[Frontiers in Bioscience 11, 2802-2827, September 1, 2006]

Response and function of skeletal muscle heat shock protein 70

Yuefei Liu, Larissa Gampert, Katja Nething, Jürgen M. Steinacker

Section Sports and Rehabilitation Medicine, Department of Cardiology, University of Ulm, Ulm, Germany

TABLE OF CONTENTS

1. ABSTRACT

In response to stress, cells produce a series of heat shock proteins (Hsps). One of the most prominent Hsps, is the 70 kDa Hsp (Hsp70). Hsp70 is a highly conserved and essential protein against stress. The skeletal muscle responds to a diverse group of stress signals namely, muscle contraction linked energy and milieu challenges, ischemia and exercise by producing Hsp70. The extent of this Hsp70 response in skeletal muscle depends on the type and intensity of the signal, and is characterized in a muscle fiber specific manner by a special time course. Hsp70 in the skeletal muscle is regulated at transcriptional, translational and posttranslational levels. Hsp70 serves as an indicator for cellular stress as a molecular chaperone, plays pivotal role in maintaining cellular homeostasis by preventing apoptosis, influences energy metabolism, facilitates cellular processes in terms of muscular adaptation and interacts with other signalling pathways. This review summarizes our current knowledge on the skeletal muscle Hsp70 response.

2. INTRODUCTION

In the evolution cells develop a number of mechanisms to protect themselves against the environmental stress or challenges. One of the mechanisms is the response of heat shock proteins (Hsps). Selye (1936) described how organisms reacted to various external stimuli (i.e., stressors). These reactions generally follow a programmed series of events and help the organism adapt to the imposed stress. The heat shock response is a common cellular reaction to external stressors, including physical activity. A characteristic set of proteins is synthesized shortly after the organism is exposed to stress. Hsp was termed according to the first observation of the heat shock response in 1962 (1). (It is generally accepted that Hsp refers to an Hsp protein while HSP refers to an Hsp family.) It is now well known that cells respond to heat shock or other stresses with a rapid synthesis of the Hsps (2-4), thus, Hsp is also termed stress protein (3). To date nearly all eukaryotic cells examined have been found to be able to produce Hsps (5). Thus, the Hsp response is considered to be a highly conserved characteristic of cells (6), and in deed, a high correspondence of Hsp amino acid residues between different species has been identified (7-9). This highly conserved characteristic suggests an essential universal role for Hsp in the response to cellular stress.

The skeletal muscle is an important system for the body, and one of the most important functions of the skeletal muscle is to facilitate movement of the body, to develop force and velocity to perform physical work, which can cause a variety of cellular changes (10), including Hsp induction (3, 11, 12). There are a number of different Hsps identified in the skeletal muscle with regard to their biological characteristics and functions, which can be classified into several groups according to their molecular mass. Among these Hsps the most prominent Hsps in the skeletal muscle are small Hsps, HSP70, Hsp60 and HSP90. Small Hsps are a group of Hsps with molecular mass ranged from 8 kDa (13, 14) to 27 kDa (15-17), including ubiquitin, Hsp20, Hsp25, Hsp27 and alpha B-crystallin. Studies have shown that the small Hsps play an important role in facilitating the targeting and removal of proteins denatured (18), muscle contraction (especially in slow-twitch muscle fibres) (19, 20), stabilization of microfilaments and cytokine signal transduction (21), and facilitating protein refolding and muscle development (15, 22, 23). Hsp60 is localized in mitochondria and considered mitochondrial Hsp (24, 25). Hsp60 is constitutively expressed in muscle in proportion to the mitochondrial content (25) and facilitates the folding and assembly of proteins as they enter the mitochondria and stabilizes pre-existing proteins under stress (26, 27). HSP90 is a family comprising of three proteins (21, 28): two closely related cytoplasmic isoforms, i.e. Hsp90α and Hsp90β, and a glucose regulated protein (GRP94). Hsp90 is considered to play a role in regulating the activity of steroid hormone receptors (29, 30). Recently, a study shows that Hsp90 is a component of the steroid receptor complex and thus functions as a regulatory protein (31, 32). HSP70 is a heat shock protein family composing of four major forms, i.e. 72 kDa, 73 kDa, 75 kDa and 78 kDa. The proteins with 75 kDa and 78 kDa are respectively termed GRP75 and GRP78 that are not specifically induced by heat shock, but by glucose deprivation, calcium influx or agents that perturb glycolysis (3). Hsp with 73 kDa (also termed heat shock cognate, Hsc73) is synthesized in most cells and is only slightly inducible (33). It is constitutively expressed in the cytoplasm under unstressed conditions, and migrates to the nucleus and nucleolus during stress (34). Hsc73 can bind with denaturing or unfolding pre-ribosome, possibly facilitating reintegration (35). The proteins with 72 kDa, or inducible Hsp70, may be the most abundant in Hsps induced in cells in response to stress. For example, the inducible form of Hsp70 can constitute up to 20% of the total cellular protein after appropriate stimulation (36). Therefore, the Hsp70 inducible form is the most widely studied Hsp among the heat shock proteins. In this article, we refer Hsp70 to the inducible form of Hsp70 (i.e. the Hsp72) when not specifically stated, and focus on the skeletal muscle Hsp70 with regard to its biology, responses and functions

Based on the essential and universal role in maintaining the cellular homeostasis, Hsp70 serves as a molecular chaperone involving in many cellular processes, so that Hsp70 may have profound impact on protein turnover, energy metabolism, muscle function, muscle regeneration, hypertrophy and adaptation (12). During the last years, scientific research on Hsp70 in terms of its role in facilitating cellular function and especially in energy metabolism has made great achievement. This review intends to update the new data on Hsps with emphasis on Hsp70 role in muscle function and adaptation to stress.

3. BIOLOGY OF HSP70

3.1. General features and structure of Hsp70

3.1.1. General features of Hsp70

As the other inducible Hsps Hsp70 is expressed in the unstressed cells at very low level and is barely detectable. In exposure to stress Hsp70 can be induced very rapidly and in a great amount (36). Up to date Hsp70 response has been shown in almost all eukaryotic cells examined and therefore characterized as highly conserved. This highly conserved characteristic can be well understood by the fact that there is a high correspondence of Hsp70 amino acid residues between different species (6, 37). For example, the human Hsp70 is 73% identical to the Drosophila protein and 50% identical to the E coli dnaK product (2).

HSP70 gene is a typical multigene family containing at least 10 different genes and/or pseudo-genes exhibiting sequence homology to the Hsp70 gene of Drosophila melanogaster which are localized on different chromosomes (38, 39). For example, human HspA1 and HspA1L are localized on chromosome 6p21.3, the human HspA-6 and-7 genes on chromosome 1, and Hsp70 gene -1 and -3 on chromosome 17. While Hsp70-1 gene encoding inducible Hsp70, the Hsp70 2A gene localized on chromosome 14 encoded Hsp70 is constitutively expressed (40).

Table 1. Factors involved in Hsp70 induction in the skeletal muscle

Exercise and contraction-related stress	• Free Ca^{2+} accumulation • Electro-mechanical coupling • Stress on intermediate filaments
	• Glycogen depletion • ATP depletion • Lactate accumulation and acidosis • ROS (reactive oxygen species)
Metabolic and stress-related messengers	• Hormones: cortisol • Cytokines: IL-6, tnfα
Perfusion and oxygen-supply	• Systemic hypoxemia • Exercise-related hypoperfusion • Ischemia
Temperature	• Hyperthermia and hypothermia

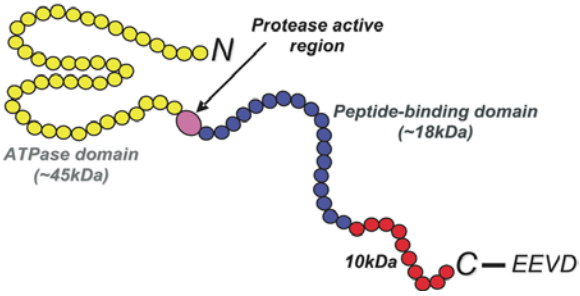

Figure 1. Schematic illustration of the molecular structure of Hsp70. Hsp70 contains three parts, i.e. the ATPase domain with about 45 kDa, the peptide-binding domain with 18 kDa and C-terminus. For details see text and ref. (41, 42).

Hsp70 has two essential functions, i.e., molecular chaperone and stress sensing. With the molecular chaperone function Hsp70 plays an important role in cellular adaptation to stress and cellular protection. With the stress sensing function Hsp70 is involved in a variety of cellular processes as immune reaction, energy metabolism and stress response.

3.1.2. Molecular Structure of Hsp70

The molecular structure of Hsp70 comprises three parts, i.e. N-terminal ATPase domain, peptide-binding site and C-terminus (Figure 1).

The N-terminal part is the mainly conserved while the C-terminal domain is the more variable domain, and the protease- sensitive site links these both regions. ATPase activity of Hsp70 can be influenced by Hsp70 cofactors and is stimulated by Hsp40 (43). The ATPase activity can be inhibited by an inhibitory protein termed HspBP1 and the ATPase domain of Hsp70 has a binding site to HspBP1 (44). HspBP1 (8 μM) inhibited approximately 90% of the Hsp40-activated Hsp70 ATPase activity. HspBP1 prevented ATP binding to Hsp70, and therefore this is the likely mechanism of inhibition. Hsp40-activated ATPase activity is essential for the renaturation activity of Hsp70. An X-ray crystallography shows that the 45 kDa fragment with the ATPase domain contains four domains forming two lobes with a deep cleft between (45). The 18 kDa peptide-binding domain consists of tow four-stranded antiparallel β-sheets and a single α-helix (46). The 10 kDa C-terminus contains an α-helix and a glycine/praline-rich aperiodic segment next to the highly conserved EEVD terminal sequence (47).

3.2. Induction of Hsp70 in skeletal muscle

The induction of Hsp70 is usually a stress-mediated process. Since the heat shock response of the cells with production of a series of newly synthesized proteins was reported about 40 years ago, a variety of stresses have been proven to be able to induce Hsp70 response, which includes insults causing protein degradation, changes in pH value, reactive oxygen species (ROS), UV irradiation or chemical stress (2, 5). For the skeletal muscle physical exercise has been shown to induce Hsp70 response (48). To date, physical exercise is considered as an established stress in terms of Hsp70 induction (12). In skeletal muscle the induction of Hsp70 by exercise is related not only to the temperature- mediated effects of exercise, i.e. hyperthermia, but also to different other physiological processes such as contraction related stress, changes in energy metabolism, induction of metabolic and stress related messengers or disturbances in perfusion and oxygen transport. These Hsp70 inducing factors can be summarized in table 1.

Hsp70 induction may be initiated by protein denaturation (49) and therefore, the denatured protein may serve as a "cellular thermometer" in terms of Hsp70 response (figure 2).

3.3. Regulation of Hsp70 response/expression

The regulation of Hsp70 response/expression is based on different levels and may represent a complex system. First, the inducers themselves can regulate Hsp70 response. Studies on Hsp70 induced by different mechanisms have shown that Hsp70 response is related to the intensity of stress (12). For example, a hyperthermia induced Hsp70 expression level is associated with the change of temperature (50). It has been reported that Hsp70 expression level in the skeletal muscle in patients with peripheral arterial occlusive disease (PAOD) is associated with the severity of the disease (51). Recently, we have observed that an ischemia induced Hsp70 expression in the skeletal muscle depends on the ischemic duration (52). The dependence of Hsp70 response on stress intensity can also be elucidated by exercise. Previous studies have shown that physical training induced Hsp70 expression in the skeletal muscle depends on the exercise intensity in human beings (53, 54), and similar results could be obtained from animal study (55).

Second, it is shown in figure 2 that Hsp70 response to stress may present an auto-regulatory 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

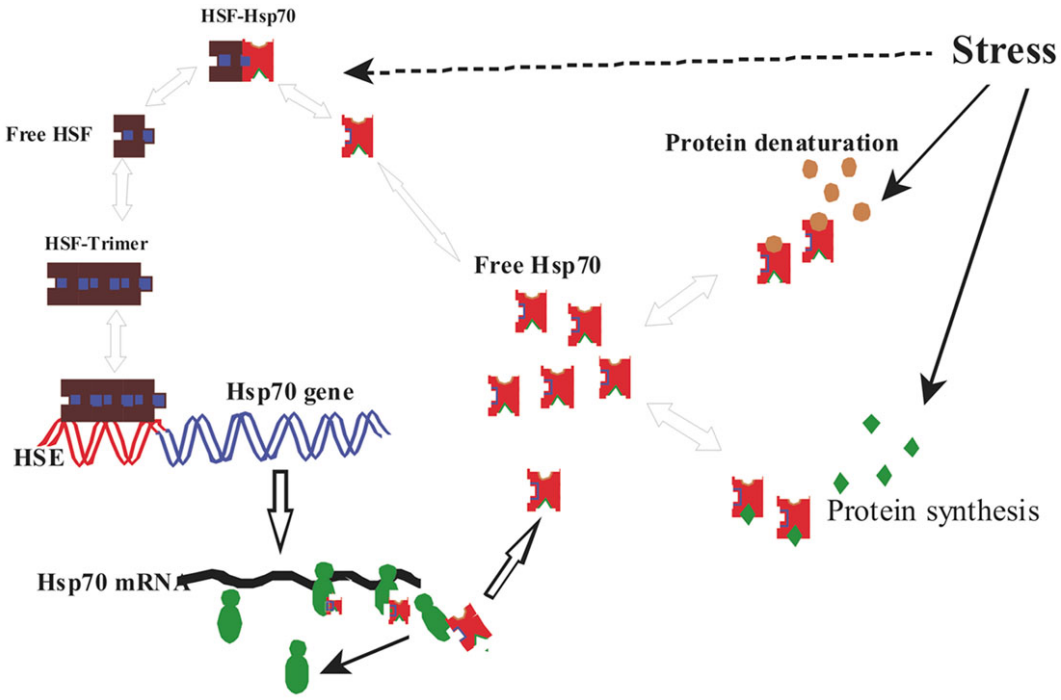

Figure 2. The process of Hsp70 induction and regulation. The stress induced cellular changes like dentured proteins are captured by Hsp70, causing a dissociation of the HSF-Hsp70 complex and triggering the gene transcription for Hsp70 production.

may also be regulated at least partly at protein level. This seems especially to be the case for the ischemia-induced Hsp70 response since Hsp70 production may be affected by protein synthesis. It has been observed that the effect of ischemia alone on Hsp70 response was smaller than that of the ischemia followed by reperfusion (56, 57), suggesting that Hsp70 response to ischemia may be influenced by protein synthesis which is limited under ischemic condition and improved by reperfusion. Furthermore, it has been shown that in the transfected cells containing the human Hsp70 gene, the heat stress induced Hsp70 response can be controlled by the 3´-untranslated region of Hsp70 gene, indicating a mechanism that regulates Hsp70 production through the posttranscriptional control (56, 58).

Third, like most of other proteins Hsp70 is also regulated at transcriptional level. A number of previous studies have shown that in response to stress muscle Hsp70 mRNA is rapidly up-regulated (12, 56, 59, 60). A 30 min running on treadmill could lead to a significant increase in Hsp70 mRNA in the human skeletal muscle within 4 minutes after exercise (61). We have previously reported that an exercise training resulted in an up-regulation of Hsp70 mRNA in well-trained human skeletal muscle and there was a discrepancy between Hsp70 mRNA and Hsp70 protein (62), suggesting that Hsp70 response may be regulated independently at protein level as well as at mRNA level.

