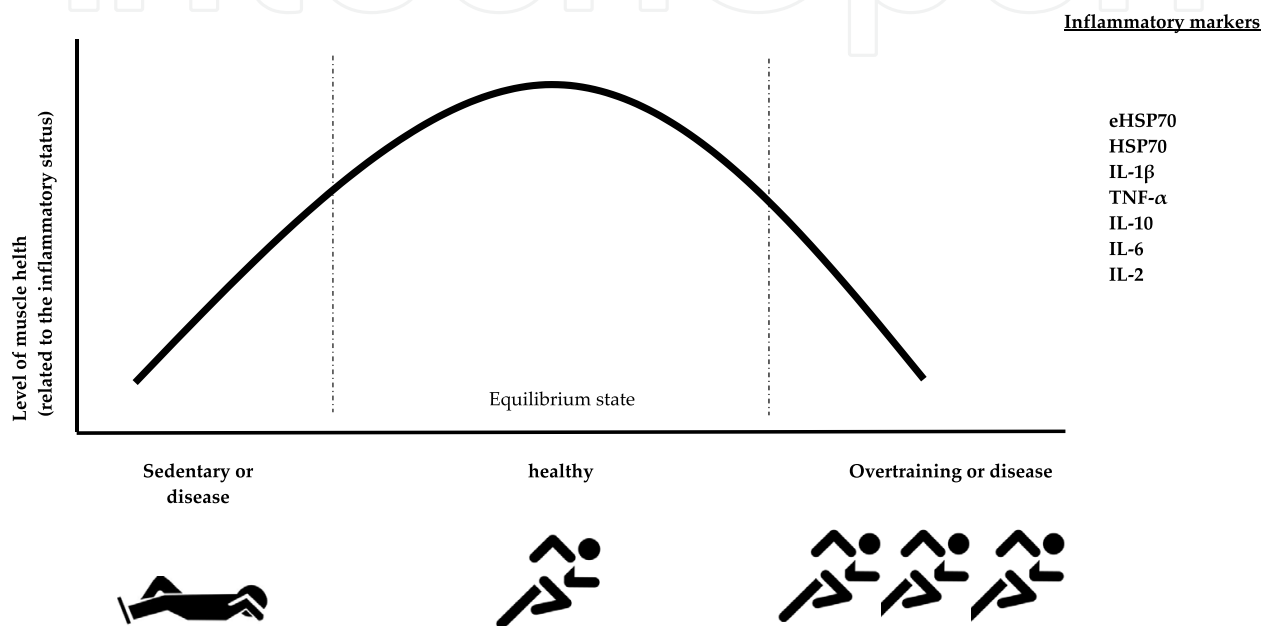

Figure 2. Relation between the degree of exercise or disease challenge and inflammatory markers: The proposed markers may represent both the inflammatory status and muscle status of health. In this way, the figure represents the hypothesis that there is an equilibrium state of many markers in health and a disequilibrium in sedentary, disease and overtraining situations. These markers include extracellular/intracellular HSP70 ratio hypothesis and many others cytokines.

There are many diseases related with higher levels of eHSP70, suggesting that serum levels of this proteins may be considered a novel important biomarker. Whereby health people have low plasmatic levels of eHSP70, the association of these proteins with illness, disease progression and mortality was hypothesized, as well as longevity and health parameter status were attributed to this lower concentration. On the other hand, a rise 3.7-fold eHSP70 circulating levels in critically ill patients was correlated with less hospital treatment period [74] and death [75].

The increase in eHSP70 during the exposure to stresses has also been demonstrated to be the result of the activation of the sympathetic nervous system via alpha-adrenergic receptors leading to eHSP70 export and increased eHSP70 serum concentration[76, 77]. Thus, even though the necrotic cell death might result in the appearance of HSP70 within the extracellular milieu, an increasing number of studies suggest that this is not the major

rule but, on the contrary, physiological effectors (e.g. fever, hypoglycemia and sympathetic stimulation) are the true excitatory signals for the eHSP70 exocytotic pathway, which suggests that highly conserved evolutionary responses are tightened to eHSP70 production, meaning that extracellular HSP70 response may have had an important evolutive role.

