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REVIEW

Role of heat shock proteins 70/90 in exercise physiology and exercise immunology and their diagnostic potential in sports

Karsten Krüger,[1] Thomas Reichel,[1] and Carsten Zeilinger[2]

[1]*Department of Exercise and Health, Institute of Sports Science, Leibniz University Hannover, Hannover, Germany; and*
[2]*Center of Biomolecular Drug Research, Leibniz University Hannover, Hannover, Germany*

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Krüger K, Reichel T, Zeilinger C. Role of heat shock proteins 70/90 in exercise physiology and exercise immunology and their diagnostic potential in sports. *J Appl Physiol* 126: 916–927, 2019. First published February 7, 2019; doi:10.1152/japplphysiol.01052.2018.—Heat shock proteins (HSPs) are molecular chaperones facilitating the unfolding or folding of secondary structures of proteins, their client proteins, in cellular stress situations. Various internal and external physiological and mechanical stress factors induce a homeostatic imbalance, followed by an increased expression of HSP70 and HSP90. Exercise is a stress factor, too, and its cumulative physiological perturbation manifests at a cellular level by threatening the protein homeostasis of various cell types. Consequently, an increase of HSP70/90 was described in plasma and mononuclear cells and various organs and tissues, such as muscle, liver, cardiac tissue, and brain, after an acute bout of exercise. The specific response of HSP70/90 seems to be strongly related to the modality of exercise, with several dependent factors such as duration, intensity, exercise type, subjects' training status, and environmental factors, e.g., temperature. It is suggested that HSP70/90 play a major role in immune regulation and cell protection during exercise and in the efficiency of regeneration and reparation processes. During long-term training, HSP70/90 are involved in preconditioning and adaptation processes that might also be important for disease prevention and therapy. With regard to their highly sensitive and individual response to specific exercise and training modalities, this review discusses whether and how HSP70 and HSP90 can be applied as biomarkers for monitoring exercise and training.

biomarkers; inflammation; recovery; stress proteins

INTRODUCTION

Exercise is a physiological stress factor that has been shown to affect the levels of heat shock proteins (HSPs). In general, HSPs are upregulated in response to cellular stress as needed to stabilize cellular functions and ensure survival. Moreover, HSPs, specifically HSP70 and HSP90, have an immunologic impact, which seems to be somewhat more differentiated and reflects a bidirectionality of HSP response (13). On one hand, HSP70/90 are upregulated and properly downregulated to control acute inflammatory conditions in parallel to their pro- and anti-inflammatory chronology. On the other hand, during conditions of chronic inflammation or excessive stress, accumulation of HSP70 and 90 seems to aggravate inflammation. Therefore, HSPs have been classified as potential therapeutic targets during inflammatory diseases (12).

Address for reprint requests and other correspondence: K. Krüger, Leibniz Univ. Hannover, Inst. of Sports Science, Dept. of Exercise and Health, Am Moritzwinkel 6, 30167 Hannover, Germany (e-mail: Karsten.krueger@sportwiss.uni-hannover.de).

During acute exercise, various HSPs, such as HSP70 and 90, are upregulated in various organs and tissues, such as blood, followed by a downregulation in the period of recovery. The term "recovery" means the physiological status after exercise as the body returns to homeostasis, its normal stable and physiological resting state. Both proteins are suggested to play a role as mediators of fatigue and biochemical stress sensors. Implicating their role as universal stress sensors, exercise represents only one of various cellular stressors offering a holistic view on the factor "stress." Accordingly, the combination of different stressors, such as exercise and lifetime stress or exercising during hot climate conditions, seems to aggravate HSP accumulation (19). Consequently, there is a higher physiological effort for the resolution of the stressful situation. In addition, in the case of uncontrolled or chronic stress, such as conditions of chronic inflammation, the body's ability for backregulation to homeostasis is threatened or exceeded, which might implicate pathological conditions such as overtraining (65).

In light of these assumptions, the present review aims to discuss these differential roles of HSP70 and 90 in exercise

physiology, with a specific focus on the bidirectional impact of these HSPs during inflammation and exercise stress. Here it seems to be of particular importance that HSPs are not only upregulated during stress but also properly downregulated during resolution of the stressful situation. Regulation of HSP70/90 in blood offers the opportunity to use these molecules as stress sensors and biomarkers. In this regard, HSPs might offer possibilities not only for quantification of stress but also for monitoring recovery processes holistically (4). Using such sensor proteins as biomarkers during exercise and recovery might help to control training processes adequately in accordance with individual abilities. In parallel, other interfering stressors, such as situations of psychological stress, are also considered, having in mind the picture of a "stressed athlete." When training stimuli are incorrectly observed because of a lack of systematics and athletes are additionally burdened by lifetime stressors, there might be an inadequate balance between stress and recovery processes (32, 44, 76, 109). Hence, situations of chronic stress and overtraining might occur, which may be of special interest for monitoring (65). Biomarkers would be of great value for checking and controlling the internal load condition, specifically with regard to their objectivity, sensitivity, reproducibility, and automation of the monitoring processes (7). It therefore seems desirable to exploit further potential biomarkers for sports practice beyond the known markers. Muscle enzymes and hormones are identified as stress-sensitive biomarkers in the blood plasma; inter alia creatine kinase (CK) and lactate dehydrogenase (LDH) are used in specific settings for training control and monitoring of regeneration. However, these parameters have been shown to have a high variance in response to a standardized training program. The partly cost-intensive measurement effort and lack of reliability of these enzymes adversely affect their application in sports practice (27, 32, 66, 109). Thus there is a requirement for innovative biomarkers that reflect an athlete's stress level.

In particular, HSP70 and 90 are key factors after stress encounter. They are both expressed in response to inherent physiological changes, for example, after exercise (84). The level of plasma HSP70/90 during exercise increases individually, dependent on intensity and duration. In particular, the enormous release of HSP70 and 90 during high-load exercise is speculated to contribute to fatigue sensations (34). Thus both HSPs are considered as potential biomarkers, which can give a variety of information on individuals' stress processing and the course of recovery processes. In the process, other interfering stress factors are also considered, culminating in a holistic reflection of stress experiences in athletes.