Fourth, in the regulation of Hsp70 response, HSF has an important role. To date there are at least four different HSF genes in mammalians encoding HSF1, 2, 3 and 4, respectively (63-65). HSF1, 2 and 4 can be classified in two isoforms, i.e. α and β, respectively. HSF1 is activated by several stressors like heat, oxidative stress or denatured proteins, mediates stress- induced Hsp70 expression and plays an essential role in the ubiquitin-proteasome-mediated pathway (66). In contrast, HSF2 is not regulated in response to classical stress stimuli, but under developmentally related conditions and is suggested to play a role in controlling development and differentiation- specific gene expression. It has been reported that HSF3 is specifically activated in unstressed proliferating cells by direct binding the c-myb proto-oncogene product (c-Myb), suggesting that the c-Myb-induced HSF3 activation may contribute to the growth-regulated expression of Hsps. This activation pathway seems to be blocked by p53 (67). Therefore, the regulation of HSF3 activity may affect Hsp70 expression during cellular proliferation and apoptosis. HSF4 is preferably expressed in human heart, skeletal muscle, brain and pancreas. The novel family member HSF4α has been reported to function as a repressor of Hsp expression while the more recently reported HSF4β has the potential for transactivating heat shock genes in yeast. In contrast to all vertebrate HSFs human HSF4 lacks the carboxyl-terminal hydrophobic segment that might be involved in the negative regulation of DNA binding activity (64, 65).

The activation of HSFs, e.g. HSF1, is a stress related process. In unstressed mammalian cells HSF1 monomers are located in the cytoplasm bound to Hsp70 members. In response to stressors like heat shock, ischemia, ATP or oxygen depletion the accumulation of denatured proteins competes Hsp70 away from Hsp70-HSF complex. HSFs are then phosphorylated by protein kinase

C or other serine/threonine kinases and form the DNA-binding homo-trimetric structure (figure 2). These trimers then enter the nucleus, bind to the promoter region of Hsp70 gene and trigger Hsp70 production. The newly synthesized Hsp70 binds to HSF and thus prevents further DNA-bind of HSFs.

The activated trimer of HSFs has high affinity to bind to the promoter gene of Hsp70, a specific DNA sequence, named the heat shock element (HSE). This highly conserved HSE includes typically 5´-nGAAn-3´ sequence, often existing multiple copies and is located in the promoter region upstream of the Hsp70 genes. The binding of HSF to HSE initiates Hsp70 production.

3.4. Basic functions of Hsp70

Hsp70 has been proven to be involved in a variety of cellular processes and exerts different functions including cellular protection against stress, protein metabolism like protein degradation, protein folding and synthesis, facilitating cellular adaptation to stress and development as well as effects on cellular energy metabolism (5, 8, 12). All these functions of Hsp70 can be attributed to the two basic functions, i.e. molecular chaperone and stress sensing.

3.4.1. Molecular chaperone

Chaperones are defined as proteins and protein assemblies that help other proteins fold into their proper native conformation, prevent them from misfolding and aggregation during de novo synthesis and under conditions of stress (68). For details see ref. (5, 12). Extensive research indicates that a major function of Hsp70 is a molecular chaperone. First, Hsp70 passes the newly synthesized, unfolded proteins to members of the HSP60 leading to folded proteins. Second, Hsp70 carries proteins for translocation into different cellular compartments. Finally, Hsp70 may serve as cohort proteins to other proteins such as glial-axon transfer proteins (41). Thus, in this consideration, Hsp70 may be useful in assisting design of drug-delivery vehicles and a potentially therapeutical target for gene therapy.

With this characteristic as molecular chaperone Hsp70 together with its co-chaperones of the J-domain protein family can prevent the aggregation of non-native proteins through association with hydrophobic patches of substrate molecules, which shields them from intermolecular interactions (´holder´activity) and assist non-native folding intermediates to fold to the native state (´folder´activity) (for detail see ref. (42)). In cooperation with ATP and other cofactors, Hsp70 can facilitate the functional restoration of the denatured proteins or the degradation of irreversible damaged proteins by transporting them to the corresponding organelles and proteasomes (41).

It has been shown that the chaperone activity of Hsp70 is essentially dependent on ATP hydrolysis which takes place in the N- terminal domain, leading to conformational changes in the C-terminal domain. There are two conformations of Hsp70 protein, i.e., the ATP-bound state and the ADP-bound state (after ATP hydrolysis). Proteins of the Hsp40/DNA-J family can increase the Hsp70 chaperone activity by enhancing the ATPase activity of Hsp70 (42).

Recently, we have observed that an over-expression of Hsp70 in the cultured Hela cells affected cellular ATP level dependently on the Hsp70 level (69), and this effect resulted mainly from augmented glycolytic activity rather than glucose oxidation. It is well known that several regulatory proteins are involved in the glucose metabolism such as glucose transporters and calcium regulatory protein like calmodulin (59, 70-72). Therefore, the Hsp70 effect on energy metabolism might be attributed to the potential mechanism that Hsp70 as molecular chaperone interacts with these regulatory proteins. Further studies are needed to clarify this issue.

3.4.2. Stress sensing

The other important basic function of Hsp70 is the stress sensing. Various stresses including hyperthermia cause primary changes with denatured proteins (49) and such proteins combined with regulatory protein can constitute a “cellular thermometer” (73). The denatured proteins can be recognized and captured by Hsp70, triggering the stress response (49). There is evidence of cellular protein denaturation within the temperature range of Hsp70 induction in both bacteria and mammalian cells (41, 74). There is also another proposal for the cellular thermometer, i.e. the HSF itself, since the biochemical environment of the HSF can cause HSF to oligomerize, presumably through effects on protein conformation caused by various stresses (49).

Hsp70 interacts with key regulators of many signal transduction pathways controlling cell homeostasis, proliferation, differentiation and cell death (42). The interaction of Hsp70 with these regulatory proteins continues in activation cycles that also involve Hsp90 and a number of co-chaperones. The regulatory proteins, called clients, are thereby kept in an inactive state from which they are rapidly activated by the appropriate signals. It is known that the steroid hormone receptors play an important role in the stress response. It has been shown that Hsp70 and Hsp40 first interact with the steroid-receptor in an ATP-dependent reaction to produce a receptor-Hsp70-Hsp40 complex that is “primed” to be activated to the steroid-binding state in a second ATP-dependent step with Hsp90, Hop, and p23. Hsp70 and Hsp90 thus repress regulators in the absence of the upstream signal and guarantee full activation after the signal transduction pathway is switched on (75). Hsp70 can be titrated away from these clients by other misfolded proteins that may arise from internal or external stresses. Consequently, through Hsp70 disturbances of the cellular system induced by environmental, developmental or pathological processes act on these signal transduction pathways (76). In this way, stress response and apoptosis are linked to each other. Hsp70 inhibits apoptosis acting on the caspase-dependent pathway at several steps both upstream and downstream of caspase activation and on the caspase-independent pathway (77, 78).

In the event of infections or immune reactions Hsp70 plays an important role in the signal transduction (79). Gene encoding Hsp70 is found to reside in the major histocompatibilty complex, and a protein binding motif of Hsp70 is very similar to the peptide-binding cleft of this complex Class I proteins. Studies have shown that Hsp70 is involved in the immune process, such as antigen presentation (80). Hsp70 can activate pro-inflammatory cytokine (e.g. IL-6, TNFα) production or other innate immune factors (i.e. NF-κB) and specifically bind with high affinity to the plasma membrane of antigen presenting cells. Furthermore extracellular Hsp70 has been shown to stimulate the complement pathway, to increase phagocytosis and to function as a powerful adjuvant for eliciting antigen-specific immune responses and anti-tumour immunity.

The significance of the stress sensing role of Hsp70 is that Hsp70 can serve as an indicator for the cellular stress. It is evident that the stress induced Hsp70 expression is related to the stress intensity (12). Studies have shown that Hsp70 can be considered as a molecular marker of thermal injury (81), neurotoxicity (82) or environmental metal strain (83). It has been reported that the serum level of Hsp70 was directly related to inflammatory status of the investigated geriatric subjects (84). Moreover, Hsp70 may be used as a marker for prostate cancer, eventually in conjunction with PSA (85). In our recent observation we found that Hsp70 expression level was associated with the training status of well trained athletes and the severity of ischemia in the skeletal muscle (52, 86). It is further evident that exogenous Hsp70 stimulated TNFα, IL-1β or IL-8 in human monocytes via CD14 and calcium dependent pathways (41). In this respect, Hsp70 can be considered as a chaperokine since it may serve on one hand as chaperone and on the other hand as a cytokine.

4. RESPONSE OF SKELETAL MUSCLE HSP70

4.1. Hsp70 response to muscle disorders and injury

4.1.1. Muscle disorders

Although it has been reported that a bacterial infection induced significantly Hsp90 expression in the skeletal muscle, but the skeletal muscle Hsp70 did not change (87), numerous studies show that changes of skeletal muscle Hsp70 are involved in a variety of muscle disorders. For example, Hsp70 is expressed in the skeletal muscle in tubular aggregates (88). The syndrome of myopathy with tubular aggregates manifested by muscle pain and stiffness may be associated with inflammation (89), and it is evident that Hsp70 may be regulated by proinflammatory cytokines like TNFα, IL-1α, and IL-6 (90, 91). There is also evidence of Hsp70 involvement in another muscle disease, Duchenne muscular dystrophy (DMD) (92, 93). DMD is a severe genetic muscle disorder caused by dystrophin deficiency, which results in muscle necrosis. Interestingly, with decreased disease severity, the associated over expression of Hsp70 was reduced (93). The presence of Hsp70 in degenerating fibres of muscles from DMD may reflect protein degradation in this situation (92).

Recently, the expression of Hsp70 in the diabetic skeletal muscle has been investigated. Bruce et al. reported that the intramuscular Hsp70 and heme oxygenase-1 (a small Hsp) mRNA were reduced in patients with type 2 diabetes (59), and the data provided new evidence that the pathogenesis of type 2 diabetes involved perturbations to the antioxidant defence mechanism within skeletal muscle. This result is consistent with that of Kurucz et al. who showed that Hsp70 mRNA was lowered in type 2 diabetes and Hsp70 mRNA level correlated with glucose uptake rate (94). Interestingly, an eight week endurance exercise training can modulate Hsp70 response in diabetes (95), so that endurance training may offset some of the adverse effects of diabetes by up-regulating tissue Hsp expression. However, in that study the endurance training induced the activation and expression of transcriptional regulatory heat shock factor-1 only in non-diabetic control, thus, diabetes may impair Hsp protection, possibly via transcriptionally mediated mechanisms.

4.1.2. Muscle injury

Muscle injury is known to be associated with inflammation and therefore can induce Hsp70 response in the skeletal muscle (96). It has been reported that muscle injury indicated by an increase in blood CK during prolonged exercise training was accompanied by an increase in skeletal muscle Hsp70 (62). Thompson et al. observed an increase in intramuscular Hsp70 expression following a single bout of eccentric exercise in humans (97). Furthermore, this same work group has examined the repeated bout effect of an eccentric exercise on Hsp70 response in the skeletal muscle (98), and they found that the intramuscular Hsp70 response to the second bout of eccentric exercise was comparable to that of the first bout exercise (four weeks apart) although the exercise-induced muscle damage indicated by CK release, muscle soreness and loss of mobility by second bout of eccentric exercise was dramatically damped. These data suggest that the skeletal muscle Hsp70 response to eccentric exercise is not only determined by the exercise-induced muscle damage. Interestingly, the authors found the basal level of Hsp70 for the second bout of exercise was lower than that for the first one, and they speculated that the lower basal Hsp70 expression might mediate the attenuation of muscle damage (98). In a further study Thompson et al. demonstrated that with regard to different muscle groups Hsp70 response to an eccentric exercise was regulated by different pathways (99), they concluded that the response of post exercise Hsp through mitogen-activated protein kinase (MAPK) was exercise-specific and local, not systemic.

4.1.3. Muscle atrophy

The expression of Hsps in muscle atrophy has been well documented (16, 100-102). The skeletal muscle atrophy may result from an activation of protein degradation and/or a suppression of protein synthesis in the muscle (103-106). Baracos et al. have found that an accelerated muscle proteolysis and muscle wasting are responsible for the muscle atrophy in tumour-bearing rats, resulting primarily from activation of the ATP-dependent pathway involving ubiquitin and the

proteasome (100). 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/Hsp70 with the polysomes, and a shift towards heavier polysomes, which may slow ribosome translation, thus slowing elongation rate and suppressing protein synthesis (107). As mentioned above, Hsp70 expression is involved in the muscle myopathy which are frequently accompanied by muscle atrophy.

Aging is a typical problem leading to muscle atrophy. It is evident that the aged skeletal muscles fail to adapt following contractile activity (108) and the attenuated Hsp70 response may play a major role in development of the age-related functional deficit that occurs in skeletal muscle (109). Interestingly, the age associated attenuation of Hsp70 response to exercise seems to be muscle fibre specific, so that Naito et al. found that the exercise-induced accumulation of Hsp70 was different between fast and slow muscles, and aging is associated with a blunted expression of Hsp70 in fast skeletal muscle in response to chronic exercise (110).

Recently, there is increasing evidence that Hsp70 is involved in the muscle atrophy resulting from mechanical unloading or inactivity. Oishi et al. observed that a nine-week hind limb suspension led to a decrease to 38% in Hsp70 content in animals and this could be over-compensated by a two- or four-week reloading (111). However, Desplanches et al. did not find any changes in Hsp70 in slow-twitch muscles after two-week unloading despite a slow-to-fast fibre transition (112). The different results stated above may be attributed to the different circumstances of the experiments.

4.1.4. Muscle hypertrophy

Hypertrophy, an increase in cell size without cell division, is a fundamental adaptive process employed by post mitotic skeletal muscle cells. The role of Hsp70 in muscle hypertrophy has also been investigated. It has been reported that a laboratory model of hypertrophy (compensatory hypertrophy or stretch hypertrophy) caused an increase in Hsp70 content in the hypertrophied muscle, while naturally work-induced hypertrophy did not result in altered Hsp70 expression (113). Interestingly, it has been found that Hsp expression in the hypertrophied myocardium may be influenced by the mechanism of hypertrophy (114). Pressure overload, for instance, results at first in an induction of cellular proto-oncogene and heat shock protein genes, and then a re-induction 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 response might represent the general pattern of growth induction to work overload by terminally differentiated cells that have lost the ability to undergo DNA replication (114).

In our previous study on human skeletal muscle, we found that a six week strength training led to a myosin heavy chain (MHC) isoform transition and an increased expression of a cardiac-like isoform (MHC Iα mRNA) in m. triceps brachii (115). In this study we have further determined the Hsp70 content, and found that there was a three-fold increase of Hsp70 at protein level and an approximately four-fold increase at mRNA level (116). In a study on patients with spinal cord injury, Willoughby et al. found that a 12-week cycling training led to a significant increase in myofibrillar protein content along with an increase in Hsp70 mRNA and a decrease in ubiquitin mRNA (117). These data suggest that in the human skeletal muscle undergoing hypertrophy through strength training Hsp70 response is induced. The role of Hsp70 in muscle hypertrophy may also be supported by the reloading experiments in which a reloading or endurance training subsequent to the unloading led to a significant increase in Hsp70 expression along with muscle growth (111, 112). However, there is also evidence that muscle hypertrophy induced by static load is not accompanied by an Hsp70 response (118). Ueno et al. reported that a six-week centrifugation (static load) led to a significant increase in the weight of antigravity muscle, but no up-regulation of Hsp70 was observed.

