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CHANGES IN SKELETAL MUSCLE HEAT SHOCK PROTEINS: PATHOLOGICAL SIGNIFICANCE

Yuefei Liu and Jürgen M. Steinacker

Department of Sports and Rehabilitation Medicine, University of Ulm, Steinhövelstr. 9, D-89070 Ulm, Germany

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1. ABSTRACT

In response to stress, cells rapidly produce a series of new proteins known as heat shock proteins (HSP). HSPs are considered to be molecular chaperones which play a universal role in maintaining cellular homeostasis. It is known that different HSPs are expressed in skeletal muscle, namely, small HSPs (including ubiquitin, alpha B-crystallin, HSP20 and HSP 27), HSP70, HSP60 and HSP90. Skeletal muscle is a complex and heterogeneous system in that its contractile proteins are made of different isoforms to form various muscle fibre types, and each type of muscle fibre has its own histochemical and functional characteristics. It seems that the induction of HSPs differs with muscle fibre type suggesting HSP expression is muscle fibre type specific. HSPs have been shown to respond in muscle diseases and following exercise. However, the molecular mechanisms of HSP induction, regulation and its role in maintaining the muscle function, are not completely understood. Relatively few studies of HSP have been conducted in human skeletal muscles. This review discusses the significance of changes of HSPs in skeletal muscle in both physiological and pathological conditions.

2. INTRODUCTION

Nearly four decades have elapsed since the heat shock response was first reported by Ritossa in 1962 (1). It is now well known that cells repond to heat shock or other stresses with a rapid synthesis of a small number of proteins, which are now called heat-shock proteins (HSP) or stress proteins (2-4). The HSP response is considered to be a highly conserved characteristic of cells (5). To date nearly all eukaryotic cells examined have been found to be able to produce HSP (6), and a high correspondence of HSP amino acid residues between different species has been identified (7-9). This highly conserved characteristic suggests a universal role for HSP in the response to cellular stress.

Though HSP has been extensively studied in the last decade (10-12), the function and mechanism of action of HSP has yet to be completely elucidated. Two established essential functions of HSP are thought to be its protective effects against stress and molecular chaperone action. A number of studies have shown that HSP confers protection against cellular stresses including hyperthermia, hypoxia, ischemia and reperfusion, which would otherwise lead to cell death (13-19). As a molecular chaperone, HSP plays an important role in facilitating protein synthesis, folding and assembly as well as in environmental adaptation and organism development (20-25).

Skeletal muscle is a quite heterogeneous system with regard to the constitutional proteins and energy metabolism of its isoforms (26). One of the most important functions of the skeletal muscle is to facilitate movement of the body, which can cause a variety of tissue changes (27), including HSP induction (4, 28, 29). A number of studies have reported that HSP is induced and upregulated in skeletal muscle in response to stress (30-35). Different HSPs may be expressed in skeletal muscle, which may have significant effects on muscular function, adaptation, or myopathy. This review discusses changes in skeletal muscle HSPs in terms of tissue physiology and pathology.

3. SKELETAL MUSCLE HEAT SHOCK PROTEINS

In response to stress, cells synthesize HSP to the extent that the latter may constitute up to 20% of the total cellular protein (36). There are many HSPs which can be expressed in the skeletal muscle (10); these range from 8 to 110 kDa in molecular mass, and can be divided into several groups based on their size and function. The most prominent HSPs in skeletal muscle are the small HSPs, HSP of 70 kDa (HSP70) and HSP of 60 kDa (HSP60) or 90 kDa (HSP90).

3.1. Small HSPs

Ubiquitin is probably the smallest HSP (molecular mass approx. 8 kDa) (37) which is reportedly expressed in human skeletal muscle (38). Ubiquitin is a highly conserved protein that plays a role in both chromatin structure and in protein degradation. Many intracellular proteins that are to be degraded are first covalently modified by the addition of ubiquitin. The increase in ubiquitin levels after stress presumably facilitates the targeting and removal of proteins denatured as a consequence of the stress event (39). The 20 kDa HSP (HSP20 or p20) has been shown in rat muscle, constituting up to 1.3% of the total celluar protein in the muscle (25), and its expression is considered to be related to muscle contraction, specifically in slow-twitch muscles (40).

Extensive investigations have shown that HSP27 is expressed in human skeletal muscle, with a homologue expressed in rat muscle (24, 41-45). HSP27 is localized in the cytosol under unstressed conditions, and translocates inside the nucleus during stress. This protein is expressed at relatively low levels in normal cells, but exhibits a 10- to 20 fold induction in the cell after stress. The major functions of HSP27 include stabilization of microfilaments, and cytokine signal transduction (20).

Another important small HSP in skeletal muscle is alpha-crystallin. There are two types of alpha-crystallin, i.e. alpha A and alpha B (46). Alpha A is specifically expressed in the lens, whilst alpha B is expressed constitutively in many different tissues, and can be induced by physiological stress. Both the alpha- crystallins share the same gene, which is known as a gene sharing effect (47). Alpha B-crystallin also shares structural homology with HSP27 (48), and can serve as a molecular chaperone, either to prevent aggregation of denatured proteins, or to facilitate protein refolding upon the removal of cellular stress (49, 50). Therefore, alpha B-crystallin may play an important role in muscle function (44, 45, 51) and development (24).