THE CHAPERONES: A SERVANT NETWORK FOR CLIENT PROTECTION

HSPs are molecular chaperones in cells that help their client proteins in the unfolding or folding of their secondary structures in cellular stress situations (106). HSPs are divided into six families (HSP100, HSP90, HSP70, HSP60, HSP20, and small HSPs). In Fig. 1 HSP90/70 are prominent, while HSP100 and the other HSPs are separated into group I and group II HSPs. Physicochemical stress or single-point mutations in the genome, which lead to amino acid exchange, cause nonfunctional or denatured proteins; HSP70/90 can determine the fate of the proteome pool under stress conditions (43). HSPs are essential chaperones that actively organize the correct folding of newly synthesized or denatured proteins in cells, thereby preventing the formation of aggregates (16, 50, 62). They do not have any information about the correct folding of the clients, but through their binding they support the proteins in their folding process by avoiding binding, aggregation or faulty interactions with other molecules. In a broad variety of species, HSPs are involved in signal transduction, in the control of the cell cycle, in stress management, and in the transport of proteins (14). Not only is the expression of some of these genes highly heat inducible, but it is also induced by other environmental factors such as UV radiation, oxygen deficiency, or the presence of ethanol or heavy metals (51, 67, 87). A major aim in HSP research is understanding the different roles of HSPs and their controlled response to stress (45, 105). Stressful situations, which can result from cancer, extreme sports, lifestyle, or aging, can produce a huge amount of damaged proteins (72) (Fig. 1). The concentration is at ~200–300 g/l, in comparison to a protein blood concentration of 80 g/l, demonstrating that there is a requirement to protect and stabilize the proteome. For all regular processes and for cells, it is energetically more advantageous to recycle proteins than to synthesize them anew (20). Several inhibitors have been developed against HSP90 and HSP70 to hinder disease-related stress response. HSP90/70 have ATP-binding and client-binding sites. HSP90 has high-affinity binding sites for cochaperones, e.g., activating AHA1, inactivating p23, p50, CDC37, and HSP70, and client proteins, whereas HSP70 function is dependent on HSP40 and nucleotide exchange factor (NEF) (Fig. 1).

The human superfamily of HSP70 consists of 13 members, 4 of which account for the largest share (15, 18, 30, 97). These include the Grp70 located in the endoplasmic reticulum (ER), the mitochondrial (mt)HSP70, the constitutively expressed HSC70, and the stress-induced HSP70. HSC70 is involved in the folding of newly synthesized proteins, the transport of proteins across the cell membrane, and the assembly of multiprotein complexes. As a result of proteotoxic stress, the protein is present at higher levels and is available to the cell as an active folding aid (18). In the promoter region of heat shock transcription factor-1 (HSF1), the regulation of the expression of heat shock elements (HSEs) is controlled by means of external stress factors.

HSP70 captures client proteins and binds directly to exposed hydrophobic regions of unfolded and misfolded proteins, preventing their aggregation and giving them the chance to refold in the absence of other proteins (15, 30, 86, 97). HSP70 seems to be the most conserved protein in evolution so far (97). There are highly conserved sequences in the relevant functional sections, such as the ATP-binding sites in the NH_2-terminal domain, for one. The HSP70 superfamily has additional roles in cell preservation, such as the dissolution of protein complexes, the transmission of cell-cell signals, and the transport of proteins between subcellular components and organelles (30).

PHYSIOLOGICAL RELEVANCE OF HSP70/90

Evolutionarily, HSP70/90 families are highly conserved and can be found in all cells from archaea, eubacteria, and eu-