It is well known that growth factors like insulin-like growth factor 1 (IGF-1) have a profound role in stimulating muscle growth and hypertrophy (119). There is evidence that disorders like insulin deficiency down-regulated Hsp60 along with blunted IGF-1 receptor signalling in diabetic myocardium (120). IGF-1 may activate the myogensis process through MAPK pathway (121), and there is evidence that exercise can induce Hsp70, MAPK and IGF-1 response (99, 122). These data suggest that Hsp70 is involved in the muscle hypertrophy. This is consistent with our previous study showing an Hsp70 response along with an up-regulation of IGF-1 and its splice variant MGF (for mechano growth factor) (123, 124).

It is also likely that the role of Hsp70 in muscle hypertrophy is attributed to the effect of Hsp70 on apoptosis. It is known that an increase in IGF-1 induced by exercise also leads to an activation of phosphatidylinositol-3-OH-kinase (PI-3k) which inhibits the process of apoptosis (125). Klomleaw et al. found that with increase in Hsp70 expression during the regressive procedure of horse lumbrical muscle there was no apoptotic gene expression (126). It is evident that Hsp70 can inhibit apoptosis by preventing recruitment of caspase-9 to the apoptotic protease activating factor 1 (APAF-1) apoptosome (127). The effect of exercise-induced Hsp70 expression on apoptosis has recently been demonstrated by Alway´s work group (128). They found that Hsp70 protein content was positively correlated to B-cell lymphoma-2 (Bcl-2) protein and mRNA contents and negatively correlated to Bcl-2-associated X (Bax) mRNA content. Since Bax is pro-apoptotic and Bcl-2 a anti-apoptotic, these data suggest that exercise training attenuates the extent of apoptosis in skeletal muscle.

4.2. Hsp70 response to heat shock

4.2.1. General response of Hsps to heat shock

Heat shock was probably the first factor observed in inducing Hsp70 response (1). With an efficacy of energy utilization at about 30%, the major portion of the energy the skeletal muscle uses to do physical work is converted

into a thermal energy and this induces a temperature change in the working skeletal muscle. Indeed, the skeletal muscle is frequently confronted by the temperature challenge during the muscle contraction. Therefore, heat shock may play an important role in Hsp70 induction and serves as a mechanism to induce skeletal muscle Hsp70 response.

There are a number of studies demonstrating that heat shock can induce Hsp70 response in the skeletal muscle (129-133). Compared to myocardium, skeletal muscle seems to be less thermal sensitive with regard to Hsp70 response. Ali et al. for example, have shown that compared to other tissues including skeletal muscle, myocardium has a lowered set point temperature for heat shock factor activation (129), suggesting that the skeletal muscle is not so thermosensitive as the myocardium. Kim et al. have observed in animals that a bout of exercise (treadmill running) can significantly elevate the rectal temperature of the animals, which seems to determine Hsp70 expression level in the working muscle (134). There is evidence that exercising in a cold environment which prevented elevations in core temperature during exercise, did not cause change in Hsp70 expression in the skeletal muscle (135). This seems to be consistent with that of Kim et al. (134). Furthermore, there is study showing that a cold stress at 8°C for 20 minutes does not induce Hsp70 induction in the skeletal muscle (136). Interestingly, heat shock induced Hsp70 in the skeletal muscle seems also to be muscle fibre specific (132). However, there are studies showing that exercise induced Hsp70 response in the skeletal muscle may be independent upon the core temperature. There is evidence that with maintained core temperature (38°C) exercise induces clearly mitochondrial Hsp70 response (50), hence the mitochondrial Hsp70 expression is not dependent on temperature. Skidmore et al. have also observed in rats that both heating and exercise are major inducers of Hsp70 response (137) and exercise induced Hsp70 expression can be independent on temperature.

4.2.2. Physiology and significance of heat shock induced Hsp70 response in skeletal muscle

The role of heat shock induced Hsp70 response in the skeletal muscle has been investigated (130), however, its significance seems less clear than that in myocardium. Study in rats shows that the heat treated muscle (41°C, 60 minutes) increases in its weight after 1 day as well as 7 days with concomitant increase in Hsp70 expression, suggesting a role of Hsp70 in muscle hypertrophy induced by heat shock (138). Since a simultaneous increase in calcineurin expression has also been observed in this study, the heat induced muscle hypertrophy may be attributed to a calcineurin pathway. Certainly, the relationship and/or interaction between Hsp70 and calcineurin needs to be clarified further. In a trial with whole body hyperthermia Naito et al. observed in the muscle undergoing atrophy due to unweighting, a heat shock could increase Hsp70 expression significantly, which attenuated clearly the loss of muscle mass although an atrophy could not be completely prevented (139). It is evident that in the muscle suffering from immobilization there is a decrease in muscle mass and no significant increase in Hsp70 expression (140). However, when the immobilized muscle is exposed to heat, Hsp70 expression increased markedly and the muscle mass can be preserved, which may be attributed to reduced oxidative stress conferred by heat treatment. The effect of Hsp70 induced by heat shock in the skeletal muscle may also be associated with decreased production of IL-6 (141). It is known that the sarcoendoplasmic reticulum Ca^{++}-ATPase (SERCA1a) is important for the muscle function and SERCA1a can be inactivated by heat stress, study shows that under heat stress increased Hsp70 binds to the fast twitch skeletal muscle SERCA1a and prevents thermal inactivation (142). Additionally, there is study demonstrating that hyperthermia increases mitochondrial oxidative enzyme activity (143), suggesting a role of Hsp70 involved in muscle energy metabolism. However, there are controversial results indicating that heat shock induced Hsp70 response does not provide protective effect on ischemia in the skeletal muscle (144), and heat shock induced Hsp70 response seems not to attenuate low-frequency fatigue (145). Thus, the role of Hsp70 induced by heat shock in the skeletal muscle needs to be clarified further.

4.3. Hsp70 response to ischemia

Ischemia is an important aspect of tissue injury (35, 146). There are studies reporting the stress response in various organs and tissues to ischemia and reperfusion (57, 147-150). The myocardium has been more extensively investigated (151) than skeletal muscle (144, 152). 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 (152). Unlike the cross-protection of Hsp70 in myocardium (153), an elevated expression of Hsp70 induced by heat shock seems not sufficient to provide resistance against ischemia-reperfusion injury in skeletal muscle (144).

4.3.1. Hsp70 response to ischemia

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 (154). All these changes may be considered as stressors inducing Hsp70 production (2, 5). In a previous study we have observed changes in MHC isoforms in the skeletal muscle of patients with PAOD (155). In this study, a shift of the MHC isoform composition from type IIb to type I was demonstrated, which may be related to Hsp70 expression. We have therefore investigated the Hsp70 expression in the involved skeletal muscles of patients with this disease (51), and found a significantly increased Hsp70 level in ischemic skeletal muscle. The levels of Hsp70 expression seemed to be related to the clinical severity of the disease. In an ischemia/reperfusion experimental model Lepore and Morrion found only a modest induction of Hsp70 in the skeletal muscle undergoing ten minute ischemia followed by 15 minute reperfusion (56). Similar study was also reported by Bushell et al. (156) in which an

ischemia/reperfusion episode induced an increase in Hsp70 along with decreased ATP level. From the three studies mentioned above, it is still unclear whether ischemia can directly induce Hsp70 induction in the skeletal muscle because in the former study factors more than ischemia are involved (for instance, inactivity, systemic changes of atherosclosis, metabolic disorders and MHC isoform transition) and in the latter two studies not only ischemia but also reperfusion are involved in the Hsp70 response. To examine whether ischemia can directly induce Hsp70 response in the skeletal muscle, we have conducted a study on ischemia-induced Hsp70 in porcine skeletal muscle and found that after two hour ischemia Hsp70 mRNA increased significantly and after four hour ischemia there war a further increase in Hsp70 mRNA (52), indicating a relationship between Hsp70 response and ischemic degree. However, the increase in Hsp70 at protein level was not statistically significant. This discrepancy between Hsp70 protein and mRNA may be attributed to attenuated protein synthesis under complete ischemia and a time delay of Hsp70 accumulation at protein level.

4.3.2. Mechanisms of Hsp70 response to ischemia

The mechanisms of Hsp70 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 (157). 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 Hsp70 induction. Anoxia/hypoxia may serve as a major role in ischemia-induced Hsp70 response in the skeletal muscle. Ramaglia and Buck have observed a time-dependent expression of Hsp70 and Hsp90 in tissues of anoxic western painted turtle (158) and found that no changes in these Hsps in the early phase of anoxia (within 12 hours), however, in the late phase of the anoxia (24 hours or later) Hsp70 as well as Hsp90 increased significantly (two to four folds). These data suggest that stress proteins play a role in promoting long-term anoxia tolerance. The role of hypoxia in inducing skeletal muscle Hsp70 response to ischemia can also be supported by our recent study (159). In this study, we have observed that the hypoxia inducible factor 1α (HIF 1α) was consistently increased with Hsp70 in the muscle of patients with PAOD undergoing an oxidative stress resulting from ischemia/reperfusion (arterial occlusive disease followed by an angioplastic intervention), and a four-week walking training could attenuate the response of HIF 1α and Hsp70 (see below). Another mechanism of the ischemia-induced Hsp70 response in the skeletal muscle may be the oxidative stress caused by ischemia. Khassaf et al. investigated effect of an antioxidant vitamin C on Hsp70 (160) and found that the antioxidant used could attenuate exercise-induced Hsp70 response in the skeletal muscle.

4.3.3. Significance of Hsp70 response to ischemia

Investigation on Hsp70 expression in ischemic skeletal muscle may be of great significance, not only because Hsp70 induced by ischemia may confer protection against ischemia and preserve the cellular functions (161), but the expression of Hsps may also serve as an indicator of cellular stress as well. In our previous study mentioned above (51), the highest Hsp70 level was found in the calf muscle in patients with PAOD at 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 at stage IV, a situation in which the muscle loses its viability. The loss of muscle viability seems to be related to the reduced expression of Hsp70 although it is not clear whether 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 Hsp70 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 PAOD (162-164). In our recent study mentioned above, two groups of patients with PAOD at Fontaine stage II were included and one group underwent a four-week walking training on treadmill after an angioplastic intervention, while the other group without training served as control. We found that the training subsequent to the angioplastic intervention led to a much greater improvement in the pain free walking distance along with augmented microperfusion in comparison with the controls. In the walking group, myogenic factors like myo D and myogenin at mRNA level increased significantly, while HIF 1α and Hsp70 expression maintained unchanged. In contrast, in the matched control group, the expression of HIF 1α and Hsp70 increased significantly, while no clear increase in myogenic factors at mRNA level was observed (159). These data strongly suggest that in consistence with HIF 1α, Hsp70 response indicates cellular stress in the event of ischemia/reperfusion, and exercise training has profound benefit on muscle function and cellular adaptation.

4.4. Hsp70 response to exercise

Over two decades ago, exercise was introduced as a stimulus to induce Hsps (48). It has been proven that exercise is a sufficient physiological stimulus to induce Hsps in skeletal muscle (3). It has been demonstrated that as in other tissues (165-167), exercise-induced Hsp70 response can take place in the skeletal muscle (12). Hsp70 induction in skeletal muscle by exercise is well-documented in animal studies, however, there are fewer studies relating to human skeletal muscle, and fortunately studies dealing with this issue in the past years were emerging.

4.4.1. General response of Hsp70 to exercise in skeletal muscle

Puntschart et al. have reported the first study on Hsp70 response in human skeletal muscle after exercise (61). In their 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 in 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 muscle by exercise remained unclear until our first study was reported (62). 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. Two years later, two studies were reported (97, 168), and showed clearly the time course of Hsp70 response in human skeletal muscle.

In our first study on human skeletal muscle Hsp70 response to training we found that Hsp70 response seemed related to the total amount of exercise (62), however, we could not clarify in that study whether the Hsp70 response was more dependent on exercise intensity or exercise volume. Therefore, 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 the previous study (62). Comparing Hsp70 response between the two groups above, the dependence of Hsp70 response on exercise amount was mainly attributed to the exercise intensity rather than exercise volume (54). This relationship between Hsp70 response and exercise intensity elucidated from human skeletal muscle is strongly supported by a later study on animals (55).

Furthermore, to investigate if there are different Hsp70 responses with respect to high intensity exercise and low intensity endurance exercise, we have conducted a third study 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 low intensity endurance training (53, 54). Since a low intensity endurance training was shown to be able to induce Hsp70 response in animals and not well-trained humans (168-170), the results of the study that an endurance training did not lead to an increase in Hsp70 in the well-trained athletes raised the question whether Hsp70 response to exercise in human skeletal muscle is influenced by the training status. Thus, we have conducted another study (86), in which a group of well-trained rowers with high performance underwent two separate bouts of exercise, i.e., a bout of high intensity strength exercise and a bout of low intensity endurance rowing, respectively. No significant changes in Hsp70 expression at both protein and mRNA level was found within six hours post each exercise bout. Therefore, Hsp70 response in the well-trained human skeletal muscle is blunted, implying the impact of training status on Hsp70 response.

Since Hsp70 response is shown to be muscle fibre type specific (see below) and most of our studies dealt with Hsp70 response in m. vastus lateralis with relatively dominant slow muscle fibre type, we have examined Hsp70 response in triceps brachii with higher composition of fast-twitch fibres (116) and found a significant Hsp70 response at protein and mRNA level in the muscle examined. This result seems to be supported by the study of Thompson et al. (99) in which an increase in Hsp70 expression was significantly induced by an eccentric exercise.

There is evidence that Hsp70 response to exercise can be affected by different factors. While mechanical loading during exercise causes Hsp70 response in the skeletal muscle, a static load by centrifugation did not induce Hsp70 expression in the antigravity muscle though a hypertrophy in this muscle was elucidated (118). Aging has a significant impact on Hsp70 response to exercise. Vasilaki et al. reported that aging reduced the binding of the transcription factor HSF1, leading to a perturbation of Hsp70 response (108, 109). Hsp70 response to exercise may be influenced by gender (171). It has been shown that oestrogen attenuates Hsp70 expression in acutely exercised male rodents (172) and post-exercise Hsp70 expression in skeletal muscle (173). There seems no study reported on human skeletal muscle with respect of gender effect on Hsp70 response to exercise.

Recently, the impact of diabetes on Hsp70 response has increasingly attracted the attention of researchers. It has been reported that induction of diabetes decreased Hsp70 expression in vastus lateralis in rats (95) and endurance training may offset some of the adverse effects of diabetes by up-regulating tissue Hsp70 expression. Kurucz et al. have observed decreased expression of Hsp70 in skeletal muscle of patients with type 2 diabetes and Hsp70 mRNA correlates with glucose uptake rate (94, 112), therefore, Hsp70 is involved in the pathogenesis of type II diabetes. Unfortunately, up to date there seems no study reported with regard to effect of diabetes on Hsp70 response to exercise.

4.4.2. Mechanisms of Hsp70 response to exercise

The mechanisms of Hsp70 response induced by exercise may be complicated and multi-factorial (for detailed review see ref. (174)). Hyperthermia occurring during exercise (175) might be partly responsible. Xu et al. reported a heat activation of Hsp-related promoter in vivo in muscles using a custom-built ultrasound-mediated hyperthermia instrument and found that an optimal activation of gene expression could be achieved at 39°C (176). This heat activation is obviously not due to ultrasound used because both continuous and pulsed ultrasound do not increase Hsp70 (177). Oishi et al. investigated the thermal effect on Hsp70 induction in the skeletal muscle (133), and found that within 4 hour recovery from 60 min hyperthermia (42°C), Hsp70 increased approximately by 2-fold. Furthermore, they observed a differential response of Hsp70 in the muscle with different fibre composition during recovery after heat stress. The different Hsp70 response to muscle temperature was verified later by the same work group (132). However, exercise-induced Hsp70 expression can be independent on changes in the body temperature (137), suggesting that

other cellular changes induced by exercise may contribute to Hsp induction.

