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Heat Shock Protein As A Complement Antioxidant Defense System: A Narrative Review

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Abstract:
Background*: Heat shock proteins (HSPs) are molecular chaperones produced in response to oxidative stress (OS). They are essential for folding newly synthesized proteins and repairing damaged or misfolded proteins. Recent research has focused on elucidating the role of HSPs in regulating oxidative stress and ischemia/reperfusion injury, conditions where reactive oxygen species (ROS) play a critical role. ROS have diverse functions, including acting as signaling molecules within cells. The study searched for the classic articles on HSP expression, which are fundamental for the chronological understanding of the experimental procedures of this class of proteins.*
Materials and Methods*: This study comprises a narrative literature review utilizing PubMed and ScienceDirect databases. It employed specific search terms like "heat shock protein," "oxidative stress," "biomarkers," and "antioxidant systems" to locate pertinent studies published in peer-reviewed scientific journals. Article selection criteria included methodological rigor, relevance of investigated biomarkers, and practicality of monitoring strategies proposed. Review articles were instrumental in offering a comprehensive, current perspective, whereas original studies provided detailed insights into the correlation between HSP expression and oxidative stress across various stress contexts.*
Results*: HSPs, recognized as molecular chaperones, serve as molecular connectors between the cell and certain proteins in the membrane. They play essential roles in protein secretion, assembly, maintenance of structural protein integrity, folding, trafficking, protein degradation, and aiding the correct folding of newly synthesized and denatured proteins. The collective impact of increased levels of free radicals on cellular function has been extensively studied. In response to ROS, which can induce oxidative stress, biological systems activate a protective mechanism by increasing the expression of tightly regulated HSPs. HSPs are celebrated for their cytoprotective abilities, shielding cells from a range of insults through their involvement in polypeptide folding, assembly, organelle translocation across membranes, repair processes, and the degradation of irreparable peptides.*
Conclusion: *It is noteworthy that HSPs work in conjunction with the antioxidant system to mitigate or counterbalance the cellular effects of ROS. Increased levels of ROS are associated with apoptosis and contribute to diverse inflammatory responses, which are hallmark features in the pathogenesis of human inflammatory diseases (HIDs). Consequently, it is reasonable to hypothesize that HSPs play a crucial role in attenuating the progression of HIDs through their cytoprotective actions. These proteins may represent promising targets for drug development aimed at immunotherapy against HIDs.*
Key Word*: Heat shock protein; Oxidative stress; Biomarkers; Antioxidant systems; Exercise.*

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I. Introduction

Cells in all organisms respond to a variety of stressful conditions through rapid transcription and subsequent translation of a series of highly conserved proteins, generically termed stress proteins [1]. The term "stress protein" has a generic character in the description of biological events, since different stimuli can induce the same cellular defense mechanism [2]. Heat Shock Proteins (HSPs) appear in the literature as one of the best-studied classes of stress proteins.

HSPs are a family of highly conserved proteins that respond to stress and are expressed in a generalized manner, but at low levels under normal physiological conditions. These HSPs act as molecular chaperones, assisting in the correct folding of proteins, preventing their aggregation and directing those misfolded to specific degradation pathways [3].

The increase in the expression of HSPs was initially evidenced in Drosophila melanogaster, by means of thermal shock (incubation at different elevated temperatures). It is known that the heat shock response is a general property of all cells, both in conditions of hyperthermia and in other states of homeostatic alteration, such as exposure to heavy metals, increase in the concentration of intracellular calcium, decrease of glucose for energy supply to cells, viral and bacterial infections, hypoxia, amino acid analogues, increase in reactive oxygen species (ROS), presence of toxins, etc. [4,5,6,7].

HSPs also play a role in several cellular processes that occur during and after exposure to oxidative stress, which are characteristic of various pathological conditions, such as ischemia, cardiovascular disease, and neurodegeneration. In these conditions, oxidative stress arises due to the imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant capacity to neutralize reactive intermediates. This imbalance can result from both the excessive production of ROS, as seen in ischemic diseases, and the reduction in the activity of antioxidant enzymes. Consequently, the normal intracellular redox environment is compromised, leading to oxidation and aggregation of vital proteins and DNA, resulting in dysfunction in cellular function. As dysfunctional oxidized proteins accumulate, activation of inflammatory pathways also occurs. In addition, changes in cellular redox state may signal activation of the apoptotic cascade [8].