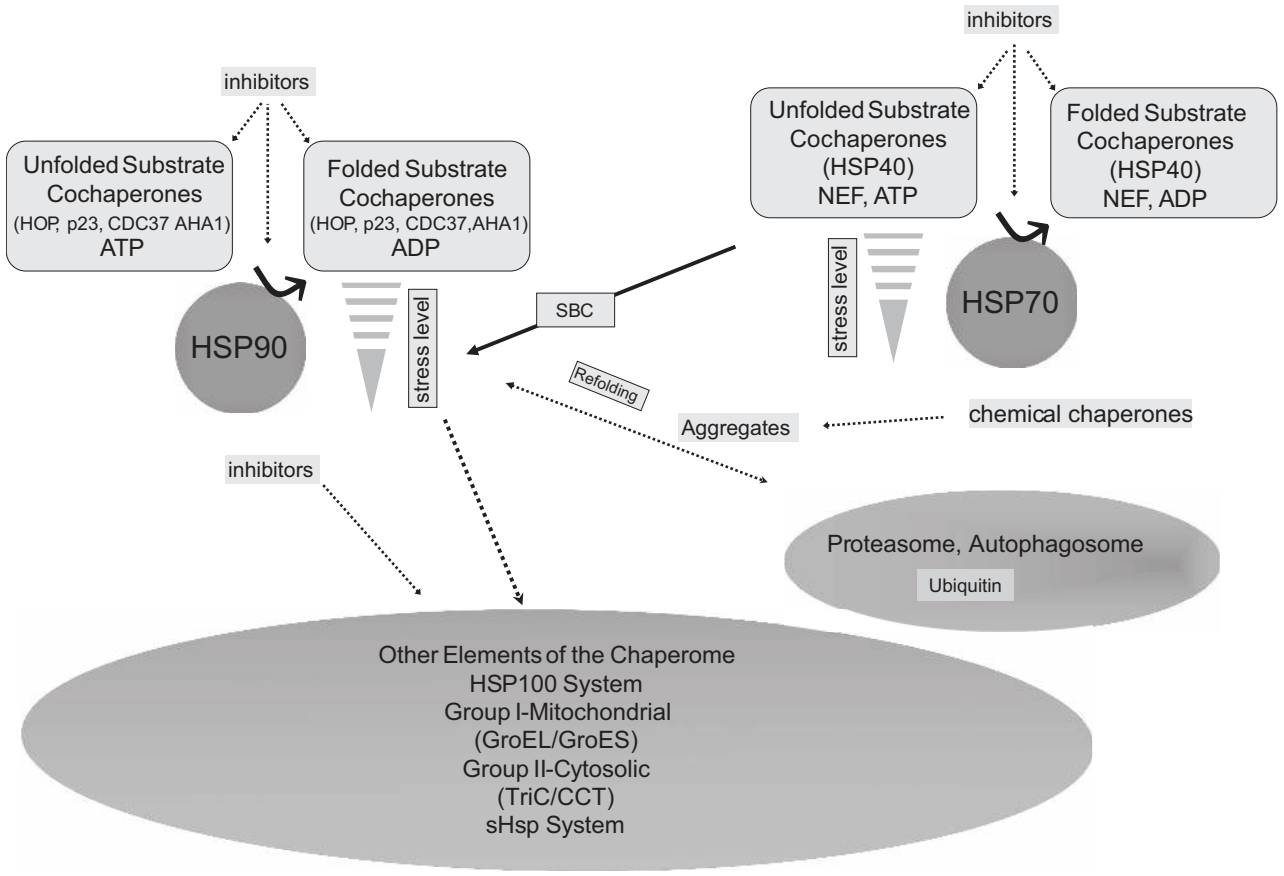

Fig. 1. Composition of the human chaperome. Stress affects expression levels of heat shock protein 90/70 with manifold consequences on the proteome; therefore there are prominent ATP-dependent stress markers in many diseases and embedded in a network of cochaperones (Hop, p23, CDC37, AHA1, HSP40). Targeting HSPs by inhibitors can stop the folding machinery directly and thereby hinder the survival of diseased cells. HOP, HSP70-HSP90 Organizing Protein; AHA1, Activator of HSP90 ATPase; NEF, nucleotide exchange factor.

karyotes (26, 40, 58, 102). In general HSP90/70 are essential for eukaryotic cells, whereas in eubacteria recent results show a dependence on environmental adaptation (58). Studies in *Saccharomyces cerevisiae* have found that 10% of all cellular proteins are directly or indirectly dependent on the proteins of the HSP90 family. They are, for one, proteins that need the chaperones to fold, stabilize, or activate but also cochaperones that regulate the functions of HSP90. The activation of the chaperoning system begins with the activation (trimerization and phosphorylation) of transcription factor HSF1 and influences basic cellular maintenance processes and molecular mechanisms like autophagy, multidrug resistance, programmed cell death, cell cycle arrest, chromatin structure, and immune response. These functions of HSF1 intervene deeply in development and physiology (31, 46, 73).

The HSP90 family can be divided into five subfamilies, four of which are present in eukaryotes and one in prokaryotes. In eukaryotes, the HSP90 genes are part of the nuclear genome, but the four different isoforms of the eukaryotic HSP90 are present in different cell compartments. In the cytosol, there are two forms, HSP90α (inducible form) and HSP90β (constitutive form). They are 85% identical and were created by gene duplication around 500 million years ago (102). The two proteins are summarized as the HSP90A subfamily and are the best-studied proteins of the HSP90 family. Under stress-free conditions HSP90 accounts for 1–2% of cytosol proteins, whereas stress can double or even triple their concentration in the cell (14). Another protein family is the glucose-regulated protein (Grp94/gp96, former Erp99), also known as HSP90B, located in the ER (also referred to as gp96), which also belongs to the HSP90 family. Except for fungi, the HSP90 family protein Grp94 is present in all eukaryotes. Grp94 supports the cell during stress and helps in breaking down misfolded proteins on the ER-associated pathway. Known Grp94-dependent proteins include insulin-like growth factor 2, immunoglobulins, and other signal transduction-mediating pattern recognition receptors (PRRs). The third subfamily contains the protein tumor necrosis factor receptor associate protein 1 (TRAP-1) (105), which protects the mitochondrion from oxidative stress. This HSP is most similar to the bacterial member of the HSP90 family, the high-temperature protein G (119). However, they are unlikely to be endosymbiotic. It seems that TRAP-1 separated early from the other three eukaryotic families and thus is very similar to the high-temperature protein G. TRAP-1 is the only representative with a unique LxCxE motif, which provides protein-protein interactions and is not found in the other members of the HSP90 family. During evolution, the proteins of the HSP90 family in eukaryotes have had a significant influence on the formation of phenotypes by genetic variations. The chaperones still have to fold proteins correctly, especially when they occur from genetic alterations of the DNA caused by stressors. In addition, HSP90s support many specific sig-

naling vectors and thus lie at the interface of many developmental pathways. In the case of great environmental stress, such as pathogen infection or mechanical injury, even HSPs become inactive and phenotypical changes occur in the organism (43). Previously hidden gene variants then come to the fore. These "disadvantaged" variants are normally not present because of HSP90 buffering. In *Drosophila melanogaster*, *Arabidopsis thaliana*, and *Danio rerio*, new phenotypes were observed after the elimination of HSP90 proteins. In the otherwise complex developmental processes, evolutionary change can thus be promoted if a new phenotype is beneficial.

IMMUNOLOGIC EFFECTS OF HSP70/90

Besides their role in maintaining the integrity of cellular proteins, HSPs have an important immunologic impact. In particular, members of the HSP70/90 family have crucial functions for inducing or balancing immune reactions and regulate acute or chronic inflammatory processes by their constitutive expression or after their intracellular or extracellular accumulation (39).

The important role of HSPs as immunologic signaling molecules became obvious after experimental administration of exogenous HSP, which induced a variety of immune functions that are currently exploited in immunotherapy of cancer, infectious diseases, and autoimmune diseases. These immune responses indicate that HSPs represent a kind of self-antigen that is present in and secreted by our own cells. Thus they are recognized by our immune system and generate proinflammatory signals. Conversely, HSPs have been shown to induce immune-regulating effects by modulating specific anti-inflammatory pathways and antiapoptotic effects (41). However, the immunologic impact of HSPs seems to depend on various factors such as the type of inflammatory response, the location of HSPs, their clearance, as well as the type of accumulated HSPs (47).