In fact, exercise causes a variety of cellular changes (54, 178, 179), all of which may result in Hsp70 induction (2). For example, challenges to energy metabolism are well known to be able to induce Hsp70 response. Glycogen and ATP depletion can induce Hsp70 response (12, 180). Recently, it has been reported that glucose ingestion can attenuate the exercise-induced increase in circulating Hsp70 and Hsp60 in humans (181). Exercise-induced decrease in pH can also result in Hsp70 response (182). Poso et al. studied effect of exercise training on Hsp70 response in skeletal muscle and found that after exercise Hsp70 mRNA correlated positively with the peak concentration of blood lactate (183). An important cellular change induced by exercise is an augmentation of oxidative stress with oxidative free radical products (36, 168), and it is well known that Hsp70 expression can be induced by free radical products (36, 184-189). This seems also to be supported by the fact that vitamin C supplements can attenuate Hsp70 expression induced by cycle ergometry (160).

During the muscle contraction, the intramuscular blood supply is temporally interrupted and recovers during the muscle relaxation, therefore, the skeletal muscle is frequently encountered by ischemia and reperfusion. As stated above, ischemia and/or reperfusion can induce Hsp70 expression. Thus, exercise-induced Hsp70 response in the working muscle may be partly attributed to ischemia/reperfusion.

Muscle injury caused by exercise may be a further mechanism underlying the Hsp70 response (190). We have previously observed that muscle injury indicated by an increase of CK release during prolonged exercise training was accompanied by an increase in skeletal muscle Hsp70 (62). Also a single bout of eccentric exercise can induce changes in inflammatory mediators, leading to Hsp70 response (96). It is likely that exercise-induced Hsp70 response is associated with IL-6 since IL-6 has been proven to be able to activate Hsp70 gene expression in human skeletal muscle (90).

4.4.3. Significance of Hsp70 response to exercise

Based on the basic functions of Hsp70 stated above, Hsp70 expression in skeletal muscle may provide useful information relating to exercise. Previous studies have shown that Hsp70 response to exercise is dependent upon exercise intensity (53-55, 62). Therefore, the expression in skeletal muscle may serve as an indicator of cellular stress relating to exercise intensity, which may help identify or prevent 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 (191). Since Hsp70 response can be activated through the inflammatory process caused by exercise (90), Hsp70 expression may provide information relating to exercise-induced muscle damage. In fact, in the skeletal muscle undergoing damage during/after exercise CK is increased along with an increase in Hsp70 expression (14, 97, 98). Furthermore, exercise-induced cellular changes including ATP and glycogen depletion, decrease in pH and increase in lactate can lead to an Hsp70 induction, this suggests that Hsp70 response induced by exercise may to certain extent reflect the cellular changes resulting from exercise.

It is well documented that Hsp70 provides protection against cellular stress and damage (49, 161). The protective role of Hsp70 against cellular damage has been extensively investigated on myocardium (148, 192, 193). However, there are only few studies addressing the skeletal muscle (56, 130, 194). Our previous study has shown that along with an increase in Hsp70 the level of CK decreased during a prolonged training (62), suggesting a potential protective effect of Hsp70. This result seems to be supported by further study from Heads et al. (195) reporting that a high level expression of Hsp70 protects cells against thermal stress. On the contrary, Cao et al. have shown that an impaired Hsp70 induction led to a decreased thermotolerance in a temperature-sensitive multinucleated cell line (196). There is evidence that Hsp70 can bind to the fast-twitch skeletal muscle sarcoplasmic reticulum Ca2+-ATPase and protect its function by stabilizing the nucleotide binding domain (197). A preconditioning in terms of Hsp70 induction seems to confer protect against muscle damage caused by the subsequent eccentric contraction (98). Similar to that for myocardium (148, 153), Hsp70 induction can play a fundamental role in protection against ischemia for skeletal muscle (130, 194). Our previous study showed that in the ischemic skeletal muscle in patients with PAOD the Hsp70 level was associated with the severity of the disease, but in patients with the most severe ischemia (Fontaine stage IV) Hsp70 was lower than that in Fontaine stage III, implicating that a failure to produce higher Hsp70 might be responsible for the loss of tissue viability in the involved skeletal muscle (51). However, it seems not clear whether the skeletal muscle can also be protected in the event of ischemia by a so-called preconditioning with Hsp70 induction although Baumeister et al. have reported that a preconditioning 24 hours prior to ischemia conferred a significant reduction in muscle injury subjected to the subsequent ischemia (198). Lepore et al. showed that an ischemic preconditioning 24 hours prior to a prolonged ischemia did not improve muscle survival after reperfusion (56). Bushell et al. investigated the mechanisms involved in an ischemic preconditioning of skeletal muscle and found that the effect of preconditioning seemed not to be attributed to Hsp70 induction, but to the infusion of adenosine to animals immediately before exposure to the four-hour period of ischemia (156). Thus, in terms of preconditioning, the role of Hsp70 in skeletal muscle ischemia has to be clarified in further studies.

Based on the biological role as molecular chaperone Hsp70 response to exercise may be important for muscle regeneration and repair. Muscle contractions may cause a variety of challenges to temperature, energy metabolism and protein turnover. During regeneration from stress a series of cellular processes occur in the skeletal muscle, in which Hsp70 can be involved. Plumier et al., for example, have found that an over-expression of Hsp70 could significantly facilitate the myocardial recovery from

ischemia (161). There is evidence that during recovery from heat shock, the skeletal muscle Hsp70 expression was significantly increased (133). In the skeletal muscle undergoing mechanical unloading (for instance, hind limb suspension in animals), an endurance training can improve the recovery or remodelling of the disused skeletal muscle, which may be attributed to an increase in Hsp70 accumulation in the muscle (112), suggesting that Hsp70 may have impact on facilitating the muscle regeneration or repair. This result seems to be supported by the study of Oishi et al. who have observed that after a prolonged unloading for nine weeks, the skeletal muscle weight decreased significantly along with a decrease in Hsp70 (about 38%), and returned to the control level after two week reloading (111). In this study after two weeks of reloading Hsp70 level increased over the control level, and then returned to the control level after eight weeks of reloading. Goto et al. have demonstrated that a regeneration of the atrophied muscle resulting from disuse (suspension) could be significantly accelerated by heat shock in which Hsp70 expression was clearly induced (199), and this consideration was strongly supported by an in-vitro study of the same work group (200). A further study has shown that during muscle regeneration from injury, Hsp70 is involved in the cell proliferation and differentiation of the myogenic cells, and the regulatory cofactor of Hsp70 (BAG-1) may promote the folding capacity of Hsp70 (201).

In one of our previous studies (53), it is shown that a high intensive exercise training phase induced Hsp70 expression was followed by a transformation of MHC isoforms with an increase in MHC IIa (202). Another study showed that an increase in Hsp70 expression was induced by a strength training along with an up-regulation of MHC Iα mRNA in the muscle undergoing hypertrophy (115, 116). It is known that the transition of MHC isoforms serves as an important mechanism of the muscular adaptation to exercise (203-205). Since protein synthesis is indispensable in the process of MHC isoform transition, it is not difficult to understand that Hsp70 plays an important role in this process due to its basic function as molecular chaperone (107, 206). There are a number of studies demonstrating that Hsp70 response is involved in the muscle fibre transition in a muscle fibre specific manner (81, 131, 207, 208).

4.5. Characteristics of skeletal muscle Hsp70 response

Though some common features like stress-responsive and highly conserved, Hsp70 response in the skeletal muscle has distinct characteristics.

4.5.1. Time course of Hsp70 response in skeletal muscle

According to the definition of Hsp response, Hsps can be generally rapidly produced in response to cellular stress. It is true that in many tissues Hsps can be induced within a short time after cells exposed to the stress (2, 5, 21, 80). In cell culture, for instance, Hsp70 response can be activated within several minutes or hours (209-211). Numerous studies show that Hsp70 can be induced very rapidly in variety of cells and tissues (87, 131, 212-214). Also in human beings, Hsp70 can be quickly induced after exposure to stress in different kinds of organs or tissues. Fehrenbach et al. have reported that in leukocytes Hsp27 and Hsp70 transcripts increased significantly immediately after acute exertion accompanied by elevated levels of corresponding proteins, and in the observed 24 hours after exercise Hsps remained high (215). An exercise-induced Hsp70 expression in human blood has been observed at 30 minutes during exercise (216), and this increase was obviously not resulted from Hsp70 release from the working muscle because the blood Hsp70 increase preceded clearly the increase in intramuscular Hsp70. This result is confirmed by the study of Febbraio et al. (217) who reported that exercise induced hepatosplanchnic release of Hsp70 in humans. However, Hsp70 response in human skeletal muscle seems to be somewhat specific in terms of the time course. Unlike that in the rodent skeletal muscle, in which Hsp70 response can be induced in minutes/hours (108, 132), Hsp70 response to stress in human skeletal muscle seems to be much slower with a time delay. This is particularly true for the Hsp70 response at protein level, and there is evidence of a time delay between cellular stress and Hsp70. Puntschart et al. showed that an exercise-induced Hsp70 expression at mRNA level could be observed within 4 minutes of the recovery from a 30 minute treadmill exercise (61), but Hsp70 expression at protein level did not increase significantly within three hours after cessation of the exercise. This is consistent with that of Khassaf et al. (168) who have studied the time course of Hsp70 responses in human skeletal muscle to oxidative stress induced by non-damaging exercise, and found that a peak level of Hsp70 induced by a single exercise bout occurred six days post-exercise. This special time course of Hsp70 response in skeletal muscle is also supported by animal study that a time-dependent expression of Hsp70 and 90 in tissues of the anoxic western painted turtle was observed and Hsp70 expression increased in the skeletal muscle only in the late phase of a long term anoxia (158).

The time course of Hsp70 response may also be attributed to the so-called discrepancy between mRNA and protein level. Our previous study has shown that training induced Hsp70 expression at protein level maintained constant over a long period while Hsp70 mRNA decreased gradually after induction (53). Actually, there have been studies demonstrating that the changes of Hsp70 mRNA are not accompanied by the corresponding changes of Hsp70 at protein level, it seems that Hsp70 mRNA response to stresses occurs within hours while those of Hsp70 protein within days (61, 168, 218). The discrepancy may also suggest that the posttranscriptional mechanisms play important role in regulating Hsp70 expression level.

4.5.2. Dependence of Hsp70 response on stress intensity

It is evident that the skeletal muscle Hsp70 response is dependent upon the degree of stress. In response to heat shock, for instance, Hsp70 expression correlates to the degree of temperature change. In the myocardium, the most thermal sensitive tissue, Hsp70 protein accumulation exposed to different temperatures was in the order: 30°C > 28°C > 26°C (129). The dose-dependent response of Hsp70 in the skeletal muscle can also be elucidated by ischemia. There is evidence that a significant induction of Hsp70 expression in the skeletal muscle can be induced not by a short term (e.g. five

minutes) but by a prolonged ischemia (10 minutes) (56). The investigation on Hsp70 expression in the ischemic skeletal muscle in patients with PAOD showed that the expression level of Hsp70 was associated with the severity of the disease (51). That is, the more severe the disease, the higher the Hsp70 expression level in the involved skeletal muscle. In our recent study it has been shown that the Hsp70 mRNA level in the ischemic skeletal muscle is different with regard to the ischemic duration (52). Since in this study a complete interruption of blood supply was introduced, the ischemic degree was dependent upon ischemic duration, so that a relationship between the degree of ischemia and Hsp70 mRNA level seems to be reasonable. Furthermore, the dose-dependent response of Hsp70 in the skeletal muscle to exercise training has also been observed. In our previous study, we could demonstrate that Hsp70 response to physical training was related to the training volume (62). Somewhat later we could further clarify that the Hsp70 production in the working muscle was dependent on the exercise intensity (54). Actually, different levels of Hsp70 expression in response to different exercise intensity as well as exercise types have also been observed (53). These data are strongly supported by a further independent study on animals (55).

The dose-dependent response of Hsp70 in the skeletal muscle to cellular stress is of great physiological as well as pathophysiological significance since it might serve as the basis for an important role of Hsp70 as a stress indicator.

4.5.3. Muscle fibre specific response of Hsp70

It is well-known that the skeletal muscle is composed of muscle fibres which principally comprise contractile proteins. Based on their immunohistochemical characteristics, MHC, one of the major components of the contractile proteins, can be identified in several isoforms (219). 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) (203). 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 (220). Different muscle fibre types differ in functional and biochemical properties, as does their expression and response of Hsps. A number of studies have demonstrated that Hsp70 response is characterized by tissue specific, that is, muscle fibre specific (12). In fact, Hsp70 expression is not only organ or tissue specific (81, 87, 131), but also muscle fibre type specific (208). Studies on Hsp70 expression and muscle fibre specificity have shown that the inducible form of Hsp70 is constitutively expressed in rat muscles comprising of type I muscle fibres, but not in those comprising of type IIb fibres. In muscles of mixed fibre type, Hsp70 content is roughly proportional to the percentage of type I fibres (207). These results suggest a specific expression of Hsp70 in type I muscle fibre. It has been further demonstrated that during muscle fibre transition produced by hypertrophy resulting in increase of type I fibres or produced by hypertrophy leading to increase of type II fibres due to thyroid hormone excess, the relationship between Hsp70 content and type I muscle fibre MHC composition is maintained, and Hsp70 content is not directly related to muscle oxidative capacity (114). 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 (208). However, the correlation of Hsp70 to MHC I seems to be only in sedentary muscles, and after training, Hsp70 content in a muscle essentially devoid of MHC I can reach levels comparable to those in a muscle high in MHC I (221). Recently, we have observed an increased Hsp70 expression in m. triceps brachii resulting from a six-week strength training (116), which seems to be accompanied by an MHC transition from IIx to IIa (115). Additionally, there is evidence that in the skeletal muscle undergoing atrophy with slow-to-fast muscle fibre type transition due to unloading, Hsp70 was not induced, while an endurance training led to an increase in Hsp70 along with normalization of the atrophied muscle (112), these results are supported by further study (111). The specific Hsp70 response with respect to muscle fibre types has been also observed under heat shock stress (132). Oishi et al. have shown that heat shock induced Hsp70 response in soleus was higher and longer than that in plantaris (133). The tissue-specific Hsp70 response in the skeletal muscle has been also observed under conditions of muscle disorders. In our previous study on patients with PAOD, it could be demonstrated that an increase in Hsp70 in the skeletal muscle was accompanied by an MHC isoform transition from MHC IIx $\rightarrow$IIa$\rightarrow$I (51, 155).

However, there are also controversial results showing that an eccentric exercise induced Hsp70 expression occurs in the m. biceps brachii (with relatively higher fast fibres) but not in the m. vastus lateralis (with relatively higher slow fibres) (99). This implies that the so-called tissue specific Hsp70 response is only relative because like the muscle with high slow fibres the muscle rich in fast fibres has also the ability to response with Hsp70 production to cellular stress.