3.2. HSP70

The HSP70 family may be the most abundant HSP induced in cells in response to stress. For example HSP72, an inducible form of HSP of molecular mass 72 kDa, can consititute up to 20% of the total cellular protein after appropriate stimulation (13). Therefore, the HSP70 family has been the most widely studied of HSPs within the heat shock field. Four major forms of HSP70 have been identified in skeletal muscle. The proteins with a molecular mass of 75 kDa and 78 kDa are respectively termed glucose-regulated proteins (GRP75 and GRP78) since they are not specifically induced by heat shock, but by glucose deprivation, calcium influx or agents that perturb glycosylation (4). GRP78 is constitutively expressed in the sarcoplasmic/endoplasmic reticulum (52), and may have a general role in the assembly of secretory proteins (53). GRP75, a mitochondrial specific isoform (54, 55), is involved in the translocation of precursor proteins across mitochondrial membranes, and their subsequent stabilization and folding within the mitochondria (56, 57).

The two most extensively studied proteins in the HSP70 family are HSC73 and HSP72 (58). HSC73, a heat shock protein of molecular mass 73 kDa, is synthesized in most cells and is only slightly inducible (59). It is constitutively expressed in the cytoplasm under unstressed conditions, and migrates to the nucleus and nucleolus during stress (60). HSC73 can bind with denaturing or unfolding pre-ribosomes, possibly facilitating renaturation (61). It is evident that the absence of HSC73 slows down ribosome translocation, thus elongating the rate of protein synthesis (62). In addition, it is possible that HSC73 can affect muscle energy metabolism (63).

In contrast to HSC73, HSP72 is present in low quantities in unstressed cells, and is thought to be principally stress-inducible (64-66). In response to stress, HSP72 can be rapidly synthesized in the cytoplasm, migrating to the nucleoli where it may bind to proteins or other structures (67). It has been suggested that the cytosolic form of HSP70 is an important factor facilitating the early steps of protein maturation (58), since it was observed that a large number of newly synthesized proteins could be isolated complexed with HSP70. Such interactions appear transient, because after 15-30 minutes, the newly synthesized proteins were no longer found complexed with HSP70. These interactions of HSP70 with newly synthesized proteins seem to take place as the target proteins were undergoing ribosomal synthesis (58). A number of studies have reported HSP70 induction in skeletal muscle (29, 33, 45, 68). The HSP70 response to heat or other stresses seems to be not only organ or tissue-specific (69, 70), but also muscle fibre type specific (71-74). The protective role of HSP70 has been well-documented, and interestingly, a hyperthermia induced HSP70 can confer protection against myocardial ischemia, suggesting that the protection of HSP70 is possibly provided in cross-protection manner (17).

There are many common physical properties of HSC73 and HSP72, suggesting similar biological functions (75, 76). It has been reported that HSC73 acts during unstressed conditions whilst HSP72 is synthesized to meet the increased demands during episodes of stress (77,) although these HSPs may have distinct physiological effects (75).

3.3. HSP60 and HSP90

Skeletal muscle is capable of producing HSP60 in response to stress (33, 78, 79). HSP60 is thought to be localized in mitochondria. There is now evidence that HSP60 is synthesized in a precursor form in cytoplasm,

and then translocated into the mitochondria, where it is processed into its mature form (54). HSP60 facilitates the folding and assembly of proteins as they enter the mitochondria, and stabilizes preexisting proteins under stress (80, 81). Newly synthesized mitochondrial proteins have been shown to interact and to coprecipitate with HSP60. Because of its chaperone-like activity HSP60 has been termed a molecular chaperone, or chaperonin (82). Like GRP75, HSP60 is also constitutively expressed in muscle in proportion to the mitochondrial content, and is increased after chronic electrical stimulation (33). Recently, it was shown that HSP60 and GRP75 increased in the skeletal muscle after prolonged endurance training, corresponding with increased mitochondrial enzyme activity (83).

HSP90 is a family comprised of three proteins: two closely related cytoplamic isoforms, i.e. HSP90*a* and HSP90*ß*, and a glucose regulated protein (GRP94). HSP90 has been identified in the cytosol, nucleus, and endoplasmic reticulum (20), and is reported to exist in many kinds of cells (84-86). Another important property of HSP90 is the binding of unoccupied steroid hormone receptors (87), including estrogen, progesterone, glucocorticoid, and androgen receptors (87-89). HSP90 may play an important role in regulating the activity of hormone receptors; in the absence of the hormone, HSP90 is thought to bind to the receptor and maintain it in an inactive form. On hormone presentation, the receptor-HSP90 complex dissociates, allowing receptor binding to DNA.