Recent studies demonstrated a strong interaction between Toll-like receptors (TLRs) and HSPs. TLRs are the critical sensors for recognition of microorganisms whose expression patterns are closely related to the immunologic function of the cells (85). On the one hand, Hsp70 is known to be a ligand for TLRs. On the other hand, all TLRs, except TLR3, are known to be client proteins of gp96. Their correct folding is dependent on dimerization of gp96 in the ER lumen. Consequently, gp96 depletion results in a loss of TLRs and unresponsiveness to TLR ligands. Knockout mice that are deficient for gp96 expression have an increased susceptibility to bacterial infection (52, 101).

HSPs and Cross-Presentation of Antigens

An important immunologic process, which is supported by HSPs, is the cross-presentation of antigens. Cross-presentation describes the ability of exogenous antigens to enter endogenous pathways of major histocompatibility complex class I molecules, followed by a priming of $CD8^+$ T cells (38). The process of cross-presentation can occur via both an endocytic pathway and a cytosolic pathway. For both pathways, constitutive expressions of HSP70 and HSP90 are required to translocate antigens into the cytosol (38). The process of peptide cross-presentation bound to HSPs represents a receptor-mediated pathway. HSP70 and gp96 have been shown to bind to several receptors, such as CD91 and LOX1, while HSP90/gp96 activate mainly scavenger receptor-A on antigen-presenting cells. Pharmacological inhibition or deletion of HSP90 abrogates antigen cross-presentation. Accordingly, HSP70 and HSP90 are specific and important regulators of antigen cross-presentation (9).

Opposing Effects of HSPs During Inflammation

In early studies, HSPs were suggested to be exclusively intracellular proteins, implying that an extracellular increase is exclusively a result of cellular damage, injury, or necrosis. These assumptions classified HSPs as "danger-associated molecular patterns" (DAMPs), which represent ligands for PRRs (3). PRRs are host sensors of the innate immune system, able to detect both molecules typical for pathogens, such as pathogen-associated molecular patterns (PAMPs), and DAMPs, which are associated with the components of the host's cells. These molecules are generally released during cell damage or death. The classification of HSPs as DAMPs implies that their signaling is distinct from the presence of pathogens, certainly also activating pathways of the innate immune response (8).

Later studies proved that HSPs are not only passively released but also actively secreted into the extracellular environment by various cell types, such as necrotic cells, lymphocytes, natural killer cells, and tumor cells, via exosomes (25, 59, 65, 71). In particular, for proteins of the HSP70 and HSP90 (gp96) families, active signaling to stimulate the innate immune response has been proven (99).

A variety of immune cells, e.g., dendritic cells, natural killer cells, and macrophages, can detect HSPs, like HSP70, via CD36, CD40, and CD91 surface receptors. Interestingly, HSP70 is also recognized by T cells through T-cell receptors during a presentation with major histocompatibility complex molecules (99, 111). For HSP90/gp96 chaperones, it has been shown that they interact with TLR2 and TLR4 molecules that can induce the activation of the NF-κB pathway, followed by the production of a variety of cytokines, such as IL-12 and TNF-α. This activation process is induced by the COOH-terminal peptide-binding region of HSP70 (49, 117). In most cases, HSP binding to one of these receptors induces the initiation of an activating, proinflammatory signaling cascade, followed by the initiation of nonspecific cytokine and chemokine production and, in parallel, upregulation of costimulatory molecules (Fig. 2) (2). Similar processes have also been shown after treatment of THP-1 cells with mycobacterial HSP70 (49, 117). However, there is also some evidence for differential regulation processes after treatment with mycobacterial HSP70 and human HSP70. These differences might be explained by different binding regions at CD40 on macrophages and dendritic cells (6). Whereas mycobacterial HSP70 seems to exclusively induce proinflammatory signals, mammalian HSP70 not only activates but also regulates the immune response by inducing anti-inflammatory signals. However, some researchers have argued that there was a complication in initial studies regarding the copurification of lipopolysaccharide as a contaminant in preparations of recombinant HSPs (110). This discussion remains controversial because in vitro studies with highly purified HSP molecules might not represent a suitable in vitro model for the complexity of HSP activities in a living organism. However, current studies suggest that HSPs might have