Oxidative stress causes inevitable damage to cellular proteins, requiring rapid refolding. The synthesis of HSP represents the oldest and most effective mechanism of cellular protection. Throughout life, cellular adaptation to various cytotoxic factors, of both xenobiotic and natural origin, has conferred on HSP a polyfunctionality: they can act as anti- or pro-apoptotic factors, in addition to regulating the activity of these factors. The crucial role of heat shock proteins in adaptation, inflammation, and immune response has also been demonstrated. Experimental and clinical studies have confirmed the significant role of HSP in the development of pathophysiological phenomena such as oxidative stress, aging, tumor formation, and immune responses [9].

The aim of this work is to present the mechanism of activation of HSPs as a result of stressful processes in the human body, highlighting the role of oxidative stress. It is hypothesized that HPS may act as a complement to the antioxidant defense system.

II. Material And Methods

The present study is a narrative literature review, using the PubMed and ScienceDirect data bases. Specifics search terms such as "heat shock protein", "oxidative stress", "biomarkers", "antioxidant systems" were used to identify relevant studies published in peer-reviewed scientific journals. The selection of articles considered criteria such as methodological quality, the relevance of the biomarkers investigated, and the applicability of the proposed monitoring strategies. Review articles have been particularly useful to obtain a comprehensive and up-to-date view of the area, while original studies have provided specific data on the relationship between HSP expression and oxidative stress under different stress conditions.

III. Result And Discussion

The study aimed to analyze the functional bases of HSPs, mechanisms of activation and action, as well as the potential of physical exercise in the expression of this class of proteins.

Function of Stress Proteins

HSPs are of fundamental importance for the survival of cells, not only in conditions of stress, where there is an increase in the synthesis of proteins aimed at repairing cellular damage, but also in transport and folding events of proteins in formation [10,11,12,13,14,15]. For this reason, they are also called "molecular chaperones" [16,17,18]. Some classes of stress proteins are constitutive and are called cognate proteins. Cognate proteins are constitutive members of stress protein families that function under normal physiological condition. For example, Hsc70 is a cognate protein of the HSP70 class. Protein families with molecular weights of 72 kDa, 73 kDa, and 90 kDa correspond to the largest classes of stress proteins expressed by the body [19].

Chaperones play an essential role in the proteostasis network by facilitating the folding or degradation of proteins, thus preventing protein aggregation. In humans, there are 332 genes that encode the chaperone and co-chaperone families, collectively forming the "chaperome". A significant subset of these chaperone genes encodes for the HSP family of molecular chaperones, initially identified due to their dramatic up-regulation under conditions of cellular stress. This highly conserved group of molecular chaperones is categorized into several classes based on their monomeric molecular mass, including Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small HSPs (sHSPs). Traditionally, these HSPs have been classified as ATP-dependent or ATP-independent chaperones. ATP-dependent chaperones, such as Hsp70, Hsp60, Hsp90, and Hsp100, utilize repeated cycles of ATP hydrolysis to actively assist in the folding or unfolding of client proteins. On the other hand, ATP-independent chaperones, such as sHsps, do not actively uncoil or fold client proteins, but recognize and bind to aggregation-prone proteins to stabilize them and prevent their aggregation [20].

Molecular chaperones can be defined as a category of proteins that advise the correct formation of other polypeptides, although they are not components of their normal functional structure [21]. The major problem of proteins in formation and those newly synthesized is the exposure of their interacting surfaces with the intracellular environment, which could facilitate contacts with other molecules, triggering changes in the already programmed structural and functional conformation of the protein. The main function of molecular chaperones is to assist the self-formation and folding of polypeptide chains, by inhibiting alternative pathways of binding other proteins to the chains that are formed, helping in the non-formation of these incorrect interactions. The following processes show the general role of chaperones in relation to the problem of interacting surfaces [21]:

- **Protein Synthesis.** The amino-terminal region of each polypeptide is synthesized before the carboxyl-terminal region, and in this condition, there may be the action of chaperones interacting with the protein in formation, in order to prevent incorrect interactions of elements of the protein structure itself or with other cellular components.
- **Protein Transport.** Proteins that enter organelles such as endoplasmic reticulum, mitochondria, plastids, and bacterial periplasms cross the membrane only in an unfolded or semi-coiled state. Proteins are synthesized by cytosolic ribosomes in the form of precursors, and transport into a given organelle can only be done through interaction with a chaperone.
- **Protein function.** In many situations, the normal functioning of oligomeric complexes involves changes in subunit-subunit interactions, so transient exposures of contact surfaces of these complexes can be mediated by molecular chaperones.
- **Stress Response.** Environmental stress can cause protein denaturation. To protect against the stress imposed, cells accumulate proteins that prevent the production of denatured protein aggregates.

As molecular binders between the cell and some proteins in the membrane, HSPs, which are well known as molecular chaperones, participate in protein secretion, assembly, maintaining the integrity of structural proteins, folding, trafficking, protein degradation, and binding to newly synthesized and denatured proteins, helping their folding to the correct information. During proteostasis, HSPs mediate immune cell functions and responses and play a critical role in cell proliferation, signal transduction, apoptosis, and angiogenesis as cancer therapeutic targets. The protective mechanisms of HSPs consist of folding nascent polypeptides, participating in suppressing proinflammatory cytokines and intracellular transport, repairing damaged proteins and eliminating unrepairable proteins. In response to injury and stress signals, HSPs are synthesized in large quantities and bind to proteins to ameliorate protein denaturation, misfolding, and aggregation by regulating cellular signaling pathways, RNA (ribonucleic acid) stabilization/translation and apoptosis. Collectively, HSPs are beneficial for cellular repair and recovery to ensure cell survival [22].

Mechanisms of Action of Stress Proteins

Due to ongoing mitochondrial oxidative respiratory reactions and various cellular and non-cellular processes - such as phagocytosis, inflammatory responses, ionizing radiation, air pollutants, physical exercise, cigarette smoking, and ozone exposure - cells frequently produce reactive oxygen species (ROS) and reactive nitrogen species (RNS). These compounds disrupt the normal balance between oxidants and antioxidants within cells, leading to oxidative stress. ROS and RNS encompass a range of oxygen-containing molecules, including superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), alkoxyl radical ($RO^{\bullet}$), peroxyl radical ($HOO^{\bullet}$), nitric oxide radical ($NO^{\bullet}$), nitrogen dioxide (NO_2), as well as potent non-radicals like hydrogen peroxide (H_2O_2), ozone (O_3), and singlet oxygen ($^1O_2$). These species are highly reactive and more powerful than ordinary oxygen and nitrogen, thereby exerting harmful effects on biological systems when accumulated in excessive amounts [23,24,25,26,27,28,29].

Oxidative stress occurs in circumstances in which there is an imbalance between the prooxidants and antioxidant systems, so that the former are predominant. Within a strategy of maintaining the redox state against oxidative conditions, the blood plays a fundamental role, transporting and redistributing antioxidants throughout the body; in this way, the antioxidant capacity in the blood can give us estimates of oxidative stress levels, allowing a less invasive method of measurement than by other routes, such as biopsy. To rid the body of the deleterious effects of ROS, there are antioxidant systems divided into two classes: the non-enzymatic system, composed mainly of β-carotene (pro vitamin A), ascorbic acid (vitamin C) and alpha tocopherol (vitamin E) and total free sulfhydryl groups (TFSG), and the enzymatic system, with catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and superoxide dismutase (SOD) as the main enzymes [9].

The cumulative impact of elevated levels of free radicals on cellular function has been extensively documented. In response to reactive oxygen species (ROS), which can precipitate oxidative stress, biological systems employ a protective mechanism by upregulating highly-regulated heat shock proteins (HSPs). HSPs are renowned for their cytoprotective capabilities, safeguarding cells against a spectrum of insults through their roles in polypeptide folding, assembly, organelle translocation across membranes, repair processes, and the degradation of irreparable peptides. Studies have shown that ROS-induced genotoxicity can lead to DNA

fragmentation, yet this effect has been mitigated by the presence of the Hsp70 family, suggesting that HSPs may protect against DNA damage caused by ROS-induced stress [22, 28,29,30,31,32,33].