4. CELLULAR MECHANISMS FOR HEAT SHOCK PROTEIN INDUCTION IN SKELETAL MUSCLE

The various functions of HSPs in the muscle cell as molecular chaperones and regulating proteins are of relevance: (i) to the basic functions of the cell in terms of protein synthesis, protein maturation and degradation; (ii) to specific exercise-related functions relating to increased metabolism-related changes in protein function and synthesis; (iii) exercise intensity and general stress-related mechanisms including hypoxemia and ischemia. Until recently, only a few studies have dealt specifically with inducers of HSPs in human skeletal muscle. More data exist for mammalian skeletal and cardiac muscles, and many data are derived from mammalian cell and tissue models which to some degree do not reflect physiological conditions. Major cellular inducers of HSPs which may be imposed on the muscular cell during exercise and training, hypoperfusion, systemic hypoxaemia and ischemia are depicted in Table 1 (10, 21, 90-92).

4.1. Principles of heat shock protein induction in skeletal muscle

The induction of HSPs is a process which has to enable HSPs to chaperone intact or damaged proteins, and newly produced polypeptides in different cellular compartments. Since HSPs ensure survival under stressful situations that would otherwise lead to serious cell damage and possibly to cell death, the sensing of actual cell stress is a vital function.

The induction of HSPs can be accomplished with remarkable speed. With exposure to hyperthermic stress in most organisms, HSPs are the most dominant proteins to be produced in the cell (6). The typical regulation of production of various HSPs defines their class of proteins, since the sometimes dramatic increase in their production rate is regulated from a specific DNA sequence, named the heat shock element (HSE), located upstream from the coding regions of various HSP genes (92, 93). HSE is a highly conserved element including typical 5´-nGAAn-3´ sequences, which often exist in multiple copies. The HSE is a binding site in the promotor region for a specific protein known as heat shock transcription factor (HSF). HSP regulation is a typical example of inducible promotor gene regulation(21, 91, 93, 94).

HSPs like glucose regulated proteins (GRP) and other chaperones are induced by different, mainly metabolic stressors and provide less (or no) protection against thermal stress. The regulation of GRPs does not involve an HSE in the promotor region of the respective gene. GRPs are induced in cells subjected to glucose-depletion, hypoxia, calcium ionophores, calcium flux or to glucose analogues (10, 90).

At least two different mammalian HSF genes have been encoded, HSF1 and HSF 2. They differ substantially (62%) at the nucleic acid level (94, 95). DNA binding and trimerization seem to be similar (94). HSF1 is activated by several stressors like heat, oxidative stress and denatured proteins, whilst HSF 2 seems to regulate protein expression and cell differentiation. The expression of HSF1is itself constitutive and not stress inducible (90, 94, 96). Two isoforms (termed a and ß) of HSF1 and HSF2 can be generated by alternative splicing in a tissue-dependent manner (97).

4.2. Activation of heat shock factor

In unstressed cells, HSF1 is present in the protoplasm and nucleus in a monomeric form that has no DNA binding activity. After exposure to heat shock or other physiological stresses, HSF1 undergoes a conversion to a trimeric state and aquires DNA-binding activity (HSF activation). HSF then accummulates in the nucleus and binds to the HSE. In addition, HSF is subject to stress-dependent phosphorylation (91, 98).

The activation of HSF, e.g. HSF1, is related to temperature (heat shock), hypoxia, metabolic inhibitors, ATP depletion, pH changes, oxidative stress and exercise (Table 1), although the mechanisms of the regulation are far from being completely understood. It has been suggested that HSP themselves may negatively regulate HSP gene expression via an autoregulatory loop. This is demonstrated in Figure 1 (21, 90, 91).

HSP70 is thought to bind on HSF1, since an increased level of denatured and misfolded proteins is correlated with an increased HSP70 transcriptional response. Furthermore, HSP70 transcription is blocked by incubation with protein synthesis inhibitors, suggesting that misfolding or aggregation of nascent proteins regulates the response (92). During episodes of stress the accumulation of misfolded or damaged proteins competes with HSP70 from HSF1, and allows the HSF1 monomers to form DNA-binding monomers (21, 90, 91, 98).

Table 1: Factors that can induce heat shock proteins

Exercise and contraction-related stress	
	- Free Calcium accumulation
	- Electro-mechanical coupling
	- Stress on intermediate filaments
Energy metabolism	
	- Glycogen depletion
	- ATP depletion
	- Lactate accumulation
	- Acidosis
	- Oxidative free radicals
Metabolic and stress related messengers	
	- Hormones: epinephrine, norepinephrine, cortisol
	- Cytokines: IL-6, TNF-a
Perfusion and oxygen-uptake	
	- Systemic hypoxaemia
	- Exercise-related hypoperfusion
	- Ischemia
Hyperthermia	

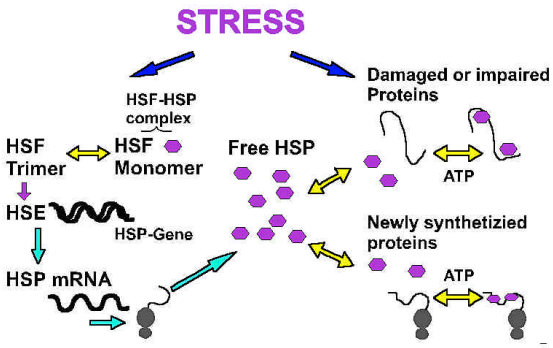

Figure 1. Stress-dependent regulation of HSP-gene expression by HSF (left), and functions of HSP as chaperone of denaturated proteins or of nascent proteins (right), and the feed-back regulation of HSP-HSF-complex (for further explanation see text).