4.5.4. Factors that affect Hsp70 response in skeletal muscle

The skeletal muscle Hsp70 response can be affected by a number of factors. In terms of muscular adaptation, Hsp70 response to exercise may be blunted. In a previous study, we have observed that the Hsp70 response to an endurance training was clearly different to that of the prior high intensity strength training (53). This seems not to result completely from the different types of exercise training because there is evidence that an endurance training could also induce significant Hsp70 expression in the skeletal muscle (112, 168-170). Recently, we could demonstrate that along with the muscular adaptation to training, Hsp70 response in human skeletal muscle to exercise is significantly blunted (86). A blunted Hsp70 response in terms of muscular adaptation to endurance training has also been elucidated from the other work group (222), they found that an intense swimming training (five times a week for three months) led to a

significant decrease in Hsp70 mRNA. These results imply that one should bear the fact in the mind that Hsp70 response is influenced by training status when Hsp70 response is considered as an indicator for cellular stress. However, a controversial result has also been reported. Thompson et al. have demonstrated that the magnitude of human Hsp70 response to two identical bouts of eccentric exercise separated by four weeks was not different (98).

Muscle disorders may have distinct impacts on Hsp70 response. Bacterial infection for instance, can induce tissue-specific Hsp70 response (87). In the diabetic skeletal muscle Hsp70 expression is significantly reduced (59, 94), suggesting that Hsp70 may be involved in energy metabolism and the pathogenesis of diabetes. In the skeletal muscle undergoing atrophy resulting from aging or unloading, Hsp70 expression is significantly reduced (108, 111, 112, 126). Naito et al. have reported that aging is associated with a blunted expression of Hsp70 in fast skeletal muscle in response to chronic exercise (110).

Additionally, Hsp70 response can be influenced by different chemical agents like glucose, insulin, vitamin C, vitamin E and oestrogen (120, 160, 171-173, 181, 212, 221). Thus, for interpretation of Hsp70 response, these factors must be taken in consideration.

5. FUNCTIONS OF HSP70 IN SKELETAL MUSCLE

As a highly conserved protein, Hsp70 plays a universal role in maintaining cellular homeostasis. With the basic function, i.e. molecular chaperone and stress sensing, Hsp70 functions may be involved in many cellular processes as protection against cellular stress, apoptosis, cellular adaptation and energy metabolism.

5.1. Protection against cellular stress

The skeletal muscle confronts steadily the challenges which may lead to cellular damage. As one of the essential mechanisms against cellular stress, cells develop response of Hsps. As mentioned above, in the working muscle, the largest portion of energy utilized is converted into the thermal energy inducing elevation of the muscle temperature (175). The thermal stress can directly or indirectly induce protein denaturation (223) and the denatured protein serves therefore as a cellular thermometer (49). Since the thermal induced denatured proteins are harmful to cells, Hsp70 can confer cellular protection by capturing the denatured proteins (12, 49). There is evidence that the preconditioned cells with Hsp70 induction significantly improve their thermal stability or thermal tolerance (224, 225).

Ischemia is an important event for the skeletal muscle in terms of Hsp70 response. Numerous studies dealing with the protective role of Hsp70 against ischemia/reperfusion have been reported. There is evidence that a prior heat shock treatment with Hsp70 induction could reduce significantly the necrotic muscle (198). Interestingly, a preconditioned somatothermal stimulation on media nerve territory increases Hsp70 production and protects rat heart against ischemia/reperfusion (226). Garramone et al. have demonstrated that a prior stress conditioning using the heat-shock response confers significant biochemical and ultrastructural protection against ischemic injury in rat skeletal muscle (227). Lepore et al. has shown that a prior heat stress is effective in protecting mature skeletal muscle in vivo against necrosis after ischemia-reperfusion and has potential for use in microsurgical procedures requiring tourniquet applications (194). In one of our previous studies, we have found that in the ischemic skeletal muscle of patients with PAOD the Hsp70 expression level in Fontaine stage IV was lower than that in stage III, and the failure to further produce Hsp70 in the involved muscle might be responsible for the loss of muscle viability (51). However, there is also controversial result showing that heat shock did not protect skeletal muscle from ischemia insult (228), this seems to be supported by a further study (144). In general, a protective role of Hsp70 in ischemic skeletal muscle needs to be clarified further.

The protective role of Hsp70 have been also shown in the events of muscle disorders. In an experiment with cell culture (L6 myotubes), Luo et al. showed that in the presentation of dexamethasone, a glucocorticoid agent, cells underwent a catabolic condition characterized by accelerated protein degradation along with down-regulation of NF-κB, and this process could be prevented by Hsp70 induction through a pre-treatment with heat shock (at 43°C for one hour) (229). This study demonstrates that the protective role of Hsp70 against glucocorticoid-induced protein degradation is attributed to the prevention of down-regulation of NF-κB. It has been shown in a cell transplantation trial that a heat shock with Hsp70 induction (at 42°C for one hour) 24 hours prior to hypoxia-reoxygeneration insult could significantly increase the transplanted cell survival both in vitro and in vivo (230), and this effect seemed to be at least in part attributed to the lower percentage of early apoptosis measured by flow cytometry with annexin V staining. In a cell culture (C2C12) muscle damage model with the calcium ionophore, A23187 and the mitochondrial uncoupler, 2,4-dinitrophenol (DNP) as damaging agents, Maglara et al. (209) have demonstrated that the cellular damage to the myotubes indicated by an increase of CK release, could be distinctly reduced by an exposure to a period of hyperthermia, and there was a clear correlation between Hsp70 content and the cellular protection. As stated above, muscle damage can also be caused by disuse. Naito et al. showed that an unweighting skeletal muscle lost muscle weight and protein (231), and a pre-treatment with whole body hyperthermia led to a significant Hsp70 induction along with clearly attenuated loss of muscle weight and protein by hind limb suspension, suggesting the protective role of Hsp70 against muscle damage resulting from atrophy.

There are also studies demonstrating that Hsp70 may have protective effect on the muscle damage caused by exercise training (12). Muscle damage can be caused by strenuous and unaccustomed exercise, especially exercise involving eccentric muscle contractions (190). In terms of

muscular adaptation to exercise training, muscle damage decreases gradually with progression of the training. One of the mechanisms might be the response of Hsp70. In our previous study it has been observed that with increase of Hsp70 expression level, the release of CK decreased during the training period (62). It is documented that Hsp70 is involved in muscle adaptation to protect muscle damage (232). In the myocardium, there was reportedly a direct correlation between Hsp70 expression level and cardio-protection (233). Like the protective role of small Hsps against contraction-induced muscle damage (234), an induction of Hsp70 in response to exercise is involved in the muscular adaptation (185). Actually, the skeletal muscle has the capacity to rapidly adapt to eccentric exercise-induced muscle damage (235), a diminished production of Hsp70 in the aged muscles may be responsible for the impaired adaptation of the muscles undergoing aging process (236).

5.2. Hsp70 and apoptosis

The role of Hsps in the apoptotic process has been reported (237). It is evident that small Hsp like B-crystallin negatively regulates apoptosis during myogenic differentiation through inhibiting caspase 3 activation (238). Similar to the small Hsp, Hsp70 also exerts a negative effect on apoptosis. Suzuki et al. have reported that in the cell culture (L6 myoblasts), a pre-treatment (heat shock at 42°C for an hour) prior to the hypoxia-reoxygeneration insult can significantly reduce the percentage of early apoptosis (230). It is suggested that glutamine exerts an anabolic effect on protein turnover in the skeletal muscle and therefore promotes an apoptosis (239). Zhou and Thompson have found that glutamine can significantly increase Hsp70 expression level (240), and the glutamine effect on protein turnover can be clearly affected by a heat shock induced Hsp70. Recently, Siu et al. investigated apoptotic adaptations from exercise training in skeletal muscle (128), and found that an exercise training (five days weekly for 8 weeks on treadmill) led to a distinct decrease in Bax mRNA levels and increase in Bcl-2. In this study, Hsp70 protein content was negatively correlated to Bax mRNA and caspase-3 activity, and positively correlated to Bcl-2 protein and mRNA. This study strongly suggests that Hsp70 has a profound impact on apoptosis. These results seem to be consistent with the results of Klomleaw et al. who have investigated the horse lumbrical muscle undergoing regression (126). Their study showed that in the regressive procedure in muscle an increase in Hsp70 was accompanied by the lack of apoptotic gene expression assessed by TUNEL, implying that Hsp70 may depress the apoptotic gene expression. These results are consistent with that of a further study demonstrating that a thermal precondition with Hsp70 induction has striking effect against apoptosis initialized by adriamycine (241). Unfortunately, although the role of Hsp70 in apoptosis is fascinating, there seems up to date no study addressing this issue on human skeletal muscle.

5.3. Hsp70 and muscle adaptation

In the muscular adaptation to stress, protein metabolism is involved (240, 242). In a study on muscle recovery from an atrophy process (hand limb suspension), Goto et al. found that heat shock significantly accelerated the recovery of the atrophied muscle, and this was closely associated with Hsp70 content (199). In their another study on cell culture (200), it was observed that Hsp70 was increased by either heat shock or cyclic stretching and the Hsp70 regulation was closely related to the cell total protein, suggesting that Hsp70 may play an important role in cell growth. In facilitating cellular adaptation, other Hsps also have important impact. For instance, glucose-regulated protein 94 is necessary for maintenance of myotube fusion competence (243).

Muscle hypertrophy is one of the important mechanisms of the skeletal muscle adaptation to cellular stress. Muscle hypertrophy has been ascribed primarily to fibre cross-sectional area (i.e. fibre hypertrophy), however, fibre hyperplasia may also contribute to the muscle mass increase (244). In an in vivo study on animals, Uehara et al. have observed that rats exposed to environmental heat stress (41°C for 60 minutes) got significantly higher ratio of soleus muscle to body weight than that in unstressed rats, and the distribution of 5-bromo-2'-deoxyuridine and proliferating cell nuclear antigen-positive nuclei, that are the indicators for the cell proliferation were also increased along with augmented Hsp70 expression (245). It is further evident that a stretch-induced muscle hypertrophy can be facilitated by heat shock with production of Hsp70 (246). A relationship between Hsp70 and myogenic cells during muscle regeneration has been elucidated (201). It has been reported that cytokine like IL-4 has impact on mammalian muscle growth (247), we have recently observed that a strength training led to an up-regulation of IL-4 mRNA in the skeletal muscle along with an increase in Hsp70 expression level (116, 248). Furthermore, we have conducted a study in patients with chronic heart failure due to coronary artery disease (249). In this study, the subjects performed a strength training (three times a week for three months), and the muscle samples were attained from m. vastus lateralis using a fine needle biopsy technique. We found that in comparison with the controls (clinically matched patients without training), the trained patients clearly improved their muscle function and clinical findings without any perturbation of the cardiac functions, which was accompanied by an up-regulation of Hsp70 mRNA. This result indicates that in human skeletal muscle undergoing training induced hypertrophy, Hsp70 is also involved.

With regard to the role of Hsp70 in muscle function, there is a study demonstrating that heat shock with subsequent expression of Hsp70 improves functional recovery of the muscle suffering from ischemia/reperfusion (250). In the transgenic mice over-expressing Hsp70 in the skeletal muscle the excitation-contraction coupling process can be influenced by Hsp70 (251).

As mentioned above, the muscle fibre transition serves as a profound function and very fine mechanism for the muscular adaptation (203-205). In general, increased neuromuscular activity such as electromechanical stimulation and exercise training leads to a fast-to-slow fibre transition and on the contrary, decreased neuromuscular activity such as denervation, aging and

events leading to atrophy like disuse results in a slow-to-fast fibre transition (205). Numerous studies have demonstrated that Hsp70 response is involved in muscle fibre transition (131, 252-254). Oishi et al. reported different response of Hsps to heat stress between soleus and plantaris (133), that is, the response of Hsp70 to heat stress in soleus with higher portion of slow muscle fibres is greater than that in plantaris with higher portion of fast muscle fibres. This difference seems also to be associated with the time course of Hsp70 response (132). In a study on the relationship between MHC isoform and Hsp70 response, Ogata et al. showed that during muscle development the level of Hsp70 in the soleus muscle gradually increased in parallel with the increment in the type I MHC isoform (255). Compared with the soleus, only a small amount of Hsp70 could be detected in the plantaris muscle throughout the developmental period. Recently, using a compensatory hypertrophy model Ogata et al. have investigated the changes in Hsp70 and calcineurin content along with the changes in MHC isoforms (256). They found that two-week functional overload through cutting the tendons of its major synergists resulted in a shift towards a slower MHC profile and two weeks of thyroid hormone (T3) administration (150 μg/kg/day) resulted in a shift towards a faster MHC profile in control rats and led to an attenuation of the shift towards a slower MHC profile induced by overload. Furthermore, they have demonstrated that the Hsp70 level increased during the MHC isoform transition towards to slower MHC profile, and decrease during the MHC isoform transition towards to faster MHC profile resulting from T3 treatment. However, although there is a lot of evidence supporting the specificity of Hsp70 response in a muscle fibre manner, our opinion is that the specificity in terms of Hsp70 response to stress is relative, because 1) the muscle rich in fast fibre is proven to be capable of Hsp70 production, and 2) there are controversial results showing that an eccentric exercise induced Hsp70 expression occurs in the musculus biceps brachii (with relatively higher fast fibres) but not in the musculus vastus lateralis (with relatively higher slow fibres) (99). Oishi et al. have also shown that the levels of Hsp70 in both slow and fast rat plantar flexor are responsive to a chronic decrease in the levels of loading and/or activation, which suggests that the neuromuscular activity level and the presence of innervation of a muscle are important factors that induce Hsp70 expression (257).

5.4. Hsp70 and energy metabolism

The energy metabolism is crucial for the cellular function and Hsp70 plays a pivotal role in maintaining cellular homeostasis and facilitating cellular adaptation (5). Therefore, Hsp70 response is closely related to energy metabolism. The relationship between Hsp70 and energy metabolism may be ascribed in two aspects.

On one hand, changes in energy metabolic status significantly induce Hsp70 response. A number of studies demonstrate that the challenges to energy metabolism including glycogen and ATP depletion, lactate accumulation and production of oxidative free radicals induce Hsp70 response (174, 258-262) (for details see ref. (2, 5)). On the other hand, Hsp70 may exert direct or indirect effects on energy metabolism. Recently, the data about Hsp70 effects on energy metabolism are emerging. It is known that Hsp70 contains an ATPase fragment (263), so that Hsp70 may have direct impact on cellular ATP level. Chen et al. have demonstrated that a previous hyperthermal treatment increases significantly mitochondrial oxidative enzyme activity like cytochrome c with concomitant increase in Hsp70 expression in the m. gastrocnemius in rats (143). Certainly, it is not clear in this study whether the effect of hyperthermia on mitochondrial oxidative enzyme activity mainly results from the increase in Hsp70 expression. To our best knowledge, there seems to be a lack of studies addressing direct effect of Hsp70 on energy metabolism. We have thus conducted a study to investigate a potential direct effect of Hsp70 on cellular ATP level using a transgenic Hela cell line over-expressing human Hsp70 (69). In this study, different cell groups with controlled Hsp70 expression at different levels (low, moderate and high level) across physiologic to supra-physiologic range were obtained, and the results showed that in comparison with the untransfected or transfected but not specifically induced cells (with baseline Hsp70, controls), the cellular ATP levels in the transfected and induced cells were higher, interestingly, the highest level of cellular ATP was derived from the cells expressing moderate Hsp70 level (comparable to that in the physiologically stressed cells), but not in the cells expressing high level (superior to the physiologically induced level). In this study we have further examined the effect of Hsp70 over-expression on glucolytic as well as oxidative metabolic pathways and found that in the cells over-expressing Hsp70 the rate of glucose consumption and lactate production was distinctly increased, while except for increased activity of citrate synthase, no significant difference in oxidative metabolic pathway in comparison with the controls was observed. Therefore, the Hsp70 effect to preserve cellular ATP level is mainly attributed to its stimulative effect on glycolysis. When Hsp70 level is too high (superior to the physiologically response level), the cellular ATP level goes down, not due to an impairment of glycolysis, but because of an accelerated ATP utilization since the rate of glucose consumption as well as lactate production in the cells expressing high Hsp70 level was comparable to that in the cells with moderate Hsp70 expression level. Thus, it can be concluded from our recent study (69) that Hsp70 enhances cellular ATP level by stimulating glycolytic activity of the transgenic cells over-expressing Hsp70.