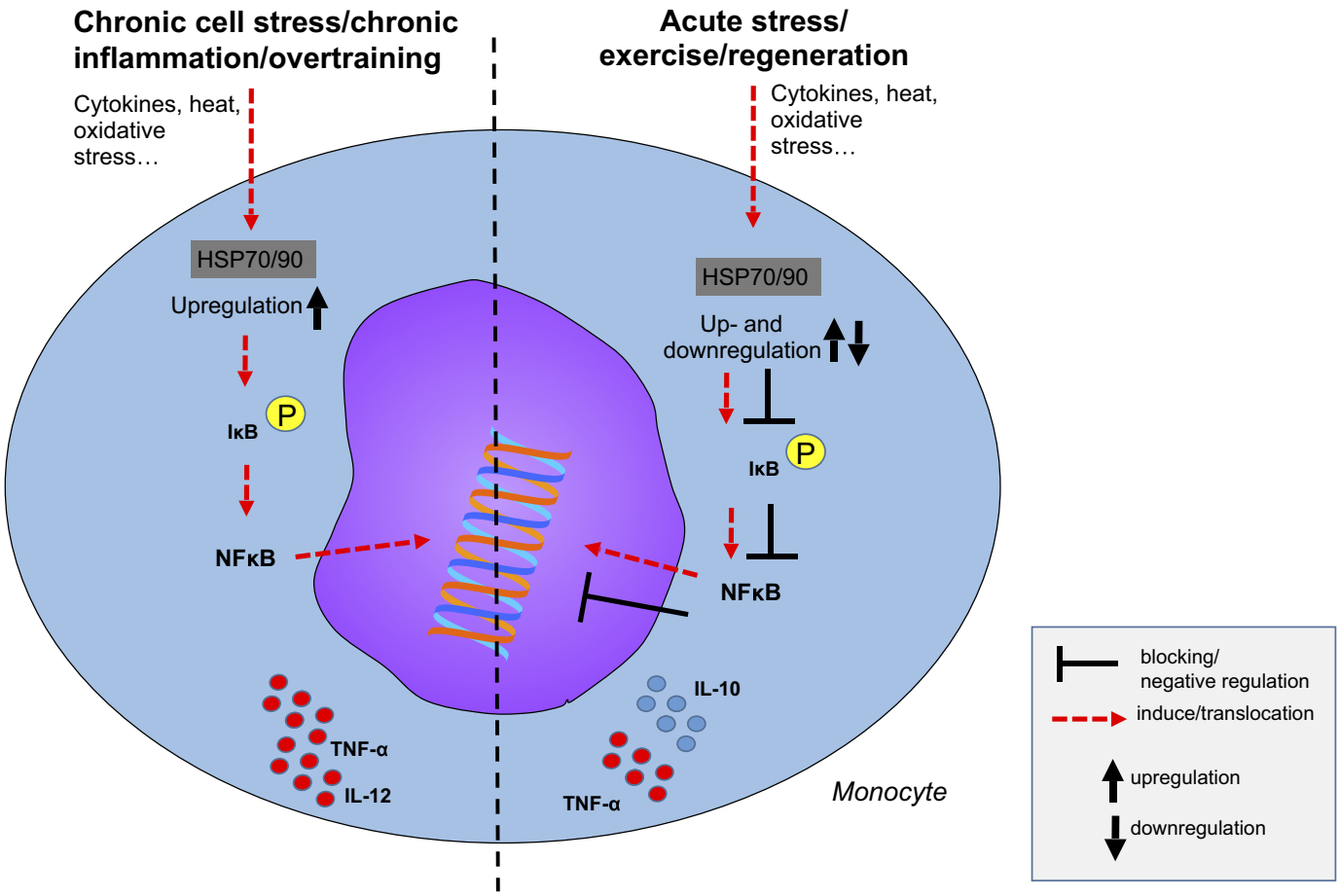

Fig. 2. Effects of chronic cell stress compared with conditions of acute stress on heat shock protein (HSP)70/90 regulation and inflammatory signaling pathways in monocytes. IκB, inhibitor of κB; NF-κB, nuclear factor-κB; P, phosphorylation.

both immune-stimulating and anti-inflammatory effects. These opposing roles are rationalized by the complexity of inflammatory processes, which have to be tightly regulated. Meanwhile, the production of alarm signals sharply diminishes after the pathogens or cellular debris has been eliminated and tissue homeostasis is restored (96). This may depend at least partly on the cellular location of HSPs. Whereas extracellular HSPs might serve primarily as danger signals, stimulating the immune response, intracellular HSPs serve as immune regulating factors (92).

These findings are supported by studies demonstrating that intracellular HSP70 accumulation induces the inhibition of NF-κB activation, something that has a profound implication for immune activation and inflammation. It was shown that HSP70 inhibits the phosphorylation of inhibitor of κB. Other studies demonstrated that tumor-derived exosome-associated HSP70 activates the mitogen-activated protein kinase cascade, one of the most ancient and evolutionarily conserved signaling pathways (17). In detail, it was found that HSPs mediate a suppressive activity of the myeloid-derived suppressor cells by activation of STAT3 and ERK, followed by IL-10 production (13, 90). IL-10 secretion is one of the central mechanisms proposed for the immune-regulatory function of HSPs (39).

There is also some evidence indicating that the immunologic effects of HSP70 or HSP90 also depend on the inflammatory milieu because HSPs are suggested as targets in chronic inflammatory diseases such as rheumatoid arthritis (115) and atherosclerosis (118). Elevated levels of HSP70 and gp96 have been found in synovial fluids from inflamed joints in rheumatoid arthritis patients and in arteriosclerotic lesions (61, 94). Accordingly, during situations of chronic or uncontrolled inflammation, HSP accumulation might intensify pathological processes by stabilizing proteins that support or induce pathological processes. These situations are characterized by chronic HSP signaling, something that might evoke completely different effects than during acute or short-term HSP increase. In contrast, during conditions of acute cellular stress, e.g., heat shock or exercise, HSP induction primarily controls immune processes, followed by a downregulation of inflammation and HSP concentration (Fig. 2) (11).

HSP70/90 RESPONSE TO EXERCISE

Exercise is a physiological stress factor accompanied by various inherent physiological alterations known to increase HSP70 and HSP90 concentration. Oxidative stress, modifications in temperature, pH, and ion concentrations, decreases in calcium concentration and intramuscular glycogen, disturbed membrane integrity, as well as glucose deprivation during and after exercise provide conditions of instability and consequent homeostatic imbalance. Also, tissue hypoxia, which presents a condition of lower than normal oxygen content and pressure in the cell, might be a trigger of HSP expression (10, 55).

Consequently, an increase of HSP70/90 was described in plasma and mononuclear cells and various organs and tissues such as muscle, liver, cardiac tissue, and the brain after an acute bout of exercise (5, 70, 98, 100) (Fig. 3). Specifically, the HSP70 response seems to strongly depend on the mode of exercise. Duration and volume of exercise, exercise type and intensity, subjects' training status, and environmental factors such as heat have been shown to affect the extent of HSP upregulation after exercise. Most studies used endurance exercise protocols and demonstrated that higher intensities seem to more strongly upregulate HSP70 production in plasma and muscle compared with activities of moderate intensity (53, 68). However, Walsh et al. (2001) proved that endurance exercise of moderate intensity also results in a significant increase in the concentration of circulating HSP70 in humans (116). In other studies, it was shown that prolonged exercise induced a more pronounced response of serum HSP70 than shorter or more intensive exercise bouts (24). A correlation was shown between preexercise HSP values and the increase in HSP70 after exercise in human muscle biopsies measured over 5–8 wk of exercise (29). After periods of intensified training, higher basal levels of HSP70 were found, which could reflect an accumulation of HSPs in leukocytes after several subsequent bouts of exercise (23). This finding is supported by the findings that HSP70 is specifically elevated after periods of intensive exercise with only short periods of recovery in muscle tissue (54). Similar observations were made in studies with rowers, where a progressive accumulation of HSP70 was found in skeletal muscle of well-trained rowers after 3 wk of high-intensity strength training (53). During a subsequent week of recovery, HSP70 values decreased to baseline values. Chronically elevated basal levels of HSP70 were found in cardiac tissue of trained mice. Interestingly, in these mice an acute bout of treadmill running did not induce a further increase, suggesting a kind of preconditioning in cardiac tissues after training (64). In contrast, lower basal HSP70 concentrations were shown within peripheral blood mononuclear cells of trained subjects, which might also reflect an adaptation to regular exercise (23). Accumulated HSP70 in muscle is suggested to work as a negative-feedback regulator for the inducible transcription of HSP70 genes. This might be an important mechanism to secure a controlled regulation of stress proteins and to maintain the capacity for an efficient recovery. Accordingly, endurance athletes might have—except in cardiac tissue—lower HSP concentrations in tissues compared with nontrained individuals, although increases in HSP were still observed in response to acute exercise (23). It was also proven that heat stress during exercise, e.g., during running in a hot environment, amplifies HSP70 concentration in muscle, cardiac tissue, and mononuclear cells, suggesting that external heat is an additional stress factor (98). Furthermore, the type of exercise affects HSP concentration. Although endurance and resistance training seem to induce HSP70 expression similarly, eccentric exercise after downhill running has been shown to be more efficient in