The interaction of cytokines or eHSP70 with the complexes of toll-like receptor (TLR2 and/or TLR4) acts as inflammatory signal to cells of the innate immune response (macrophage/dendritic cells/neutrophils). Under stimulation of TLRs, eHSP70 signalizes to the increase of the signal transduction of NF-κB dowstream pathways. Asea and co-workers have shown that eHSP70 induces NF-κB activation and the production of inflammatory cytokines in a process that requires CD14, in addition to TLR2 and TLR4 that are expressed in muscle cell surface [78-81].

By definition, cytokines are proteins secreted by cells with regulatory effects on other cells. Therefore, in addition to its function as an intracellular molecular chaperone, HSP70 in the extracellular milieu acts as a powerful cytokine, affecting the functional properties of immunocompetent cells. This dual role, as both a chaperone and cytokine, helps to elucidate recent findings indicating that heat-shock proteins can be potent adjuvant for many inflammatory related diseases [79].

5. Conclusion

In summary, HSP70s have physiological proprieties that are involved in maintenance of muscle muscle function by the interaction with molecular entities inside the skeletal muscle cell and also by via cell surface receptor. Exercise-induced increase in HSP70 expression and eHSP70 concentration have important role in the regulation of the inflammatory pathways that can be activated during high intensity exercise as well as in the course of atrophy process.

Author details

Thiago Gomes Heck and Mirna Stela Ludwig
Regional University of Northwertern Rio Grande do Sul State (UNIJUÍ), Ijuí, RS, Brazil.

Sofia Pizzato Scomazzon and Paulo Ivo Homem de Bittencourt Jr.
Laboratory of Cellular Physiology, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

6. References

[1] Smith H. From fish to philosopher. 3 ed: Nabu Press; 1961.

[2] Silbernagl S, Despopoulos A. Color Atlas of Physiology, 6 edition; Thieme, 2008

[3] Hochachka PS, GN. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. 1 edition ed: Oxford University Press; 2002.

[4] Selye H. Forty years of stress research: principal remaining problems and misconceptions. Can Med Assoc J. 1976 Jul 3;115(1):53-6.

[5] Lieber R. Skeletal Muscle Structure, Function, and Plasticity: The Physiological Basis of Rehabilitation. 2nd edition ed: Lippincott Williams & Wilkins; 2002.

[6] Morimoto R, Tissieres A, Georgopoulos C. Stress Proteins in Biology and Medicine. New York: Cold Spring Harbor 1990.

[7] Morimoto RI. Cells in stress: transcriptional activation of heat shock genes. Science. 1993 Mar 5;259(5100):1409-10.

[8] Ritossa F. Discovery of the heat shock response. Cell Stress Chaperones. 1996 Jun;1(2):97-8.

[9] Ritossa F. A new puffing pattern induced by temperature shock and DNP in *Drosophila*. Experientia. 1962;18:571-3.

[10] Tissieres A, Mitchell HK, Tracy UM. Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. J Mol Biol. 1974 Apr 15;84(3):389-98.

[11] Henderson B. Integrating the cell stress response: a new view of molecular chaperones as immunological and physiological homeostatic regulators. Cell Biochem Funct. 2010 Jan;28(1):1-14.

[12] Tavaria M, Gabriele T, Anderson RL, Mirault ME, Baker E, Sutherland G, et al. Localization of the gene encoding the human heat shock cognate protein, HSP73, to chromosome 11. Genomics. 1995 Sep 1;29(1):266-8.

[13] Tavaria M, Gabriele T, Kola I, Anderson RL. A hitchhiker's guide to the human Hsp70 family. Cell Stress Chaperones. 1996 Apr;1(1):23-8.

[14] Vydra N, Winiarski B, Rak-Raszewska A, Piglowski W, Mazurek A, Scieglinska D, et al. The expression pattern of the 70-kDa heat shock protein Hspa2 in mouse tissues. Histochem Cell Biol. 2009 Sep;132(3):319-30.

[15] Vydra N, Malusecka E, Jarzab M, Lisowska K, Glowala-Kosinska M, Benedyk K, et al. Spermatocyte-specific expression of constitutively active heat shock factor 1 induces HSP70i-resistant apoptosis in male germ cells. Cell Death Differ. 2006 Feb;13(2):212-22.

[16] Widlak W, Vydra N, Malusecka E, Dudaladava V, Winiarski B, Scieglinska D, et al. Heat shock transcription factor 1 down-regulates spermatocyte-specific 70 kDa heat shock protein expression prior to the induction of apoptosis in mouse testes. Genes Cells. 2007 Apr;12(4):487-99.