We speculate that the Hsp70 effect on glycolysis may be associated with glucose transport. This idea seems to be supported by a variety of studies. For instance, there is evidence that in type II diabetic skeletal muscle, a typical phenomenon with elevated insulin resistance and thus impaired glucose transport, Hsp70 is significantly reduced

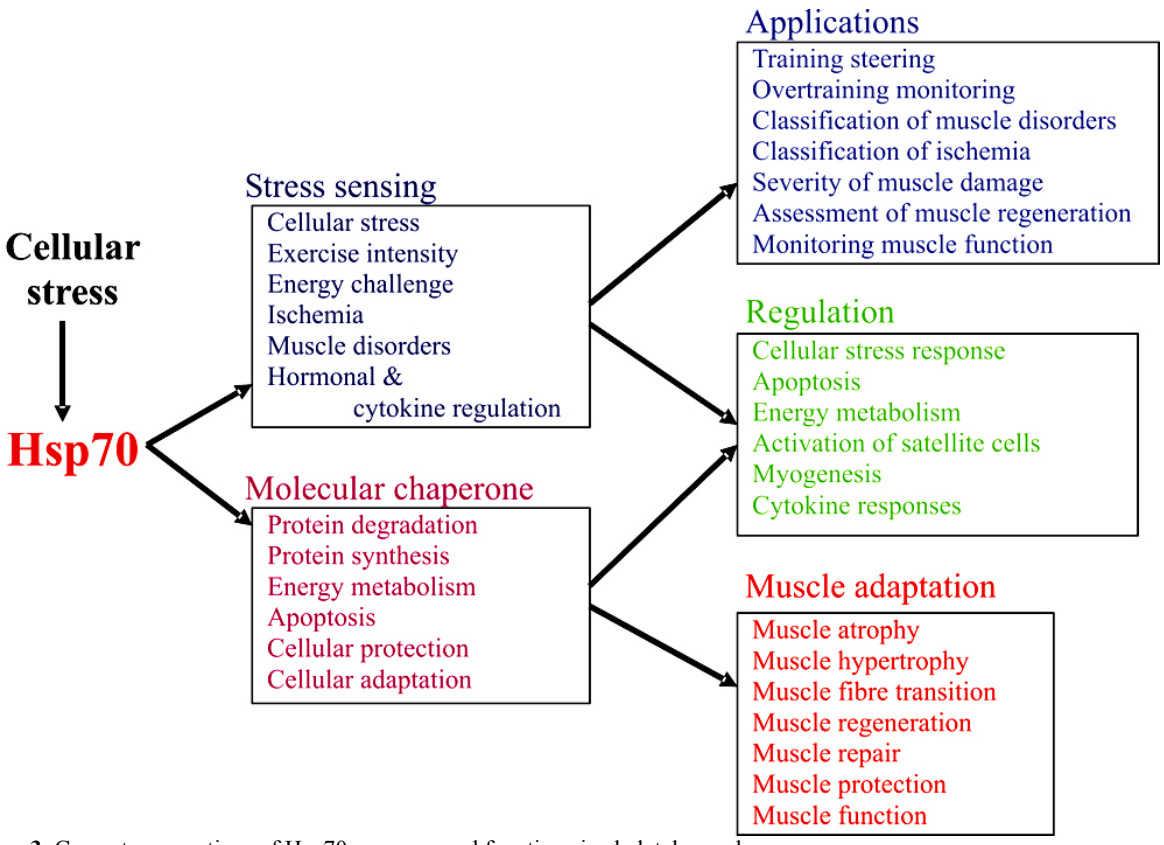

Figure 3. Current perspectives of Hsp70 response and functions in skeletal muscle.

(59). It has been reported that Hsp70 correlates with metabolic status of diabetes (264). A further study has demonstrated that decreased Hsp70 expression in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance (94), and the exercise resulting typically in restoration of insulin sensitivity can modulate Hsp70 response and improve glucose metabolism (95). A possible relation between Hsp70 and glucose transport proteins has been described in embryonic development (265). Actually, Hsp70 is associated with increased glucose flux (266). There is also evidence that heat shock stress induces Hsp70 accompanied by an up-regulation of sodium-dependent glucose transporter (70).

The other possible mechanism is regulation of calmodulin, a calcium regulatory protein, which is found to be associated with Hsp70. Calmodulin is known to interact with hexokinase, which is the only glycolytic enzyme that binds to mitochondria (72) and plays a role in hydrogen exchange (267). It has been reported that clotrimazole, a calmodulin-antagonist, reduces glycolysis (268). Indeed, glycolysis is controlled by allosteric enzymes like PFK and their reversible binding to the cytoskeleton (269). Furthermore, it has been shown that the (Ca^{2+} + Mg^{2+})-ATPase from erythrocyte ghosts catalyzed the hydrolysis of ATP together with the synthesis of ATP. This is controlled by high- and low-affinity calcium-binding sites asymmetrically located on the enzyme, and calmodulin stimulates overall turnover of the enzyme (71). In this context, Hsp70 may play a regulatory role in the glycolysis rather than a direct involvement in the glycolytic process. To clarify this point, further study is needed. It is also likely that Hsp70 effect on glycolysis is associated with its role in preventing the inactivation of lactate dehydrogenase caused by cellular stress (270). It is evident that heat shock increases glycogen synthesis and heat-shock-induced glycogenesis appears to be mediated via PI-3k/Akt-dependent glycogen synthase kinase-3 beta inactivation as well as PI-3k-independent glycogen-associated protein phosphatase 1 activation (271).

6. PERSPECTIVES

The skeletal muscle responses with Hsp70 induction to a variety of cellular stressful conditions, which include muscle disorders, exercise linked muscle contraction, temperature change, ischemia and reperfusion as well as cellular energy challenge. In this respect, Hsp70 serves as an indicator for cellular stress and plays an important role in signal transduction by its role in stress sensing in the muscle. There are evidences that Hsp70 response is involved in many cellular processes relating to muscle function, muscle regeneration and repair as well as muscle hypertrophy in terms of muscular adaptation. Herein Hsp70 plays an important role as a molecular chaperone. Figure 3.

However, although studies on Hsp70 response in skeletal muscle are emerging continuously, the role of Hsp70 response in the muscle is far from being completely

understood. As the stress indicator, the relationship between muscle disorders and Hsp70 response has not distinctly been clarified. In the induction of muscular Hsp70, cellular stresses linked to muscle contraction like calcium, temperature challenge, hypoxia and ischemia/reperfusion are among the important inducers. The interaction between Hsp70, hypoxia induced factor, and the sensor of cellular energy like kir6.2 of the ATP-dependent potassium channel. Thus, the role of Hsp70 in stress sensing, especially its relation to other cellular processes such as energy metabolism, hormonal and cytokine receptor regulation, myogenesis and apoptosis, has to be established further.

In terms of molecular chaperone, the role of Hsp70 in protein metabolism has been extensively investigated. Recently, it is increasingly recognized that in the process of muscular adaptation, apoptosis as well as myogenesis play a pivotal role. Studies now available have indicated some hints that Hsp70 may have profound impact on these cellular processes. However, there is lack of studies on the potentially direct effect of Hsp70 in facilitating cellular processes including apoptosis, vessel growth and myogenesis attributed to satellite cell activation. In particular, there seems a lack of studies dealing with the relationship between Hsp70 response and muscle function. With modern techniques like transgenic cell culture model we have begun to study the effect of Hsp70 on energy metabolism. Further studies are necessary in this direction, especially in vivo studies.

7. REFERENCES

1. Ritossa F: A new puffing pattern induced by temperature shock and DNP in Drosophila. *Experentia* 18, 571-573 (1962)
2. Lindquist S: The heat-shock response. *Ann Rev Biochem* 55, 1151-1191 (1986)
3. Locke M & E. G. Noble: Stress proteins: the exercsie response. *Can J Appl Physiol* 20, 155-167 (1995)
4. Tissieres A, H. K. Mitchell & U. M. Tracy: Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromsome puffs. *J Mol Biol* 84, 389-398 (1974)
5. Lindquist S & E. A. Craig: The heat-shock proteins. *Ann Rev Genet* 22, 631-677 (1988)
6. Hunt C & R. I. Morimoto: Conserved features of eukaryotic HSP70 genes revealed by comparison with the nucleotide sequence of human HSP70. *Proc Natl Acad Sci U S A* 82, 6455-6459 (1985)
7. Ingolia T. D, E. A. Craig & B. McCarty: Sequence of three copies of the gene for the major Drosophila heat shock induced protein and their flanking regions. *Cell* 21, 669-676 (1980)
8. Lindquist S & R. Petersen: Selective translation and degradation of heat-shock messenger RNAs in Drosophila. *Enzyme* 44, 147-166 (1990)
9. Pelham H. R: A regulatory upstream promoter element in the Drosophila hsp70 heat-shock gene. *Cell* 30, 517-528 (1982)
10. Steinacker J. M: Physiological aspects of training in rowing. *Int J Sports Med* 14, S3-S10 (1993)
11. Fehrenbach E & A. M. Niess: Role of heat shock proteins in the exercise response. *Exerc Immunol Rev* 5, 57-77 (1999)
12. Liu Y & J. M. Steinacker: Changes in skeletal muscle heat shock proteins: Pathological significance. *Front Biosci* 6, D12-D25 (2001)
13. Bond D. G & M. J. Schlesinger: Ubiquitin is a heat shock protein in chicken embryo fibroblasts. *Mol Cell Biol* 5, 949-956 (1986)
14. Thompson H. S & S. P. Scordilis: Ubiquitin changes in human biceps muscle following exercise-induced damage. *Biochem Biophys Res Commun* 204, 1193-1198 (1994)
15. Benjamin I. J, J. Shelton, D. J. Garry & J. A. Richardson: Temporospatial expression of the small HSP/alpha B-crystallin in cardiac and skeletal muscle during mouse development. *Dev Dyn* 208, 75-84 (1997)
16. Sakuma K, K. Watanabe, T. Tosuka & K. Kato: Pathological changes in levels of three small stress proteins, aB crystallin, HSP27 and P20, in the hindlimb muscle of dy mouse. *Biochem Biophys Acta* 1406, 162-168 (1998)
17. Stokoe D, K. Engel, D. G. Campbell, P. Cohen & M. Gaestel: Identification of MAPKAP kinase 2 as a major enzyme responsible for the phosphorylation of the small mammalian heat shock proteins. *FEBS Lett* 313, 307-313 (1992)
18. Carlsen N, S. Roger & M. Rechsteiner: Microinjection of ubiquitin: changes in protein degradation in Hela cells subjected to heat-shock. *J Cell Biol* 104, 547-555 (1987)
19. Inaguma Y, K. Hasegawa, K. Kato & Y. Nishida: cDNA cloning of a 20-kDa protein (p20) highly homologous to small heat shock proteins: developmental and physiological changes in rat hindlimb muscles. *Gene* 178, 145-150 (1996)
20. Neufer P. D & I. J. Benjamin: Differential expression of aB- crystallin and HSP27 in skeletal muscle during continuous contractive activtiy. Relationship to myogenic regulatory factors. *J Biol Chem* 271, 24089-24095 (1996)
21. Moseley P. L: Heat shock proteins and heat adaptation of the whole organism. *J Appl Physiol* 83, 1413-1417 (1997)
22. Horwitz J: Alpha-crystallin can function as a molecular chaperone. *Proc Natl Acad Sci U S A* 89, 10449-10453 (1992)
23. Jakob U, M. Gaestel, K. Engel & J. Buchner: Small heat shock proteins are molecular chaperones. *J Biol Chem* 268, 1517-1520 (1993)
24. Ellis J: Proteins as molecular chaperones. *Nature* 328, 378-379 (1987)
25. Ornatsky O. I., M. K. Connor & D. A. Hood: Expression of stress proteins and mitochondrial chaperonins in chronically stimulated skeletal muscle. *Biochem J* 311 (Pt 1), 119-123 (1995)
26. Cheng M. Y, F. U. Hartl, J. Martin, R. A. Pollock, F. Kalousek, W. Neupert, E. M. Hallberg, R. L. Hallberg & A. L. Horwich: Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast. *Science* 337, 620-625 (1989)
27. Martin J, A. L. Horwich & F. U. Hartl: Prevention of protein denaturation under heat shock stress by the chaperonin HSP60. *Science* 258, 995-998 (1992)
28. Montel V, F. Gardrat, J. L. Azanza & J. Raymond: Heat-shock protein 90: intrinsic peptidase activity and in