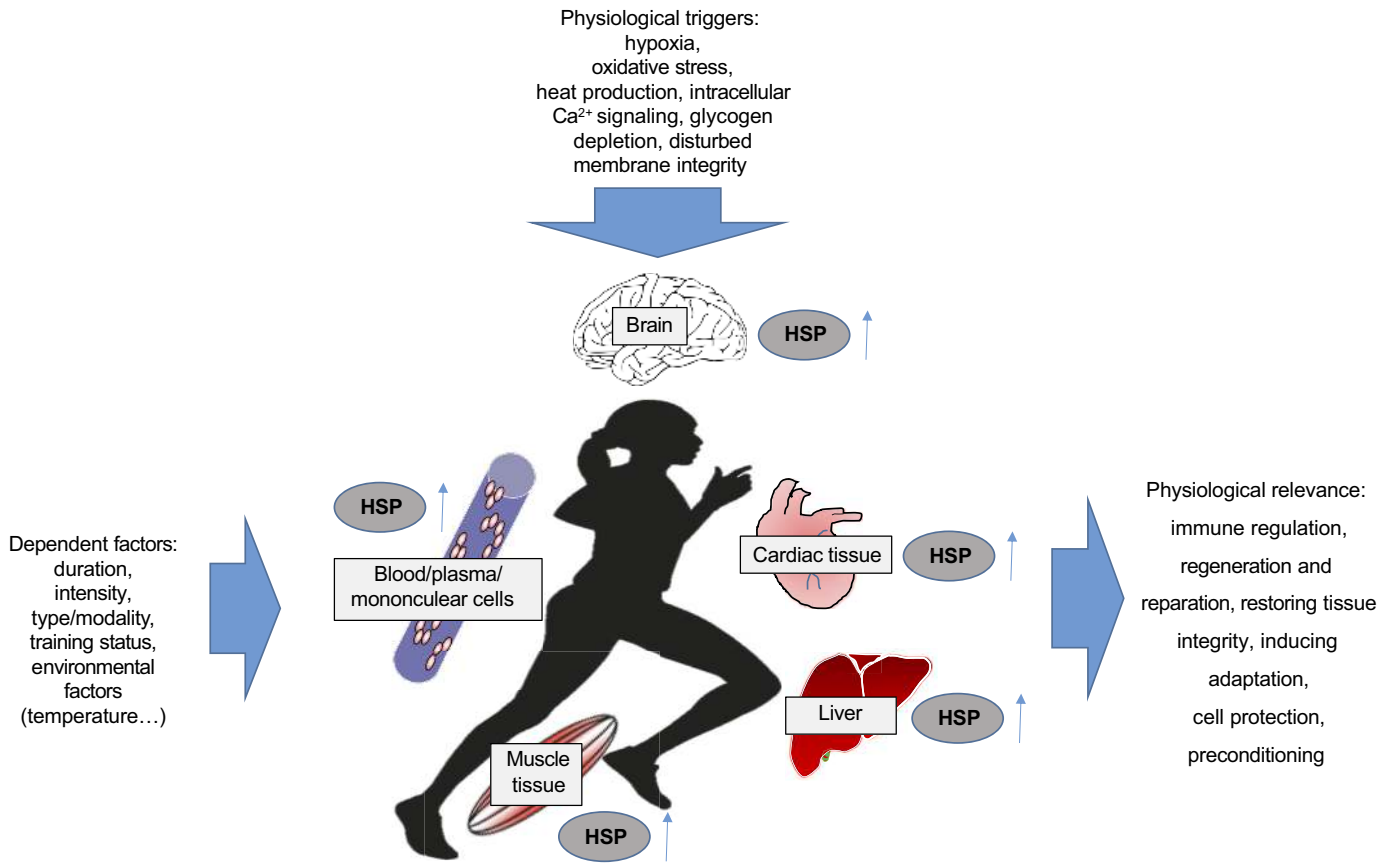

Fig. 3. Overview of increased expression of heat shock proteins (HSPs) in different organs and tissues during acute exercise and their physiological triggers, exercise-dependent factors, and potential physiological relevance.

raising the HSP70 response in muscles of rats than horizontal running (57).

Mechanisms of HSP Expression During Exercise

In response to acute exercise, transcriptional expression of the HSP70 gene is regulated primarily through HSF1. After activation, HSF1 trimerizes and translocates into the nucleus and binds to the promoter region of the HSP70 gene. In parallel, exercise-induced activation of the downstream adrenergic receptor-mediated signaling kinase (protein kinase A) occurs, which inhibits ERK1/2 activation and therefore is permissive to increase HSP70 concentrations.