[17] Kroeger PE, Morimoto RI. Selection of new HSF1 and HSF2 DNA-binding sites reveals difference in trimer cooperativity. Mol Cell Biol. 1994 Nov;14(11):7592-603.

[18] Kroeger PE, Sarge KD, Morimoto RI. Mouse heat shock transcription factors 1 and 2 prefer a trimeric binding site but interact differently with the HSP70 heat shock element. Mol Cell Biol. 1993 Jun;13(6):3370-83.

[19] Sistonen L, Sarge KD, Morimoto RI. Human heat shock factors 1 and 2 are differentially activated and can synergistically induce hsp70 gene transcription. Mol Cell Biol. 1994 Mar;14(3):2087-99.

[20] Sistonen L, Sarge KD, Phillips B, Abravaya K, Morimoto RI. Activation of heat shock factor 2 during hemin-induced differentiation of human erythroleukemia cells. Mol Cell Biol. 1992 Sep;12(9):4104-11.

[21] Sarge KD, Park-Sarge OK, Kirby JD, Mayo KE, Morimoto RI. Expression of heat shock factor 2 in mouse testis: potential role as a regulator of heat-shock protein gene expression during spermatogenesis. Biol Reprod. 1994 Jun;50(6):1334-43.

[22] Sarge KD, Zimarino V, Holm K, Wu C, Morimoto RI. Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. Genes Dev. 1991 Oct;5(10):1902-11.

[23] Nakai A, Morimoto RI. Characterization of a novel chicken heat shock transcription factor, heat shock factor 3, suggests a new regulatory pathway. Mol Cell Biol. 1993 Apr;13(4):1983-97.

[24] Nakai A, Tanabe M, Kawazoe Y, Inazawa J, Morimoto RI, Nagata K. HSF4, a new member of the human heat shock factor family which lacks properties of a transcriptional activator. Mol Cell Biol. 1997 Jan;17(1):469-81.

[25] Morimoto RI. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev. 1998 Dec 15;12(24):3788-96.

[26] Morimoto RI, Kline MP, Bimston DN, Cotto JJ. The heat-shock response: regulation and function of heat-shock proteins and molecular chaperones. Essays Biochem. 1997;32:17-29.

[27] Maresca B, Lindquist S. Heat Shock. Berlin: Springer-Verlag; 1991.

[28] Hu B, Mayer MP, Tomita M. Modeling Hsp70-mediated protein folding. Biophys J. 2006 Jul 15;91(2):496-507.

[29] Hu B, Tomita M. The Hsp70 chaperone system maintains high concentrations of active proteins and suppresses ATP consumption during heat shock. Syst Synth Biol. 2007 Mar;1(1):47-58.

[30] Noble EG, Milne KJ, Melling CW. Heat shock proteins and exercise: a primer. Appl Physiol Nutr Metab. 2008 Oct;33(5):1050-65.

[31] 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(3):239-46.

[32] Febbraio MA, Ott P, Nielsen HB, Steensberg A, Keller C, Krustrup P, et al. Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. J Physiol. 2002 Nov 1;544(Pt 3):957-62.

[33] Milne KJ, Noble EG. Exercise-induced elevation of HSP70 is intensity dependent. J Appl Physiol. 2002 Aug;93(2):561-8.

[34] Thompson HS, Clarkson PM, Scordilis SP. The repeated bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans. Acta Physiol Scand. 2002 Jan;174(1):47-56.

[35] Thompson HS, Scordilis SP, Clarkson PM, Lohrer WA. A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. Acta Physiol Scand. 2001 Feb;171(2):187-93.

[36] Silver JT, Kowalchuk H, Noble EG. hsp70 mRNA temporal localization in rat skeletal myofibers and blood vessels post-exercise. Cell Stress Chaperones. 2011 Jan;17(1):109-20.

[37] Silver JT, Noble EG. Regulation of survival gene hsp70. Cell Stress Chaperones. 2011 Jan;17(1):1-9.

[38] Paulsen G, Vissing K, Kalhovde JM, Ugelstad I, Bayer ML, Kadi F, et al. Maximal eccentric exercise induces a rapid accumulation of small heat shock proteins on myofibrils and a delayed HSP70 response in humans. Am J Physiol Regul Integr Comp Physiol. 2007 Aug;293(2):R844-53.