vitro long-term self-processing. *Life Sci* 67, 1585-1600 (2000)
29. Catelli M. G, N. Binart, I. Jung-Testas, J. M. Renoir, E. E. Baulieu, J. R. Feramisco & W. J. Welch: The common 90-kd protein component of non-transformed ´8S´steroid receptors is a heat-shock protein. *EMBO* J4, 3131-3135 (1985)
30. Dalman F. G, I. C. Scherrer, I. P. Taylor, H. Akil & W. B. Pratt: Localization of the 90-kDa heat shock protein-binding site within the hormone-binding domain of the glucocorticoid receptor by peptide competition. *J Biol Chem* 266, 3482-3490 (1991)
31. Botos J, W. Xian, D. F. Smith & C. L. Smith: Progesterone receptor deficient in chromatin binding has an altered cellular state. *J Biol Chem* 279, 15231-15239 (2004)
32. Tago K, F. Tshukahara, M. Naruse, T. Yoshioka & K. Takano: Regulation of nuclear retention of glucocorticoid receptor by nuclear Hsp90. *Mol Cell Endocrinol* 213, 131-138 (2004)
33. Sorger P. K & H. R. Pelham: Cloning and expression of a gene encoding HSC73, the major HSP70-like protein in unstressed rat cells. *EMBO J* 6, 993-998 (1987)
34. Welch W. J & J. R. Feramisco: Nuclear and nucleolar localization of the 72,000- dalton heat shock protein in heat-shocked mammalian cells. *J Biol Chem* 259, 4501-4513 (1984)
35. Welch W. J & J. P. Suhan: Cellular and biochemical events in mammalian cells during and after recovery from physiological stress. *J Cell Biol* 103, 2035-2052 (1986)
36. Donati Y. R. Y, D. O. Slosman & B. S. Polla: Oxidative injury and the heat shock response. *Biochm Pharmacol* 40, 2571-2577 (1990)
37. Voellmy R, A. Ahmed, P. Schilller, P. Bromley & D. Rungger: Isolation and functional analysis of a human 70,000-dalton heat shock protein gene segment. *Proc Natl Acad Sci U S A* 82, 4949-4953 (1985)
38. Hunt C. R, D. L. Gasser, D. D. Chaplin, J. C. Pierce & C. A. Kozak: Chromosomal localization of five murine HSP70 gene family members: HSP70-1, Hsp70-2, Hsp70-3, Hsc70t, and Grp78. *Genomics* 16, 193-198 (1993)
39. Mues, G.I, T. Z. Munn & J. D. Raese: A human gene family with sequence homology to Drosophila melangaster HSP70 heat shock genes. *J Biol Chem* 261, 874-877 (1986)
40. Bonnycastle L. L, C. E. Yu, C. R. Hunt, B. J. Trask, K. P. Clancy, J. L. Weber, D. Patterson & G. D. Schellenberg: Cloning, sequencing, and mapping of the human chromosome 14 heat shock protein gene (HSPA2). *Genomics* 23, 85-93 (1994)
41. Kiang J. G & G. C. Tsokos: Heat shock protein 70 kDa: Molecular biology, biochemistry, and physiology. *Pharmacol Ther* 80, 183-201 (1998)
42. Mayer M. P & B. Bukau: Hsp70 chaperones: Cellular functions and molecular mechanism. *Cell Mol Life Sci* 62, 670-684 (2005)
43. Ballinger C. A, P. Connell, Y. Wu, Z. Hu, L. J. Thompson, L. Y. Yin & C. Patterson: Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol Cell Biol* 19, 4535-4545 (1999)
44. Raynes D. A & V. Guerriero, Jr.: Inhibition of Hsp70 ATPase activity and protein renaturation by a novel Hsp70-binding protein. *J Biol Chem* 273, 32883-32888 (1998)
45. Bork P, C. Sander & A. Valencia: An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, and hsp70 heat shock proteins. *Proc Natl Acad Sci U S A* 89, 7290-7294 (1992)
46. Morshauser R. C, H. Wang, G. C. Flynn & E. R. P. Zuiderweg: The peptide-binding domain of the chaperone protein hsc70 has an unusual secondary structure topology. *Biochemistry* 34, 6261-6266 (1995)
47. Hightower L. E, S. E. Sadis & I. M. Takenaka: Interactions of vertebrate hsc70 and hsp70 with unfolded proteins and peptides. 179-207 (1994)
48. Hammond G. L, Y. K. Lai & C. L. Markert: Diverse forms of stress lead to new patterns of gene expression through common and essential pathway. *Proc Natl Acad Sci U S A* 79, 3485-3488 (1982)
49. Hightower L. E: Heat shock, stress proteins, chaperones, and proteotoxicity. *Cell* 66, 191-197 (1991)
50. Mitchell C. R, M. B. Harris, A. R. Cordaro & J. W. Starnes: Effect of body temperature during exercise on skeletal muscle cytochrome c oxidase content. *J Appl Physiol* 93, 526-530 (2002)
51. Liu Y, M. Lehmann, C. Baur, M. Storch, L. Sunder-Plassmann & J. M. Steinacker: HSP70 expression in skeletal muscle of patients with peripheral arterial occlusive disease. *Eur J Vasc Endovasc Surg* 24, 269-273 (2002)
52. Gampert L, A. Gebhardt, H. Reinelt, S. Schmidt, T. Marx & J. M. Steinacker: Ischemia induces HSP70 expression in porcine skeletal muscle. *Med Sci Sports Exerc* 36, S318-S319 (2004)
53. Liu Y, W. Lormes, L. Wang, S. Reißnecker & J. M. Steinacker: Different skeletal muscle HSP70 responses to high-intensity strength training and low-intensity endurance training. *Eur J Appl Physiol* 91, 330-335 (2004)
54. Liu Y, W. Lormes, S. Baur, A. Opitz-Gress, AltenburgmD., M. Lehmann & J. M. Steinacker: Human skeletal muscle HSP70 response to physical training depends on exercise intensity. *Int J Sports Med* 21, 351-355 (2000)
55. Milne K. J & E. G. Noble: Exercise-induced elevation of HSP70 is intensity dependent. *J Appl Physiol* 93, 561-568 (2002)
56. Lepore D & W. Marrison: Ischemic preconditioning: lack of delayed protection against skeletal muscle ischemia-reperfusion. *Microsurgery* 20, 350-355 (2000)
57. Nishizawa J, A. Nakai, T. Higashi, M. Tanabe, S. Nomoto, K. Matsuda, T. Ban & K. Nagata: Reperfusion causes significant activation of heat shock transcription factor 1 in ischemic rat heart. *Circulation* 94, 2185-2192 (1996)
58. Moseley P. L, E. S. Wallen, J. D. McCafferty, S. Flanagan & J. A. Kern: Heat stress regulates the human 70-kDa heat-shock gene through the 3´-untranslated region. *Am J Physiol* 264, L533-L537 (1993)
59. Bruce C. R, A. L. Carey, J. A. Hawley & M. A. Febbraio: Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism. *Diabetes* 52, 2338-2345 (2003)
60. Dupont-Versteegden E. E, J. D. Houle, R. A. Dennis, J. Zhang, M. Knox, G. Wagoner & C. A. Peterson: Exercise-

induced gene expression in soleus muscle is dependent on time after spinal cord injury in rats. *Muscle Nerve* 29, 73-81 (2004)
61. Puntschart A, M. Vogt, H. R. Widmer, H. Hoppeler & R. Billeter: Hsp70 expression in human skeletal muscle after exercise. *Acta Physiol Scand* 157, 411-417 (1996)
62. Liu Y, S. Mayr, A. Opitz-Gress, C. Zeller, W. Lormes, S. Baur, M. Lehmann & J. M. Steinacker: Human skeletal muscle HSP70 response to training in highly trained rowers. *J Appl Physiol* 86, 101-104 (1999)
63. Morimoto R. I, P. E. Kroeger & J. J. Cotto: The transcriptional regulation of heat shock genes: a plethora of heat shock factors and regulatory conditions. *Experentia* 77, 139-163 (1996)
64. Nakai A, Y. Kawazoe, M. Tanabe, K. Nagata & R. I. Morimoto: The DNA-binding properties of two heat shock factors, HSF1 and HSF3, are induced in the avian erythroblast cell lime HD6. *Mol Cell Biol* 15, 5268-5278 (1995)
65. Nakai A, M. Tanabe, Y. Kawazoe, J. Inazawa, R. I. Morimoto & K. Nagata: HSF4, a new member of the human heat shock factor family which lacks peptides of a transcriptional activator. *Mol Cell Biol* 17, 469-481 (1997)
66. Pirkkala L, P. Nykanen & L. Sistonen: Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. *FASEB J* 15, 1118-1131 (2001)
67. Tanikawa J, E. Ichikawa-Iwata, C. Kanei-Ishii, A. Nakai, S. Matsuzawa, J. C. Reed & S. Ishii: p53 suppresses the c-Myb-induced activation of heat shock transcription factor 3. *J Biol Chem* 275, 15578-15585 (2000)
68. Zheng Z & M. A. Yenari: The application of HSP70 as a target for gene therapy. *Front Biosci* 11, 699-707 (2006)
69. Steinacker J. M, L. Wang, L. Gampert, Y. Liu, K. Nething & O. Prokopchuk: Effects of overexpression of heat shock protein 70 on energy metabolism. *Med Sci Sports Exerc* 37, S455-(2005)
70. Ikari A, M. Nakano, K. kawano & Y. Suketa: Up-regulation of sodium-dependent glucose transporter by interaction with heat shock protein 70. *J Biol Chem* 277, 33338-33343 (2002)
71. Mas-Oliva J: Synthesis of ATP catalyzed by the (Ca^{2+} Mg^{2+})-ATPase from erythrocyte ghosts. Energy conservation in plasma membranes. *Biochem Biophys Acta* 812, 163-167 (1985)
72. Penso J & R. Beitner: Clotrimazole and bifonazole detach hexokinase from mitochondria of melanoma cells. *Eur J Pharmacol* 342, 113-117 (1998)
73. Craig E. A & C. A. Gross: Is hsp70 the cellular thermometer? *Trends Biochem Sci* 16, 135-139 (1991)
74. Lepock J. R, H. E. Frey, A. M. Rodahl & J. Kruuv: Thermal analysis of CHL V79 cells using differential scanning calorimetry: impliactions for hyperthermic cell killing and the heat shock response. *J Cell Physiol* 137, 14-24 (1988)
75. Njemini R, C. Demannet & T. Mets: Inflammatory status as an important determinant of heat shock protein 70 serum concentrations during aging. *Biogerontol* 5, 31-38 (2004)
76. Matranga V, R. Bonaventura & G. Di Bella: HSP70 as a stress marker of sea urchin coelomocytes in short term cultures. *Cell Mol Biol* 248, 345-349 (2002)
77. Jäättelä M, D. Wissing, K. Kokholm, T. Kallunki & M. Egeblad: Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases. *EMBO J* 17, 6124-6134 (1998)
78. Jäättelä M: Escaping Cell Death: Survival Proteins in Cancer. *Exp Cell Res* 248, 31-43 (1999)
79. Kaufmann S. H. E & B. Schoel: Heat shock proteins as antigenes in immunity against infection and self. In: The Biology of Heat Shock Proteins and Molecular Chaperones. Eds: Morimoto,R.I, Tissieres,A, Georgopoulos,C, Cold Spring Harbor Laboratory Press, Plainview, 495-531 (1994)
80. Moseley P. L: Exercise, stress, and the immune conversation. *Exerc Sport Sci Rev* 28, 128-132 (2000)
81. Flanagan S. W, A. J. Pyan, C. V. Gisolfi & P. L. Moseley: Tissue-specific HSP70 response in animals undergoing heat stress. *Am J Physiol* 268, R28-R32 (1995)
82. Pratt W. B & D. O. Toft: Regulation of Signalling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp Biol Med* 228, 111-133 (2003)
83. Lepock J. R, H. E. Frey & W. E. Inniss: Thermal analysis of bacteria by differential scanning calorimetry: relationship of protein denaturation in situ to maximum growth temperature. *Biochem Biophys Acta* 1055, 19-25 (1990)
84. Minami Y, J. Höhfeld, K. Ohtsuka & F. U. Hartl: regulation of the heat-shock protein 70 reaction cycle by the mammalian DNAJ homolog, Hsp40. *J Biol Chem* 271, 19617-19624 (1996)
85. Abe M, J. B. Manola, W. K. Oh, D. L. Parslow, D. J. George, C. L. Austin & P. W. Kantoff: Plasma levels of heat shock protein 70 in patients with prostate cancer: a potential biomarker for prostate cancer. *Clin Prostate Cancer* 3, 49-53 (2004)
86. Nething K, L. Wang, Y. Liu, W. Lormes & J. M. Steinacker: Blunted HSP70 response to acute exercise in well-trained skeletal muscle. *Med Sci Sports Exerc* 36, S318-(2004)
87. Ramaglia V, G. M. Harapa, N. White & L. T. Buck: Bacterial infection and tissue-specific Hsp72, -73 and -90 expression in western painted turtles. *Comp Biochem Physiol C Toxicol Pharmacol* 138, 139-148 (2004)
88. Martin J. E, K. Mather, M. Swash & A. B. Gray: Expression of heat shock protein epitopes in tubular aggregates. *Muscle & Nerve* 14, 219-225 (1991)
89. Rosenberg N. L, H. E. Neville & S. P. Ringel: Tubular aggregates. Their association with neuromuscular disease, including the syndrom of myalgia/cramps. *Arch Neurol* 42, 973-976 (1985)
90. Febbraio M. A, A. Steensberg, C. P. Fischer, C. Keller, N. Hiscock & B. K. Pedersen: IL-6 activates HSP72 gene expression in human skeletal muscle. *Biochem Biophys Res Commun* 296, 1264-1266 (2002)
91. Schett G, K. Redlich, P. Xu, P. Bizan, M. Gröger, M. Tohidast-Akrad, H. Kiener, J. Smolen & G. Steiner: Enhanced expression of heat shock protein 70 (hsp70) and heat shock factor 1 (HSF1) activation in rheumatoid arthritis synovial tissue. *J Clin Invest* 102, 302-311 (1998)
92. Ardle A. M & M. J. Jackson: Heat shock protein 70 expression in skeletal muscle. *Biochem Soc Trans* 24, 485S-(1996)
93. Bornman L, B. S. Polla, B. P. Lotz & G. S. Gericke: Expression of heat-shock/stress proteins in Duchenne muscular dystrophy. *Muscle & Nerve* 18, 23-31 (1995)

94. Kurucz I, A. Morva, A. Vaag, K. F. Eriksson, X. Huang, L. Groop & L. Koranyi: Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance. *Diabetes* 51, 1102-1109 (2002)
95. Atalay M, N. K. Oksala, D. E. Laaksonen, S. Khanna, C. Nakao, J. Lappalainen, S. Roy, O. Hanninen & C. K. Sen: Exercise training modulates heat shock protein response in diabetic rats. *J Appl Physiol* 97, 605-611 (2004)
96. Hirose L, K. Nosaka, M. Newton, A. Laveder, M. Kano, J. Peake & K. Suzuki: Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exerc Immunol Rev* 10, 75-90 (2004)
97. Thompson H. S, S. P. Scordilis, P. M. Clarkson & W. A. Lohrer: A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. *Acta Physiol Scand* 171, 187-193 (2001)
98. Thompson H. S, P. M. Clarkson & S. P. Scordilis: The repeated bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans. *Acta Physiol Scand* 174, 47-56 (2002)
99. Thompson H. S., E. B. Maynard, E. R. Morales & S. P. Scordilis: Exercise-induced HSP27, HSP70 and MAPK responses in human skeletal muscle. *Acta Physiol Scand* 178, 61-72 (2003)
100. Baracos V. E, C. DeVivo, D. H. Hoyle & A. L. Goldberg: Activation of the ATP-ubiquitin-proteasome pathway in skeletal muscle of cachectic rats bearing a hepatoma. *Am J Physiol* 268, E996-1006 (1995)
101. Medina R, S. S. Wing & A. L. Goldberg: Increase in levels of polyubiquitin and proteosome mRNA in skeletal muscle during starvation and denervation atrophy. *Biochem J* 307, 631-637 (1995)
102. Piatigorsky J, M. Kantorow, R. Gopal-Srivastava & S. I. Tomarev: Recruitment of enzymes and stress proteins as lens crystallins. *EXS* 71, 241-250 (1994)
103. Beck S. A, K. L. Smith & M. J. Tisdale: Anticachectic and antitumor effect of eicosapentaenoic acid and its effect on protein turnover. *Cancer Res* 51, 6089-6093 (1991)
104. Costelli P, N. Carbo, L. Tessitore, G. J. Bagby, F. J. Lopez-Soriano, J. M. Argiles & F. M. Baccino: Tumor necrosis factor- a mediates changes in tissue protein turnover in a rat cachexia model. *J Clin Invest* 92, 2783-2789 (1993)
105. Strelkov A. B. S, A. L. A. Fields & V. E. Baracos: Effect of systemic inhibition of prostaglandin production on protein metabolism in tumor-bearing rats. *Am J Physiol* 257, C261-C269 (1989)
106. Tessitore L, P. Costelli & F. M. Baccino: Hormonal medication for cachexia. *Br J Cancer* 67, 15-23 (1993)
107. Ku Z, J. Yang, V. Menon & D. B. Thompson: Decreased polysomal HSP70 may slow polypeptide elongation during skeletal muscle atrophy. *Am J Physiol* 268, C1369-C1374 (1995)
108. Vasilaki A, L. M. Iwanejko, F. McArdle, C. S. Broome, M. J. Jackson & A. McArdle: Skeletal muscles of aged male mice fail to adapt following contractile activity. *Biochem Soc Trans* 31, 455-456 (2003)
109. Vasilaki A, M. J. Jackson & A. McArdle: Attenuated HSP70 response in skeletal muscle of aged rats following contractile activity. *Muscle Nerve* 25, 902-905 (2002)
110. Naito H, S. K. Powers, H. A. Demirel & J. Aoki: Exercise training increases heat shock protein in skeletal muscles of old rats. *Med Sci Sports Exerc* 33, 729-734 (2001)
111. Oishi Y, K. Taniguchi, H. Matsumoto, F. Kawano, A. Ishihara & Y. Ohira: Upregulation of HSP72 in reloading rat soleus muscle after prolonged hindlimb unloading. *Jpn J Physiol* 53, 281-286 (2003)
112. Desplanches D, L. Ecochard, B. Sempore, M. H. Mayet-Sornay & R. Favier: Skeletal muscle HSP72 response to mechanical unloading: influence of endurance training. *Acta Physiol Scand* 180, 387-394 (2004)
113. Kilgore J. L, B. F. Timson, D. K. Saunders, R. R. Kraemer, R. D. Klemm & C. R. Ross: Stress protein induction in skeletal muscle: comparison of laboratory models to naturally occurring hypertrophy. *J Appl Physiol* 76, 598-601 (1994)
114. Izumo S, B. Nadal-Ginard & V. Mahdavi: Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. *Proc Natl Acad Sci U S A* 85, 339-343 (1988)
115. Liu Y, A. Schlumberger, K. Wirth, D. Schmidtbleicher & J. M. Steinacker: Different effects on human skeletal myosin heavy chain isoform expression: strength training vs combination training. *J Appl Physiol* (2003)
116. Brkic M, Y. Liu, A. Schlumberger, K. Wirth, D. Schmidtbleicher & J. M. Steinacker: Arm muscle HSP70 response to strength training. *Med Sci Sports Exerc* 36, S318-(2004)
117. Willoughby D. S, J. W. Priest & M. Nelson: Expression of the stress proteins, ubiquitin, heat shock protein 72, and myofibrillar protein content after 12 weeks of leg cycling in persons with spinal cord injury. *Arch Phys Med Rehabil* 83, 649-654 (2002)
118. Ueno S, K. Yokoyama, M. Okuno, R. S. Wang, Y. Fujioka & Y. Kobayashi: Effects of static load on the weight and protein content in the leg muscles of the mouse: a simulation of prolonged standing in the workplace. *Ind Health* 42, 401-407 (2004)
119. Adams G. R: Exercise effects on muscle insulin signalling and action. Invited review: Autocrine/paracrine IGF-1 and skeletal muscle adaptation. *J Appl Physiol* 93, 1159-1167 (2002)
120. Chen H. S, Y. X. Shan, T. L. Yang, H. D. Lin, J. W. Chen, S. J. Lin & P. H. Wang: Insulin deficiency downregulated heat shock protein 60 and IGF-1 receptor signalling in diabetic myocardium. *Diabetes* 54, 175-181 (2005)
121. Coolican S. A, D. S. Samuel, D. Z. Ewton, F. J. McWade & J. R. Florini: The mitogenic and myogenic actions of insulin-like growth factors utilize distinct signalling pathways. *J Biol Chem* 272, 6653-6672 (1997)
122. Hameed M, R. W. Orrell, M. Cobbold, G. Goldspink & S. D. R. Harridge: Expression of IGF-1 splice variants in young and old human skeletal muscle after high resistance exercise. *J Physiol* 547, 247-254 (2003)
123. Liu Y, M. Heinichen, L. Gampert, A. Schlumberger, d. Schmidtbleicher & J. M. Steinacker: Strength training induced expression of mechano-growth factor in human skeletal muscle. *Med Sci Sports Exerc* 36, S52-(2004)
124. Necker A, Y. Liu, A. Schlumberger, K. Wirth, d. Schmidtbleicher & J. M. Steinacker: IGF-1 Response in