Since acute and chronic exercise elicit several physiological alterations that might mediate HSP70/90 induction, more than one factor of homeostatic imbalance is suggested to affect HSP concentration. Increase in body temperature seems a rather obvious signal to induce the expression of HSPs. During exercise, core body temperature in humans can reach ~40°C during heavy exertion (88). That heat production is certainly a contributing mechanism for HSP expression during exercise, which is supported by the pronounced expression during physical activity in a hot environment. However, in some tissues, such as skeletal muscle, an increase in HSP70 concentration was found without any thermal stimulus. Accordingly, the mechanisms of HSP70 expression might be tissue specific (70, 98, 100). Studies on HSP70 expression in muscles and liver provided evidence that increased oxidative stress was associated with increased HSP70 concentrations (89). The increased production of reactive oxygen species during exercise leads to nonspecific reactions with cellular components bringing about damage to proteins, lipids, and nucleic acids. In muscle cells, several enzymatic defense mechanisms, which involve superoxide dismutase, glutathione peroxidase, and catalase in addition to nonenzymatic antioxidants, are active to prevent oxidative stress. In parallel, synthesis of the protective HSP70 starts to maintain myofibrillar integrity and stabilizes cellular proteins (108). In skeletal muscle, HSP70 concentration was positively associated with compromised glucose concentrations (22). The relevance of substrate availability for HSP70 levels is supported by a set of data demonstrating a blunted HSP response after supplementation of glucose (22).

It was also demonstrated that hypoxia is a trigger of HSP expression (10, 55). In this regard, it was shown that the activity of both HSPs and hypoxia-inducible factor-1α increased after second heat or hypoxia. This upregulation of both factors in response to different stressors is designated as a process of cross-tolerance. Thus HSPs lead to a kind of preconditioning that aims to confer tolerance to subsequent different cellular stressors. In line with these findings, a repeated bout of exercise has been shown to increase the level of endogenous HSP70 as well as muscle HSP70 but to a lesser extent than after the first bout. Interestingly, before the second bout of exercise, basal levels of HSP70 were decreased compared with the levels before the first bout (21, 60, 107).

Another trigger of HSP70 expression in muscles seems to be elevated levels of intracellular Ca^{2+}. Cell culture experiments using muscle cell lines showed an increased HSP70 expression concomitantly with action potentials and membrane polarization after electrical stimulation. This effect was blocked in the presence of Ca^{2+} inhibitors, suggesting calcium signals as the main trigger (42).

Role of HSPs in Exercise Physiology

HSP70/90 expression and secretion during exercise might have multiple functions. The release of HSP70 into the bloodstream might be a danger signal induced after exposure to physiological stress. Possibly, it is aimed at supporting immunologic processes associated with regeneration and reparation of tissue damage, restoring tissue integrity, or inducing adaptation (12). Some researchers speculate that increasing levels of peripheral HSP70 molecules participate in fatigue perception. For intracellular HSPs, a protective role against injury, such as ischemia-reperfusion damage, is supposed, which comprises the immune-regulating and antiapoptotic potentials of HSP70/90 (34, 93). Senf et al. (2013) found that extracellular HSP70 has a role in muscle repair and fiber adaptation by restoring the recruitment of muscle cells involved in the inflammatory response (95). The expression of HSP70 in certain locations might have protective effects against the death of motor neurons and muscle cells (77). In studies by Paulsen et al. (2007), an association was found between muscular force generation and HSP70 levels. Probably, HSPs' function as stabilizers of disrupted myofibrillar structures is to contribute to the reparation and adaptation processes by refolding of denatured proteins and folding of newly synthesized proteins (80). The authors also speculated that HSP70 blunts inflammatory processes in muscle, something that might support regeneration processes. It is also speculated that HSP production contributes to long-term adaptations by preventing muscle damage after repeated bouts of eccentric exercise ("repeated bout effect"). This effect describes the phenomenon that plasma CK was elevated after exercise at the beginning of conditioning programs and decreased in response to a repeated bout of exercise (10).

With regard to the preventive and therapeutic effects of exercise, animal experiments suggest an important role of HSPs for exercise preconditioning and prehabilitation. For example, preconditioning processes induced an increased HSP70 concentration in cardiac tissue, which reduced myocardial infarction and ischemia-reperfusion injury and increased survival time during heatstroke in rats (36, 37, 56, 82). Accordingly, HSP expression is suggested to be a mechanism that may at least partially account for the preventive effect of exercise (7).

BIOMARKERS IN EXERCISE PHYSIOLOGY

The early detection of biomarkers is very important in the cases of cancer, cardiovascular disorders, and other pathological conditions but also for monitoring normal physiological processes with the aim of analyzing stress levels and understanding stress limits. Biomarkers in sport physiology help to monitor training success and analyze nutrition and metabolic health, hydration status, muscle status, endurance performance, injury status, and inflammation (5). These intrinsic data help to structure training processes and should be underpinned by high accuracy and precision. Thus these markers should give a comprehensive and powerful understanding of individual physiological balance (35, 103). However, these biomarkers have appropriate evidence supporting their use but also have crucial

limitations (32). CK as a popular recovery marker mirrors muscle fiber damage and shows increased values in studies with intensive exercise bouts. However, measurements of this biomarker observe large intraindividual and interindividual variabilities, and a poor temporal relationship with muscle recovery exists (91, 114). Furthermore, biochemical, hormonal, and immunologic biomarkers such as blood lactate, inflammatory cytokines, cortisol, or C-reactive protein have an immense fluctuation width and are questionable as to providing valid and reliable data on fatigue and recovery parameters (44, 48, 109). These value variabilities depend on factors like age, sex (74), time of day (33), and individual training status (78). From the results in many studies, it can be deduced that no single parameter could represent an adequate sensitive and reproducible fatigue/recovery status of athletes (35). Studies that were carried out with multiple biomarkers indicate that combinations are a better predictor to monitor fatigue/recovery processes than a single marker (104). Thus it is necessary to identify more universal and consistent biochemical training markers of multiple aspects of athlete health and performance.

HSP70/90 as Potential Biomarkers to Monitor Training

HSP70 and 90 might represent some of these promising biomarkers for reflecting levels of exercise stress, state of systemic recovery, and long-term training load (63). Both markers manifest high values after acute exercise, correlate well with fatigue status, and might be predictors for the efficiency of regeneration (113).