The metabolic pathway of induction of HSP70 can be explained by reduced ATP levels to a critical level at which HSP70 remains complexed to proteins and cannot be recycled. This results in a decrease of the pool of free HSP70, and competition between proteins and HSF1 for HSP70, leading to an increase in HSF1 trimers. A similar effect may show different affinity of HSPs to unfolded proteins and HSF (90, 91).

When the HSP gene is activated, increased levels of HSP are generated, which increases the pool of unbound HSP and the possibility of complex formation with activated HSF. Therefore, the binding of HSP to HSF will inhibit further DNA-binding of HSF and accordingly, will decrease HSP transcription. In this model, the activation of HSF is thought to reflect the equilibrium of `free´ and `substrate bound´ forms of HSP (90, 96). HSF activation was detectable in skeletal muscle following heat shock regardless of HSP70 content. However, muscles comprised predominantly of slow type fibres (soleus) demonstrated greater and faster HSF activation and inactivation than muscles comprised predominantly of fast type fibres (white gastrocnemius) (96).

Calcium-mediated and activity-stretch mediated transcriptional pathways during exercise result in increased protein synthesis, the degradation of proteins due to the exercise-induced transition of protein isoforms like myosin heavy chains, and may therefore change the free to bound HSP ratio (90). Free calcium levels also increase the effects of protein kinase C dependent pathways on HSP accumulation (99). Therefore, HSF activation was demonstrated in exercising heart muscle (100) and skeletal muscle (96). It may be speculated that this represents the dominant pathway of HSP regulation during low to moderate metabolic activity like endurance training.

Although it has been shown in cultured rat or Drosophila cells, that HSF-deactivation to the non-DNA-binding state after prolonged heat stress or during recovery could be accelerated by increased HSP70 levels, the induction of HSP was not significantly inhibited by already increased HSP-levels, suggesting that other mechanisms may additionally control the oligomeric transition of HSF1 (95).

4.3. Genes encoding heat shock proteins

The nucleotide sequences of different eukaryotic HSP70 genes were reported by Hunt and Morimoto (5) and Voellmy et al. in 1985 (101). They demonstrated that out of an uninterrupted transcript of 2440 nucleotides encoding the HSP of 69,800 Daltons, 72% of the human and drosophila nucleotide sequences were identical, with 50% correspondence between human HSP gene and E. coli dnaK (bacterial HSP gene). Thus, HSP70 can be regarded as a highly conserved gene. This has been proven for many HSP genes (5, 6, 10). Furthermore, a whole family of HSP genes have been determined. Two HSP70 genes (termed A and B) have been identified on chromosome 14 (102) with the HSP2B gene encoding small HSP´s in skeletal muscles (47). The GRP 60 gene is localized on chromosome 15 (103).

4.4. Molecular mechanisms controlling heat shock protein response

There is evidence that regulation of the muscular stress response is involved in transcription, translation and posttranslational protein modification (6, 90, 104).

At normal temperatures, eukaryotic unstressed cells express only low or undectable levels of HSP70 mRNA. After 15 min of heat shock, cardiac HSP mRNA increases rapidly, peaks after 2 to 4 hours and returns to control levels after 6 h (105). A single 5-min coronary occlusion doubled the expression of HSP 70, whereas four cycles of 5 min of ischemia/5 min of reperfusion resulted in a threefold increase in HSP 70 mRNA which peaked after one hour. HSP 70 mRNA returned to baseline levels 24 h after ischemia (106). HSP70 mRNA concentration in human skeletal muscle was significantly increased four-fold 4 minutes after a 30-minute run; similarly high levels

were also observed 30 min and 3 h after exercise (107). This indicates a different transcriptional regulation of muscular heat shock, ischemia and exercise response. After heat shock, HSP levels increase rapidly, whereas during training in humans, there seems to be a time lag of several days until HSP protein levels increase substantially (29, 108).

Posttranscriptional control of HSP mRNA may be related to adenine, uracil-rich sequences in the 3´-untranslated region (3´-UTR) that targets mRNAs for rapid turnover. The 3´-UTR has been shown to influence HSP70 production, and a reporter gene linked to the 3´-UTR has demonstrated increased activity in heat shocked COS-1 cells (109). Posttranscriptional regulation in the form of HSP70 mRNA stabilization provides an additional mode of heat shock gene regulation that is likely to be of significant importance in certain forms of stress (110). A study on bovine skeletal muscle showed that there were different isoforms of HSP70s, and that their corresponding genes were identical, suggesting that the presence of multiple HSP70 isoforms may be the product of post-translational modifications to the HSP70 proteins (111).