Interestingly, HSPs collaborate with the antioxidant system to counteract or neutralize the cellular impacts of ROS. Elevated ROS levels are implicated in apoptosis and contribute to various inflammatory reactions, characteristic of the pathogenesis of human inflammatory diseases (HIDs). Therefore, it is plausible to propose that HSPs play a pivotal role in mitigating the development of HIDs by exerting cytoprotective effects. These proteins could potentially serve as targets for drug development aimed at immunotherapy against HIDs [34].

Vigh et al. [35] also suggested that changes in the physical properties (fluidity and viscosity) of biological membranes due to the increase in internal temperature could influence the expression of heat shock genes. However, this homeostatic response of membrane organization seems to be transient, lasting a few hours; the accumulation of specific HSPs in the alteration regions would induce an increase in membrane stiffness, so that it can recover its normal fluidity characteristics; after the reestablishment of pre-stress conditions, the membrane would inactivate the disturbance signals and terminate the synthesis of HSPs. In this sense, Ryan et al. [36] proposed that the amount of HSP70 stress proteins synthesized seems to be dependent on the severity of the stress submitted to the organism and the cellular level of HSPs existing prior to the stress condition. They also postulated that HSP70 synthesis in heat-exposed cells is reduced when the same cells are subsequently exposed to a second heat stress. This adaptive condition was called acquired thermotolerance [37], and is an important environmental thermal regulation response in mammals. The onset of attenuation of cellular changes that may be induced by excess heat appears to be dependent on the presence of constitutive HSPs in the pre-stress state [38].

Welch [6] reports that radical oxygen species under a stress condition oxidative factors, may participate as signaling agents in the synthesis of HSPs. signaling the synthesis of HSPs would be, possibly, the fact that radical species promote exposure of hydrophobic amino acids from membrane proteins: hydrophobic amino acids would act as binding regions to HSPs, which could lead to transcription of more stress proteins.

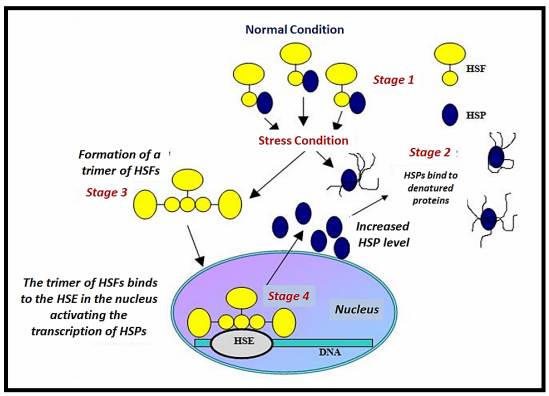
And what would be the molecular nature of the "cellular thermometers" that signal the transcription of HSPs? Hightower [39] described three possible mechanisms:

- Studies with Escherichia coli have revealed that cell damage is repaired as a function of three HSPs - DnaK, DnaJ and GrpE, by increasing the amount of the "heat shock transcription factor s32". The stress protein DnaK is the bacterial homologue of the eukaryotic HSP70 family. It is proposed that there is a linkage between the transcription factor s32 and the constitutive stress proteins, providing a regulatory complex to monitor the demand for HSPs.
- The ribosome could be a control sensor for stress responses, generating the synthesis of partially folded polypeptide chains. Such polypeptide chains would be identified as defective or denatured proteins, inducing the expression of HSPs.
- The regulation of HSP transcription could be controlled by the heat shock factor (HSF), present in the cytoplasm, which would have the ability to bind with the heat shock element (HSE), located in the nucleus. This, apparently, is the most accepted hypothesis and is presented in Figure 1.

According to this proposal, under normal homeostatic conditions, HSPs are linked to the heat shock factor (HSF) (stage 1). HSF can be defined as a transcriptional activating factor, present in the cytoplasm in a monomeric state and without DNA-binding activity. In response to hyperthermia or another stress condition there is an increase in denatured proteins in the cytoplasm, inducing an uncoupling of the HSP-HSF complex. The uncoupling occurs because the constitutive HSPs seek to bind with the denatured proteins, since their function is to prevent such denatured proteins from aggregating in deleterious interactions with other proteins, which could cause a greater stress condition (stage 2).