[39] Oishi Y, Taniguchi K, Matsumoto H, Kawano F, Ishihara A, Ohira Y. Upregulation of HSP72 in reloading rat soleus muscle after prolonged hindlimb unloading. Jpn J Physiol. 2003 Aug;53(4):281-6.

[40] Dodd S, Hain B, Judge A. Hsp70 prevents disuse muscle atrophy in senescent rats. Biogerontology. 2009 Oct;10(5):605-11.

[41] Takeda I, Fujino H, Murakami S, Kondo H, Nagatomo F, Ishihara A. Thermal preconditioning prevents fiber type transformation of the unloading induced-atrophied muscle in rats. J Muscle Res Cell Motil. 2009;30(3-4):145-52.

[42] Touchberry C, Le T, Richmond S, Prewitt M, Beck D, Carr D, et al. Diathermy treatment increases heat shock protein expression in female, but not male skeletal muscle. Eur J Appl Physiol. 2008 Feb;102(3):319-23.

[43] Johnson JD, Fleshner M. Releasing signals, secretory pathways, and immune function of endogenous extracellular heat shock protein 72. J Leukoc Biol. 2006 Mar;79(3):425-34.

[44] Heck TG. eHSP70/iHSP70 ratio as marker and immunological signaling of exercise intensity. Porto Alegre, Universidade Federal do Rio Grande do Sul; 2011. PhD Thesis. http://www.lume.ufrgs.br/bitstream/handle/10183/33312/000790090.pdf?sequence=1 Porto Alegre: Universidade Federal do Rio Grande do Sul; 2011.

[45] Heck TG, Schöler CM, de Bittencourt PI. HSP70 expression: does it a novel fatigue signalling factor from immune system to the brain? Cell Biochem Funct. 2011 Apr;29(3):215-26.

[46] Gutierrez LL, Maslinkiewicz A, Curi R, de Bittencourt PI, Jr. Atherosclerosis: a redox-sensitive lipid imbalance suppressible by cyclopentenone prostaglandins. Biochem Pharmacol. 2008 Jun 15;75(12):2245-62.

[47] Beere HM. Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways. J Clin Invest. 2005 Oct;115(10):2633-9.

[48] Beere HM. "The stress of dying": the role of heat shock proteins in the regulation of apoptosis. J Cell Sci. 2004 Jun 1;117(Pt 13):2641-51.

[49] Homem de Bittencourt PI, Jr., Curi R. Antiproliferative prostaglandins and the MRP/GS-X pump role in cancer immunosuppression and insight into new strategies in cancer gene therapy. Biochem Pharmacol. 2001 Oct 1;62(7):811-9.

[50] Homem de Bittencourt PI, Jr., Lagranha DJ, Maslinkiewicz A, Senna SM, Tavares AM, Baldissera LP, et al. LipoCardium: endothelium-directed cyclopentenone prostaglandin-based liposome formulation that completely reverses atherosclerotic lesions. Atherosclerosis. 2007 Aug;193(2):245-58.

[51] Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, et al. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. Nature. 2000 Jan 6;403(6765):103-8.

[52] Santoro MG. Heat shock factors and the control of the stress response. Biochem Pharmacol. 2000 Jan 1;59(1):55-63.

[53] Santoro MG. Heat shock proteins and virus replication: hsp70s as mediators of the antiviral effects of prostaglandins. Experientia. 1994 Nov 30;50(11-12):1039-47.

[54] Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, et al. HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci U S A. 2008 Feb 5;105(5):1739-44.

[55] Palermo-Neto J, Costa-Pinto FA. Immune-neural cognition: relationships between behavior and immunity. Neurociências. 2009;5(4):219-30.

[56] Li YP, Reid MB. NF-kappaB mediates the protein loss induced by TNF-alpha in differentiated skeletal muscle myotubes. Am J Physiol Regul Integr Comp Physiol. 2000 Oct;279(4):R1165-70.

[57] Costa-Pinto FA, Cohn DW, Sa-Rocha VM, Sa-Rocha LC, Palermo-Neto J. Behavior: a relevant tool for brain-immune system interaction studies. Ann N Y Acad Sci. 2009 Feb;1153:107-19.

[58] Nunes RB, Tonetto M, Machado N, Chazan M, Heck TG, Veiga AB, et al. Physical exercise improves plasmatic levels of IL-10, left ventricular end-diastolic pressure, and muscle lipid peroxidation in chronic heart failure rats. J Appl Physiol. 2008 Jun;104(6):1641-7.