Physical exercise, physiological stress and metabolic imbalance not only impact at the cellular level, but lead to complex regulatory changes in tissues, organs and the whole body. The respective messengers are cytokines at the cellular level and hormones at the whole-body level. It is likely that these messengers have also an impact on HSP transcriptional regulation, most possibly at the receptor level (108, 112). It has been already shown, that interleukin-6 activates HSP70 and HSP90ß-transcription in human tumor cells (113).

5. SIGNIFICANCE OF SKELETAL MUSCLE HEAT SHOCK PROTEIN CHANGES

As the stress response represents a basic celluar defense mechanism, it is not surprising that investigators all agree that this response is important (35, 44, 83, *114, 115). S*tress proteins HSPs not only serve as an indicator of muscle stress but also play a role in the processes of disease or injury.

5.1. Heat shock protein expression in myopathy

It is evident that HSP70 may be regulated by proinflammatory cytokines liks TNF-*a*, IL-1*a*, and IL-6 (116), which partly explains why the expression of HSPs is in tubular aggregates (114). The syndrome of myopathy with tubular aggregates consists of muscle pain and stiffness, and may be associated with inflamation (117). In myopathy with tubular aggregates, heat shock protein epitopes are expressed in association with the tubular abnormalities, suggesting that heat shock proteins have a role in the modulation of the tertiary structure of proteins, and may be involved in the pathogenesis of tubular aggregates and other microtubular abnormalities in muscle (114). Vajsar et al. reported a rare familial desminopathy in which the small HSP, ubiquitin, is unusually highly expressed (118). The desmin-related myopathy was also reported to be associated with another small HSP, alpha B-crystallin (119).

The involvement of HSPs in another muscle disease, Duchenne muscular dystrophy (DMD), has been reported by several authors (71, 78, 120). DMD is a severe genetic disorder caused by dystrophin deficiency, which results in muscle necrosis. Various stressful metabolic events which are likely to induce HSPs occur in DMD. Interestingly, with decreased disease severity, the associated overexpression of HSP70 was reduced, while other HSP (HSP90, GRP75) levels remained unchanged (120). The presence of HSP70 in degenerating fibres of muscles from DMD may reflect protein degradation in this situation (71). In another muscle disease, myotonic dystrophy, a novel member of the small HSPs, i. e. myotonic dystrophy protein kinase binding protein, is selectively upregulated in skeletal muscle (121).

The expression of HSPs in muscle atrophy has been reported (44, 62, 122, 123). The skeletal muscle atrophy may result from an activation of protein degradation and /or a suppression of protein synthesis in the muscle (124-127). Baracos et al. have found that accelerated muscle proteolysis and muscle wasting are responsible for the muscle atrophy in tumor-bearing rats, resulting primarily from activation of the ATP-dependent pathway involving ubiquitin and the proteasome (122). Furthermore, in rats suffering from starvation or denervation, polyubiquitin and proteosomal mRNAs are inceased in atrophying muscle, which is associated with activation of the ATP-dependent proteolytic process (123). The expression of HSPs in atrophying muscle seems to be muscular fibre type specific. For instance, Sakuma et al. (44) have observed that the small HSPs, including alpha B-crystallin, HSP27 and p20, are increased in tibialis anterior muscle (fast-twitch type) but reduced in soleus muscle (slow-twitch type) of the dy mouse (similar to DMD, but the mutated genes are localized in chromosome 10). Another mechanism responsible for muscle atrophy is the suppression of protein synthesis. Ku et al. have demonstrated that in non-weight-bearing muscle there was an ATP concentration increase, leading to a decreased association of HSC/HSP-70 with the polysomes, and a shift toward heavier polysomes, which may slow ribosome translocation, thus slowing elongation rate and suppressing protein synthesis (62).

The role of HSPs in hypertrophy has also been investigated. It has been found that a laboratory model of hypertrophy (compensatory hypertrophy and stretch hypertrophy) showed an increase in HSP72 content in hypertrophed muscle, while naturally work-induced hypertrophy did not result in altered HSP72 expression (128). Hypertrophy, an increase in cell size without cell devision, is a fundamental adaptive process employed by postmitotic skeletal muscle cells. Interestingly, it has been found that HSP expression in the hypertrophed myocardium may be influenzed by the mechanism of hypertrophy (129). Pressure overload, for instance, results at first in an induction of cellular protooncogenes and heat shock protein genes, and then a reinduction of the genes normally expressed only in perinatal life, such as fetal isoforms of contractile proteins. Such changes have not been observed in cardiac hypertrophy produced by thyroid

hormone excess. These two types of responses might represent the general pattern of growth induction to work overload by terminally differentiated cells that have lost the ability to undergo DNA replication (129).