In the specific case of HSP70, the stress response may have a self-regulation mechanism. In unstressed status HSP70 binds to a regulatory protein termed heat shock factor (HSF), which prevents a trimer formation of HSF. Under stressful condition the free HSP70 captures the denatured proteins, and this can cause a dissociation of HSP-HSF complex and allow a formation of HSF trimer, and thus trigger an Hsp70 production. Previous studies have shown that there is a discrepancy in Hsp70 between protein level and mRNA level in response to stress, suggesting that HSP70 response [40].

Figure 1. Speculative model for the activation of the heat shock factor (HSF) under stress conditions.



Source: prepared by the author.

However, in order for HSF to trigger the process of HSP synthesis, it must enter the cell nucleus and bind to the heat shock element (HSE); the entry of HSF into the nucleus is only possible through its transition from a monomeric (deactivated) state to an oligomeric (activated) state (stage 3). HSPs regulate the oligomerization state of HSF (and thus its ability to bind to DNA) by concealing the trimerization surface; once the constitutive HSPs bind to denatured proteins in the cytoplasm, the trimerization surface is exposed, making the formation of HSF trimers possible. At a subsequent stage, these trimers enter the nucleus and immediately bind to the DNA HSEs, activating the transcription of HSPs (stage 4). The synthesis of HSPs induced by a state of hyperthermia is proportional to the duration and severity of stress [41,42,43,44,45].

Several studies have demonstrated that muscle Hsp70 mRNA is promptly upregulated in response to stress. For instance, just 30 minutes of treadmill running can lead to a notable increase in Hsp70 mRNA levels in human skeletal muscle within 4 minutes post-exercise. Our previous findings indicated that exercise training resulted in elevated Hsp70 mRNA levels in well-trained human skeletal muscle. Interestingly, we observed a disparity between Hsp70 mRNA and Hsp70 protein levels, suggesting that the response of Hsp70 may be independently regulated at both the protein and mRNA levels [40].

Stress Proteins and Physical Exercise

HSPs exhibit remarkable versatility both inside and outside cells. They play crucial roles in responding to various stress conditions and providing protection against subsequent insults. Exercise, due to its physiological stresses, prominently stimulates increased expression of various HSPs in multiple tissues. The combination of physiological stresses induced by exercise and the interplay between signaling pathways in different tissues suggests that HSP expression is likely induced by a combination of stressors, with reactive oxygen species (ROS) potentially playing a significant role [46,47].

Although ROS can cause oxidative stress by disrupting the balance between their production and antioxidant levels, leading to damage to lipids, proteins, and nucleic acids, moderate levels of ROS also act as important regulatory mediators in signaling pathways. Many responses mediated by ROS actually help cells protect themselves against oxidative stress and restore redox homeostasis.

The understanding of the mechanisms that induce HSPs in physical exercise is still being studied. Many biochemical and physiological conditions, along with temperature elevation, could stimulate the expression of stress proteins in exercise, including glucose depletion, hypoxia, elevated intracellular Ca2+ concentration, and decreased pH [1]. There is also evidence that ROS, under oxidative stress, could also participate as signalers in the synthesis of HSPs [6]. Hernando and Manso [48] reported that sedentary rats submitted to intense exercise had significant increases in HSP72 in soleus muscle. Locke et al. [1] also showed that the stress induced by an exhaustive exercise on a treadmill would be a sufficient stimulus to induce synthesis of HSP72 and HSP90 in soleus muscle cells, spleen cells and lymphocytes.