[59] Pedersen BK. The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control. Essays Biochem. 2006;42:105-17.

[60] Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev. 2008 Oct;88(4):1379-406.

[61] Pedersen BK, Fischer CP. Beneficial health effects of exercise--the role of IL-6 as a myokine. Trends Pharmacol Sci. 2007 Apr;28(4):152-6.

[62] Pedersen BK, Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev. 2000 Jul;80(3):1055-81.

[63] Pedersen BK, Nieman DC. Exercise immunology: integration and regulation. Immunol Today. 1998 May;19(5):204-6.

[64] Pedersen BK, Steensberg A, Keller P, Keller C, Fischer C, Hiscock N, et al. Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. Pflugers Arch. 2003 Apr;446(1):9-16.

[65] Ament W, Verkerke GJ. Exercise and fatigue. Sports Med. 2009;39(5):389-422.

[66] Noakes TD, St Clair Gibson A. Logical limitations to the "catastrophe" models of fatigue during exercise in humans. Br J Sports Med. 2004 Oct;38(5):648-9.

[67] Noakes TD, St Clair Gibson A, Lambert EV. From catastrophe to complexity: a novel model of integrative central neural regulation of effort and fatigue during exercise in humans: summary and conclusions. Br J Sports Med. 2005 Feb;39(2):120-4.

[68] Periard JD, Ruell P, Caillaud C, Thompson MW. Plasma Hsp72 (HSPA1A) and Hsp27 (HSPB1) expression under heat stress: influence of exercise intensity. Cell Stress Chaperones. 2012 Jan 6.

[69] Walsh RC, Koukoulas I, Garnham A, Moseley PL, Hargreaves M, Febbraio MA. Exercise increases serum Hsp72 in humans. Cell Stress Chaperones. 2001 Oct;6(4):386-93.

[70] Febbraio MA, Mesa JL, Chung J, Steensberg A, Keller C, Nielsen HB, et al. Glucose ingestion attenuates the exercise-induced increase in circulating heat shock protein 72 and heat shock protein 60 in humans. Cell Stress Chaperones. 2004 Winter;9(4):390-6.

[71] Fehrenbach E, Niess AM, Voelker K, Northoff H, Mooren FC. Exercise intensity and duration affect blood soluble HSP72. Int J Sports Med. 2005 Sep;26(7):552-7.

[72] Febbraio MA, Steensberg A, Walsh R, Koukoulas I, van Hall G, Saltin B, et al. Reduced glycogen availability is associated with an elevation in HSP72 in contracting human skeletal muscle. J Physiol. 2002 Feb 1;538(Pt 3):911-7.

[73] Pedersen BK, Toft AD. Effects of exercise on lymphocytes and cytokines. Br J Sports Med. 2000 Aug;34(4):246-51.

[74] Ziegler TR, Ogden LG, Singleton KD, Luo M, Fernandez-Estivariz C, Griffith DP, et al. Parenteral glutamine increases serum heat shock protein 70 in critically ill patients. Intensive Care Med. 2005 Aug;31(8):1079-86.

[75] Gelain DP, de Bittencourt Pasquali MA, C MC, Grunwald MS, Ritter C, Tomasi CD, et al. Serum heat shock protein 70 levels, oxidant status, and mortality in sepsis. Shock. 2011 May;35(5):466-70.

[76] Lancaster GI, Febbraio MA. Mechanisms of stress-induced cellular HSP72 release: implications for exercise-induced increases in extracellular HSP72. Exerc Immunol Rev. 2005;11:46-52.

[77] Lancaster GI, Moller K, Nielsen B, Secher NH, Febbraio MA, Nybo L. Exercise induces the release of heat shock protein 72 from the human brain in vivo. Cell Stress Chaperones. 2004 Autumn;9(3):276-80.

[78] Asea A. Heat shock proteins and toll-like receptors. Handb Exp Pharmacol. 2008(183):111-27.

[79] Asea A. Initiation of the Immune Response by Extracellular Hsp72: Chaperokine Activity of Hsp72. Curr Immunol Rev. 2006 Aug;2(3):209-15.

[80] Asea A. Stress proteins and initiation of immune response: chaperokine activity of hsp72. Exerc Immunol Rev. 2005;11:34-45.

[81] Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, et al. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. J Biol Chem. 2002 Apr 26;277(17):15028-34.