5.2. Heat shock proteins and tissue specificity

It is well-known that the skeletal muscle is composed of muscle fibres which principally comprise contractile proteins. Based on their immunohistochemical characteristics, myosin heavy chain (MHC, one of the major components of the contractile proteins), can be identified in several isoforms (130). MHC I for example, is present in slow-twitch fibre types with high oxidative capacity, while MHC IId/x is characteristic of fast-twitch fibre types with high glycolytic capacity (the intermediate isoform of MHC is MHC IIa) (26). Skeletal muscle is a very complex and heterogeneous system since it comprises different muscle fibre types, and even a single fibre may contain different isoforms of contractile proteins (131). Different muscle fibre types differ in functional and biochemical properities, as does their expression and response to HSPs.

In fact, HSP expression is not only organ or tissue specific (69, 70), but also muscle fibre type specific (74). Inaguma et al. have observed progressive changes caused by denervation in hindlimb muscle. They have also found the small HSP p20 to be increased rapidly during rat development, and decreased after denervation leading to a loss of slow-twitch fibres (40). They suggested that the expression of p20 in rat hindlimb muscle is related to muscle contraction, and specifically in slow-twitch muscles. Alpha B- crystallin is abundantly expressed in tissue with high oxidative capacity (45). It was found that chronic low frequency motor nerve stimulation, which typically causes a muscle fibre type transition from fast-twitch to slow-twitch (132), rapidly evokes the induction of alpha B- crystallin mRNA and protein in the contracting skeletal muscle (45). Serial sections subjected to myosin immunohistochemistry revealed that alpha B- crystallin expression was confined to slow-twitch type I fibres and a subpopulation of fast-twitch type II fibres (IIa), indicating the expression of alpha B- crystallin in a muscle fibre type specific fashion. A further study has shown that the small HSPs including alpha B- crystallin, HSP27 and p20 were induced in the fast-twitch muscle fibres, and diminished in the slow-twitch muscle fibres, in mice with myopathy (44).

Studies on HSP70 expression and muscle fibre specificity have shown that the inducible form of HSP70 is constitutively expressed in rat muscles comprised of type I muscle fibres, but not in those comprised of type IIb fibres. In muscles of mixed fibre type, HSP70 content is roughly proportional to the percentage of type I fibres. These results suggest a specific expression of HSP70 in type I muscle fibre (73). It has been further demonstrated that during muscle fibre transition produced by hypertrophy resulting from increase of type I fibres or produced by hypertrophy resulting from increase of type II fibres due to thyroid hormone excess, the relationship between HSP72 content and type I muscle fibre MHC composition is maintained, and HSP72 content is not directly related to muscle oxidative capacity (129). There is also evidence that the increased HSP70 expression in muscles rich in type I fibres is accompanied by an increase in HSP60 (mitochondrial HSP), suggesting the muscle fibre type specificity of HSP induction (74). It is likely that HSP72 content is only correlated to that of MHC I in sedentary muscles. After training, HSP72 content in a muscle essentially devoid of MHC I can reach levels comparable to those in a muscle high in MHC I (72).

We have recently observed the changes of MHC isoforms in the calf muscles of patients with peripheral arterial occlusive disease (133) and HSP70 expression (unpublished data). The relative content of MHC I increased with the clinical severity of the disease, which coincided with an increase in HSP70 inducible form, suggesting a relation between HSP70 expression and MCH I content in skeletal muscle.

The reason for the tissue specific expression of HSPs in muscle is not understood completely, though HSP content is related to the oxidative capacity and mitochondrial content (13). However, there is evidence that HSP72 is constitutively expressed at high levels in predominantly type I muscle, but is present at very low levels in rat myocardium, a very highly oxidative tissue with high mitochondrial content. In another study, the constitutive expression of HSP72 was detected independently of MHC specific types, and no heat shock factor activation was observed (134). Further studies are necessary to explore the precise mechanisms for the tissue specificity of HSP expression in skeletal muscle.

5.3. Ischemia and heat shock proteins in skeletal muscle

Ischemia is an important aspect of tissue injury (10). There are studies reporting the stress response in various organs and tissues to ischemia and reperfusion (106, 135-138). The myocardium has been more extensively investigated (139) than skeletal muscle (140, 141). An increase of HSP expression following skeletal muscle ischemia/reperfusion has been observed, which was accompanied by a decreased ATP utilization, demonstrating a mean reduction of 60% in muscle necrosis caused by ischemia/reperfusion (140). Unlike the cross-protection HSP in myocardium (17), an elevated expression of HSP70 induced by heat shock is not sufficient to provide resistance against ischemia-reperfusion injury in skeletal muscle (141).