Given the potential of ROS to damage intracellular proteins during repetitive muscle contractions, it has been suggested that when this production exceeds the defense capacity, the synthesis of HSPs could complement the body's pre-existing antioxidant enzymatic defense [49]. In this sense, Antunes Neto et al. [9] showed an increase in the concentration of carbonylated proteins and a decrease in the activity of the antioxidant enzymes catalase and glutathione reductase in the soleus muscle of sedentary rats two hours after an exhaustive exercise protocol, indicating that exhaustion was related to an increase in oxidative stress levels. At the same time, a significant increase in HSP72 expression was detected in the soleus muscle, reinforcing the idea that HSP synthesis could be a complementary mechanism of protection against exercise-induced oxidative stress when the antioxidant enzyme system is decreasing in activity [50]. The signaling for the synthesis of HSPs would possibly be the exposure of hydrophobic amino acids of the defense enzymes themselves; hydrophobic amino acids would act as binding regions to HSPs, a signal for the synthesis of more stress proteins.

There seems to be a situation of duality when analyzing the action of ROS in the organism; at the same time that they initiate the process of lipid peroxidation of membranes, they also signal the synthesis of HSPs, to protect the cell from cell damage. Increase in the production of ROS, in a condition where there is a high demand for ATP, such as in repetitive muscle contractions, it may cause muscle fatigue due to the denaturation of proteins of the calcium-releasing mechanism in the sarcoplasmic reticulum; At the same time, the free radicals will participate in signaling pathways for transcriptional induction of HSPs, with the objective of reducing the damage caused by the increase in oxidative stress levels in sarcoplasmic reticulum in a similar subsequent contractile activity [49].

There is experimental evidence that only muscles composed of type I fibers have constitutive expression of HSP70; Mixed-fiber muscles contain constitutive HSPs according to the proportion of existing type I fibers. The increased level of constitutive HSP70 in type I fibers is possibly related to the fact that these fibers are continuously subjected to more stressful conditions than other types of fibers [1,51].

Ebisuda et al. [52] demonstrated significant increases in HSP-70, PGC-1α, HIF-1α, and PDK4 mRNA levels following high-intensity exercise in hot conditions. These gene expression enhancements likely promote a protective response against heat stress, support mitochondrial biogenesis, and facilitate fatty acid oxidation. Consequently, our findings suggest that acclimatization or acclimation to heat for competitions and races in hot conditions effectively enhances aerobic energy metabolism and mitigates heat stress. Regular exposure to heat stress through training in natural or artificially heated environments may therefore represent an effective strategy for athletes preparing for events held in hot climates.

Antunes Neto et al. [9] developed a strenuous exercise protocol with progressive loading for 60 minutes with rats running on a treadmill. It was observed that maximal oxidative stress occurred between 2 and 3 hours after exercise, with a considerable increase in protein carbonylation levels. However, the increase in the levels of erythrocyte antioxidant enzymes, Catalase and Glutathione Reductase, and leukocyte HSP suggests a systematic and conjugated action of these cell types in attenuating the consequences of oxidative stress induced by an acute session of exhaustive exercise. The peak of taurine in plasma coincided with increased lipoperoxidation and the release of the enzyme Creatine Kinase, suggesting that oxidative stress may increase the membrane permeability of muscle cells.

IV. Conclusion

The processes of adaptation to the stress imposed by continuous and repetitive exercise have their regulatory activities occurring, predominantly, in the recovery interval between training sessions (immediately after exercise up to 24 hours after the end of contractile activity), through specific expression of genes that induce synthesis of HSPs. These results reveal that the action of HSPs is sequential after a continuous contractile activity, being as a cellular compensation mechanism to the possible damage caused by oxidative stress, reinforcing that the recovery time from one training session to another is of fundamental importance for the occurrence of anabolic processes.

The role of Hsp70 in protein metabolism has been extensively studied within the realm of molecular chaperones. Recently, there has been growing recognition of its potential impact on cellular processes crucial to muscular adaptation, such as apoptosis and myogenesis. Emerging studies suggest that Hsp70 may significantly influence these processes. However, there is a notable gap in research concerning the direct effects of Hsp70 on facilitating cellular activities like apoptosis, vascular growth, and myogenesis, particularly through satellite cell activation. Specifically, there is a lack of studies exploring the relationship between the Hsp70 response and muscle function. Utilizing modern techniques such as transgenic cell culture models, initial investigations have begun to explore how Hsp70 affects energy metabolism. However, further in vivo studies are essential to deepen our understanding in this area.

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