A high sensitivity of serum HSP70 has been shown in particular for the process of regeneration. Accordingly, during recovery from exercise several physiological processes occur, including repair processes of damaged tissues, downregulation from inflammation, filling up energy stores, and decreasing oxidative stress, all of which affect HSP70 concentration (58, 74) Correspondingly, HSPs seem to be a suitable marker to monitor the progress and time frame of regeneration. Following this idea, researchers suggested that HSP70 represents a signaling molecule for exercise-induced fatigue (10). Otherwise, chronically elevated levels of HSPs implicate a lack of recovery and thus are a kind of precursor for pathophysiological states, such as overtraining. In this regard, other studies support the idea that overreaching or overtraining can be mainly defined by an inability to recover (58). A chronic increase of HSPs might reflect a reduced space for a further upregulation in response to an additional acute stress event. This assumption is supported by data from patients with chronic fatigue syndrome, in which acute HSP70 responses are suppressed (10). Consequently, it is speculated that the reduction of HSP concentration during physiological regeneration creates space for newly applied acute stress events (Fig. 4). Considering the clinical relevance and the key role in physiological and pathobiochemical adaptations, it becomes obvious that HSP70/90 emerge as a potential biomarker for monitoring not only exercise but specifically the course of recovery.

Classical Diagnostic Strategies and Innovative Detection Options

The use of HSPs as a biomarker in tissue and bodily fluids, such as blood and urine, requires techniques for easy and fast detection. Newer results indicate that HSP90α is a good biomarker for liver cancer in the early stage with an ELISA technique for detection (28). This method might also be employed to evaluate limits for endurance exercises as well as for personalized records. The main problems are the detection limits, sensitivity, and artificial quenching by foreign compounds, e.g., albumin, etc. (81, 112). To circumvent limitations and provide the highest reliable sensitivities, biomarkers need to be identified by different techniques. In general, the presence of HSPs can be monitored by immune blots, ELISA, mass spectrometry, surface plasmon resonance or protein microarrays, and also Raman-based detection systems (75). ELISA and related systems use the affinity of antibodies or aptamers as detection sensors while the signal is enhanced and detected electrically or optically. Because of the high miniaturization grade, protein microarrays are a powerful tool in monitoring the presence of HSP biomarkers, using a known fixed probe (antibody, aptamer, ligand) against unknown multiple samples (69). These technologies are under continuous optimization and, in some cases, can detect picograms of biomarkers. Required are innovative technologies that detect physiological and simple analytic biomarkers such as DNA, microRNA, protein, peptides, as well as small molecules rapidly during endurance; most of these have been endorsed by the US Food and Drug Administration and explored for the improvement

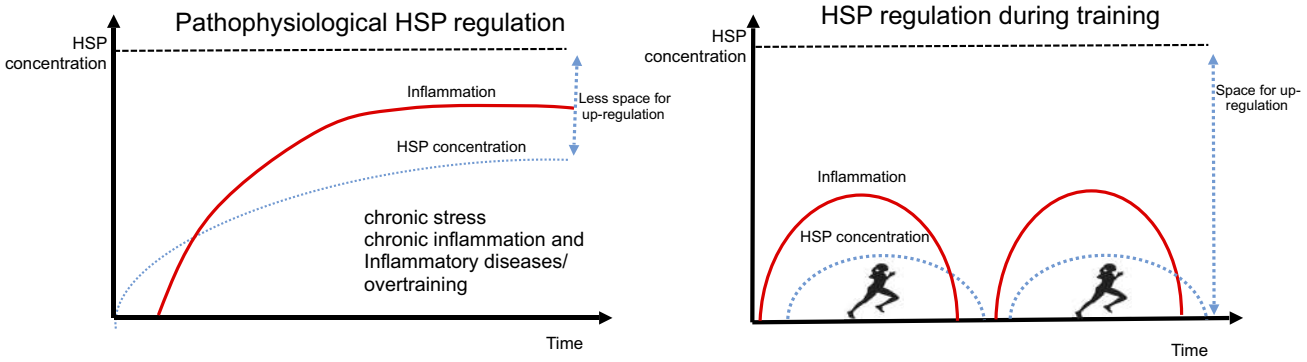

Fig. 4. Concept of heat shock protein (HSP) regulation during pathophysiological conditions (e.g., chronic stress) and during exercise training. During a condition of chronic stress, HSP concentration progressively increases, which supports a condition of chronic inflammation. The body loses its ability to downregulate HSP concentration in response to internal or external stressors. During conditions of regular exercise training, HSP concentration is up- and downregulated, indicating a stress and recovery process. Accordingly, a downregulation of HSPs is suggested to create "space" for an effective upregulation during additional or repeated stressful stimuli.

and cost reduction of health care services. These technologies comprise a ~664 noninvasive molecular biomarkers and the 592 potential minimally invasive blood molecular biomarkers available for monitoring, diagnostic, or theragnostic purposes (83). Technologies like microfluidics or microelectrical sensors for protein biomarker detection can be developed for personalized applications as well as for point-of-care diagnostics of disease markers, which in future could provide reliable and low-cost sensors for monitoring physiological status (1, 79).

PERSPECTIVES

Current knowledge suggests that both HSP70 and HSP90 might have important functions for cytoprotection, immune regulation, regeneration, and adaptation processes during exercise and training. Considering their sensitive and individualized response to various physiological changes during exertion and regeneration, these proteins can be considered as biomarkers to identify the specific response of an individual to exercise and additional stress factors and for monitoring stress/recovery cycles during training periods in athletes. In this framework, specifically the capacity for a controlled downregulation of HSP concentration might be an important physiological signal, since pathophysiological, immune-dysregulated, or overstrained systems seem unable to regulate HSP expression back to baseline level (65). Accordingly, HSP70/90 expressions may also be considered as potential markers for the early detection of overtraining syndromes in athletes. Similarly, the induction of these proteins in different tissues and cell types after exercise seems to be a physiological mechanism that mediates some of the preventive and therapeutic effects of regular exercise for the treatment of various diseases. Concurrently, their expression might be a suitable marker for finding an effective dosage to calculate exercise duration, intensity, and frequency in patients with differential exercise capacities.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.K. and C.Z. analyzed data; C.Z. interpreted results of experiments; K.K., T.R., and C.Z. prepared figures; K.K., T.R., and C.Z. drafted manuscript; K.K., T.R., and C.Z. edited and revised manuscript; K.K., T.R., and C.Z. approved final version of manuscript.

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