Ischemia is known to produce a number of cellular changes which include increased intracellular calcium, altered osmotic control, membrane damage, free radical production, decreased intracellular pH, depressed ATP levels, oxygen depletion and decreased intracellular glucose levels (142). All these changes may be considered as stressors inducing HSP production (3). In addition, we have recently observed changes in myosin heavy chain isoforms in the skeletal muscle of patients with peripheral arterial occlusive disease (133). In this study, a shift of the myosin isoform composition from type IIb to type I was demonstrated, which may be related to HSP expression. We

have therefore investigated the HSP expression in the relevant skeletal muscles of patients with peripheral arterial occlusive disease (unpulished data). In this study, a significantly increased HSP70 level was found in ischemic skeletal muscle, compared to non-ischemic muscle, and the levels of HSP70 expression seemed to be related to the clinical severity of the disease.

The mechanisms of HSP induction by ischemia are not thoroughly understood and may be very complex. The cellular changes induced by ischemia may stress the cells to survive such conditions by producing protective HSPs. Changes in cellular energy charge or redox potential due to diminished oxidative metabolism may destabilize the structure of certain proteins and trigger the same pathways induced by heat shock (143). Reperfusion certainly plays a role in the induction of HSPs. In addition, protein degradation as well as muscle fibre transition caused by ischemia may contribute to HSP induction.

Investigation of HSP expression in ischemic skeletal muscle may be of great significance, not only because HSP induced by ischemia may confer protection against ischemia and preserve the cellular functions (144), but the expression of HSPs may also serve as an indicator of cellular stress. In our recent study (unpublished data), the highest level of HSP70 was found in the calf muscle in patients with Fontaine stage III, in which the muscle suffers from ischemia at rest but remains viable; in contrast, the HSP70 level in the calf muscle was lowered in patients with stage IV, a situation in which the muscle losses its viability. The loss of muscle viability seems to be related to the reduced expression of HSP70 although it is not clear that muscle inviability results from the attenuated HSP70 response, or vice versa. These results suggest that HSP70 can indeed provide information on the level of cellular stress. It would be clinically significant if the investigation of HSP expression in ischemic skeletal muscle could shed light on the mechanism of cellular changes, particularly in the lack of established methods to assess blood supply to skeletal muscle in patients with peripheral arterial occlusive disease (145-147).

5.4. Exercise and heat shock proteins in skeletal muscle

Nearly two decades ago, exercise was introduced as a stimulus to induce HSPs (148). It has been proven that exercise is a sufficient physiological stimulus to induce HSPs in skeletal muscles (4). It has been demonstrated that exercise-induced HSP70 production can take place in blood (149, 150), liver, heart and skeletal muscle (30). To date, different HSPs have been induced in skeletal muscle by exercise. In one study, the small HSP32 (heme oxygenase-1) mRNA was increased seven- and four-fold immediately after 1h of exhaustive running, and after 3h of muscle contractions induced by nerve stimulatoin, respectively, and the increase in HSP32 mRNA was found to be dependent on an active tension generation (151). During recovery from exercise, there is an increase in c-fos, alpha B- crystallin and HSP70 mRNA. The recovery is also associated with specific transient changes in the expression of stress protein genes, suggesting that the products of these genes may have specific roles in the remolding process evoked by repeated bouts of contractile activities (152). It has been shown that mitochondrial HSPs including HSP60 and GRP75 can also be induced in skeletal muscles by exercise, and the expression of individual mitochondrial HSPs is independently regulated and uncoordinated (33). Again, the induction of HSPs in skeletal muscle by exercise may be muscle fibre type specific (45, 74).

The mechanisms of HSP induction by exercise may be complicated and multifactorial (for detailed review see ref. 90). Hyperthermia occurring during exercise (153) might be partly responsible. However, exercise-induced HSP70, for example, can be independent of changes in the body temperature (31), suggesting that other cellular changes induced by exercise may contribute to HSP induction. In fact, exercise causes a variety of cellular changes (155-157), all of which may result in HSP induction (3).

Although HSP induction in skeletal muscle by exercise is well-documented in animal studies, there are very few studies relating to human skeletal muscle. Puntschart et al. have reported HSP70 expression in human skeletal muscle after exercise (107). In this study, HSP70 mRNA concentration was significantly increased at four minutes into recovery from exercise, and this increase persisted three hours after exercise. However, the increase of HSP70 mRNA was not accompanied or followed by an increase of HSP70 protein within three hours after cessation of exercise. The explanation for this may be that the single exercise performed in this study was not sufficient to have an effect on the already high basal level of HSP70 protein, or that the period of observation was too short for a significantly increased accumulation of HSP70 protein to occur. Therefore, whether HSP70 at the protein level could be induced in human skeletal muscles by exercise had remained unclear until a recent report (29). In this study, a prolonged training programme was conducted in well-trained rowers, and HSP70 in exercised skeletal muscle was determined over four weeks. The results showed that HSP70 at the protein level increased significantly in response to rowing training, and the HSP70 response to training seemed related to the total amount of exercise. In this study, however, it can not be determined whether the HSP70 response was more dependent on exercise intensity or exercise volume.

We have conducted a further study in which two groups of rowers underwent different training strategies with reference to the exercise intensity and volume. It could be shown that HSP70 increased with amount of exercise as reported in a previous study (29). Comparing HSP70 response between the two groups above, the dependence of HSP70 response on exercise amount was mainly dependent upon the exercise intensity rather than exercise volume (158). Since the exercise volume in this study was regulated by endurance training (which contributed to a greater extent to the exercise volume) the question remained whether HSP70 is induced during lower intensity endurance training.

To answer this question, a third study was conducted, in which the enrolled rowers performed a training programme with two phases, i.e., high intensity and low intensity endurance training, respectively. We found that HSP70 increased significantly during the high intensity training, but remained unchanged during the endurance training. It can therefore be concluded that muscle HSP70 is not induced in well-trained rowers during low intensity endurance training (158, 159). This result differed from the study by Mattson et al. (83). In their study, HSP60, a mitochondrial HSP, was induced by endurance treadmill training, accompanied by an increase in mitochondrial content. Since the HSPs and study subjects in these two studies are quite different, their results may not be suitable for direct comparison.

The investigation of HSP induction in skeletal muscle (especially in man) by exercise may be of phyiological as well as clinical interest. On one hand, as an indicator of stress, HSP may provide information about the cellular changes induced by exercise. This may be of special interst for monitoring overtraining (160), and may be useful in the subsequent direction of training. On the other hand, in its protective role, HSP may preserve muscle function in cases of overtraining or muscle injury caused by exercise. In our first study on human skeletal muscle HSP70, we found that blood creatine kinase activity which serves as a biomarker for muscle injury, increased significantly after the beginning of training, but decreased with the production of HSP70 (29). So far we have not been able to show a relationship between HSP expression and physical performance, which would be of great physiological interest.

6. PERSPECTIVE

Due to their universal role as molecular chaperones, HSPs have been extensively studied. The general protective role of HSPs during episodes of stress has been extensively studied. It also has been suggested that HSPs may serve as an indicator of cellular stress. However, the mechanisms by which HSPs provide protection against cellular stress are not thoroughly understood. For instance, it is not yet clear how the expression of HSPs in cells is regulated, and the regulation of HSP expression in skeletal muscle has yet to be clarified. It is possible that HSP expression can be regulated by cytokines, as in rheumatoid arthritis synovial tissue (116). In fact, cytokines may play a role in immune reaction, metabolism and cellular signal trasduction. It is therefore worthwhile to investigate the potential role of cytokines in the regulation of HSP expression. Though HSP may play an important role in the regulation of hormone receptor activity, whether hormones affect the regulation of HSP expression is not clear. We have recently observed changes of hormones in blood plasma and HSP70 expression in human skeletal muscle undergoing a prolonged training. No significant relation was found between HSP70 and the levels of stress hormones like cortisol, leptin, insulin and thyroid hormones (unpublished data). These results suggest that the regulation of HSP70 expression in skeletal muscle (if any) does not simply follow hormonal levels in blood plasma. The activity of hormonal receptors might be more relevant to the HSP expression.

There is evidence that following HSP induction, cellular function can be preserved under stressful conditions, which is well-documented in the myocardium. However, few studies have dealt with skeletal muscle. Since one of the most important functions of skeletal muscle is in exercise, and exercise ability is determined by muscular performance, examination of the role of HSP in physical performance of muscles may prove of value. To date, no relationship between HSP expression in skeletal muscle and the performance has been established.

As an indicator of cellular stress, HSP expression in skeletal muscle may provide useful information relating to exercise, especially training and overtraining. Overtraining is thought to involve an imbalance between training and regeneration, resulting in a variety of changes in hormones, neuromuscular excitability, metabolism and performance. There is no report on the expression of HSP in skeletal muscle in the case of overtraining, although we could demonstrate HSP70 expression in training. Whether the expression of HSP during training differs from that in overtraining remains to be determined.

Finally, although there are a number of studies on HSP expression in skeletal muscle, most of these studies were conducted in animals, rather than man. Since HSP expression may be quite different between species, the data derived from animal studies may not extrapolate to human skeletal muscle, and further studies are necessary to investigate the regulation of HSP expression, the role of HSP in muscle function and the protection against stress in skeletal muscle, especially in man.

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Abbreviations: DMD: duchenne muscular dystrophy, GRP 75 and 78: Glucose-regulated protein respectively with molecular mass 75 and 78 kDa, HSC73: constitutive heat shock protein (heat shock cognate) with molecular mass 73 kDa, HSE: Heat shock element, HSF: Heat shock transcription factor, HSP: Heat shock protein, HSP20, 27, 60 70 and 90: Heat shock protein respectively with molecular mass 20, 27, 60, 70 and 90 kDa, IL: Interleukin, MHC: Myosin heavy chain, TNF: Tumor necrosis factor, UTR: Untraslated region

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Send correspondence to: Dr. med. Yuefei Liu, Department of Sports and Rehabilitation Medicine, University of Ulm, Steinhövelstrasse 9, D-89070 Ulm, Germany, Tel: +49 731 50 26966, Fax: +49 731 50 26686, E-Mail: yuefei.liu@medizin.uni-ulm.de