human skeletal muscle to strength training. *Med Sci Sports Exerc* 36, S184-(2004)
125. Pa´ rrizas M, A. R. Saltiel & D. LeRoith: Insulin-like growth factor 1 inhibits apoptosis using the phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways. *J Biol Chem* 272, 154-161 (1997)
126. Klomkleaw W, Y. Kasashima, G. A. Fuller, A. Kobayashi, T. Yoshihara, M. A. Oikawa, Y. Izumisawa & M. Yamaguchi: Horse lumbrical muscle: possible structural and functional reorganization in regressive muscle. *Anat Histol Embryol* 31, 85-98 (2002)
127. Beere H. M, B. B. Wolf, K. Cain, D. D. Mosser, A. Mahboubi, T. Kuwana, P. Tailor, R. I. Morimoto, G. M. Cohen & D. R. Green: Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol* 2, 469-475 (2000)
128. Siu P. M, R. W. Bryner, J. K. Martyn & S. E. Alway: Apoptotic adaptations from exercise training in skeletal and cardiac muscles. *FASEB J* 18, 1150-1152 (2004)
129. Ali A, P. Fernando, W. L. Smith, N. Ovsenek, J. R. Lepock & J. J. Heikkila: Preferential activation of HSF-binding activity and hsp70 gene expression in Xenopus heart after mild hyperthermia. *Cell Stress Chaperones* 2, 229-237 (1997)
130. Lepore D. A, K. R. Knight, R. L. Anderson & W. A. Morrison: Role of priming stresses and Hsp70 in protection from ischemia-reperfusion injury in cardiac and skeletal muscle. *Cell Stress Chaperones* 6, 93-96 (2001)
131. Manzerra P, S. J. Rush & I. R. Brown: Tissue-specific differences in heat shock protein hsc70 and hsp70 in the control and hyperthermic rabbit. *J Cell Physiol* 170, 130-137 (1997)
132. Oishi Y, K. Taniguchi, H. Matsumoto, A. Ishihara, Y. Ohira & R. R. Roy: Differential responses of HSPs to heat stress in slow and fast regions of rat gastrocnemius muscle. *Muscle Nerve* 28, 587-594 (2003)
133. Oishi Y, K. Taniguchi, H. Matsumoto, A. Ishihara, Y. Ohira & R. R. Roy: Muscle type-specific response of HSP60, HSP72, and HSC73 during recovery after elevation of muscle temperature. *J Appl Physiol* 92, 1097-1103 (2002)
134. Kim K. B, M. H. Kim & D. J. Lee: The effect of exercise in cool, control and hot environments on cardioprotective HSP70 induction. *J Physiol Anthropol Appl Human Sci* 23, 225-230 (2004)
135. Harris M. B & J. W. Starnes: Effects of body temperature during exercise training on myocardial adaptations. *Am J Physiol Heart Circ Physiol* 280, H2271-H2280 (2001)
136. Locke M & C. Celotti: Cold stress does not induce stress proteins SP 25 and SP 72 in rat skeletal muscle. *Cryobiology* 43, 54-62 (2001)
137. Skidmore R, J. A. Gutierrez, V. Guerriero, Jr. & K. C. Kregel: HSP70 induction during exercise and heat stress in rats: role of internal temperature. *Am J Physiol* 268, R92-7 (1995)
138. Kobayashi T, K. Goto, A. Kojima, T. Akema, K. Uehara, H. Aoki, T. Sugiura, Y. Ohira & T. Yoshioka: Possible role of calcineurin in heating-related increase of rat muscle mass. *Biochem Biophys Res Commun* 331, 1301-1309 (2005)
139. Naito H, S. K. Powers, H. A. Demirel, T. Sugiura, S. L. Dodd & J. Aoki: Heat stress attenuates skeletal muscle atrophy in hindlimb-unweighted rats. *J Appl Physiol* 88, 359-363 (2000)
140. Selsby J. T & S. L. Dodd: Heat treatment reduces oxidative stress and protects muscle mass during immobilization. *Am J Physiol Regul Integr Comp Physiol* 289, R134-R139 (2005)
141. Kim I, H. M. Shin & W. Baek: Heat-shock response is associated with decreased production of interleukin-6 in murine aortic vascular smooth muscle cells. *Naunyn Schmiedebergs Arch Pharmacol* 371, 27-33 (2005)
142. Tupling A. R, A. O. Gramolini, T. A. Duhamel, H. Kondo, M. Asahi, S. C. Tsuchiya, M. J. Borrelli, J. R. Lepock, K. Otsu, M. Hori, D. H. MacLennan & H. J. Green: HSP70 binds to the fast-twitch skeletal muscle sarco(endo)plasmic reticulum Ca2+ -ATPase (SERCA1a) and prevents thermal inactivation. *J Biol Chem* 279, 52382-52389 (2004)
143. Chen H. W, S. C. Chen, J. L. Tsai & R. C. Yang: Previous hyperthermic treatment increases mitochondria oxidative enzyme activity and exercise capacity in rats. *Kaohsiung J Med Sci* 15, 572-580 (1999)
144. Lille S, C. Y. Su, T. Schoeller, H. Suchy, S. Lyons, R. C. Russell, M. Neumeister & C. C. Lai: Induction of heat-shock protein 72 in rat skeletal muscle does not increase tolerance to ischemia-reperfusion injury. *Muscle Nerve* 22, 390-393 (1999)
145. Thomas J. A & E. G. Noble: Heat shock does not attenuate low-frequency fatigue. *Can J Physiol Pharmacol* 77, 64-70 (1999)
146. Welch W. J: Mammalian stress response: Cell physiology, structure/function of stress proteins, and implications for medicine and disease. *Physiol Rev* 72, 1063-1081 (1992)
147. Benjamin I. J, B. Kröger & R. S. Williams: Activation of the heat shock transcription factor by hypoxia in mammalian cells. *Proc Natl Acad Sci U S A* 87, 6263-6267 (1990)
148. Donnelly T. J, R. S. Sievers, F. L. J. Vissern, W. J. Welch & C. L. Wolfe: Heat shock protein induction in rat hearts. A role for improved myocardial salvage after ischemia and reperfusion? *Circulation* 85, 769-778 (1992)
149. Knowlton A. A, P. Brecher & C. S. Apstein: Rapid expression of heat shock protein in the rabbit after brief cardiac ischemia. *J Clin Invest* 87, 139-147 (1991)
150. Son M, A. R. Shahed, P. M. Werchan & J. C. Lee: C-fos and Hsp70 gene expression in rat brains in high gravitation-induced cerebral ischemia. *Neurosci Lett* 200, 81-84 (1995)
151. Mestril R & W. H. Dillmann: Heat shock proteins and protection against myocardial ischemia. *J Mol Cell Cardiol* 27, 45-52 (1995)
152. Liauw S. K, B. B. Rubin, T. F. Lindsay, A. D. Romaschin & P. M. Walker: Sequential ischemia/reperfusion results in contralateral skeletal muscle salvage. *Am J Physiol* 270, H1407-H1413 (1996)
153. Cornelussen R, W. Spiering, J. H. G. Webers, L. De Bruin, R. S. Reneman, G. J. Van der Vusse & L. H. E. H. Snoeckx: Heat shock improves ischemic tolerance of hypertrophed rat hearts. *Am J Physiol* 267, H1941-H1947 (1994)
154. Bonventre J. V: Mediators of ischemic renal injury. *Ann Rev Med* 39, 531-544 (1988)

155. Steinacker J. M, A. Opitz-Gress, S. Baur, W. Lormes, L. Sunder-Plassmann, F. Liewald, M. Lehmann & Y. Liu: Expression of myosin heavy chain isoforms in skeletal muscle of patients with peripheral arterial occlusive disease. *J Vasc Surg* 31, 443-449 (2000)
156. Bushell A. J, L. Klenerman, H. Davies, I. Grierson, A. McArdle & M. J. Jackson: Ischaemic preconditioning of skeletal muscle 2. Investigation of the potential mechanisms involved. *J Bone Joint Surg Br* 84, 1189-1193 (2002)
157. Storz G, L. A. Tartaglia & B. N. Ames: Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. *Science* 243, 189-194 (1990)
158. Ramaglia V & L. T. Buck: Time-dependent expression of heat shock proteins 70 and 90 in tissues of the anoxic western painted turtle. *J Exp Biol* 207, 3775-3784 (2004)
159. Nething K, L. Gampert, A. Necker, Y. Liu & J.M. Steinacker: Influence of exercise on myogenic markers and stress proteins in ischemic human skeletal muscle. *Finn Sports and Exerc Med e-Magazine – The International XVIII Puijo Symposium special issue* (2005)
160. Khassaf M, A. McArdle, C. Esanu, A. Vasilaki, F. McArdle, R. D. Griffiths, D. A. Brodie & M. J. Jackson: Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. *J Physiol* 549, 645-652 (2003)
161. Plumier J. C. L, B. M. Ross, R. W. Currie, C. E. Angelidis, H. Kazlaris, G. Kollias & G. N. Pagoulatos: Transgenic mice expressing the human heat shock protein 70 have improved post-ischemic myocardial recovery. *J Clin Invest* 95, 1854-1860 (1995)
162. Liu Y, A. Opitz-Gress, A. Rott, F. Liewald, L. Sunder-Plassmann, M. Lehmann & J. M. Steinacker: Effect of felodipine on regional blood supply and collateral vascular resistance i patients with peripheral artereial occlusive disease. *Vasc Med* 2, 13-18 (1997)
163. Liu Y, J. M. Steinacker & M. Stauch: Transcutaneous oxygen tension and Doppler ankle pressure during upper and lower body exercise in patients with peripheral arterial occlusive disease. *Angiology* 56, 689-698 (1995)
164. Liu Y, J. M. Steinacker, A. Opitz-Gress, M. Clausen & M. Stauch: Comparison of whole-body thallium imaging with transcutaneous PO_2 in studying regional blood supply in patients with peripheral arterial occlusive disease. *Angiology* 47, 879-886 (1996)
165. Fehrenbach E, A. M. Niess, R. Veith, H. H. Dickhutt & H. Northoff: Changes of HSP72-expression in leukocytes are associated with adaptation to exercise under conditions of high environmental temperature. *J Leukoc Biol* 69, 747-754 (2001)
166. Ryan A. J, C. V. Cisolfi & P. L. Moseley: Synthesis of 70k stress protein by human leukocytes - Effect of exercise in the heat. *J Appl Physiol* 70, 466-471 (1991)
167. Salo D. C, C. M. Donovan & K. J. Davies: HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. *Free Radic Biol Med* 11, 239-246 (1991)
168. Khassaf M, R. B. Child, A. McArdle, D. A. Brodie, C. Esanu & M. J. Jackson: Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise. *J Appl Physiol* 90, 1031-1035 (2001)
169. Samelman T. R, L. J. Shiry & D. F. Cameron: Endurance training increases the expression of mitochondrial and nuclear encoded cytochrome c oxidase subunits and heat shock proteins in rat skeletal muscle. *Eur J Appl Physiol* 83, 22-27 (2000)
170. Samelman T. R: Heat shock protein expression is increased in cardiac and skeletal muscles of Fischer 344 rats after endurance training. *Exp Physiol* 85, 92-102 (2000)
171. Paroo Z & E. G. Noble: Isoproterenol potentiates exercise-induction of Hsp70 in cardiac and skeletal muscle. *Cell Stress Chaperones* 4, 199-204 (1999)
172. Paroo Z, P. M. Tiidus & E. G. Noble: Estrogen attenates HSP72 expression in acutely exercised male rodents. *Eur J Appl Physiol* 80, 180-184 (1999)
173. Paroo Z, E. S. Dipchand & E. G. Noble: Estrogen attenuates postexercise HSP70 expression in skeletal muscle. *Am J Physiol* 282, C245-C251 (2002)
174. Locke M: The cellular stress response to exercise: role of stress proteins. *Exerc Sport Sci Rev* 25, 105-136 (1997)
175. Brooks G. A, K. J. Hilttelman, J. A. Faulkner & R. E. Beyer: Tissue temperatures and whole-animal oxygen consumption after exercise. *Am J Phsiol* 221, 427-431 (1971)
176. Xu L, Y. Zhao, Q. Zhang, Y. Li & Y. Xu: Regulation of transgene expression in muscles by ultrasound-mediated hyperthermia. *Gene Ther* 11, 894-900 (2004)
177. Locke M & E. Nussbaum: Continuous and pulsed ultrasound do not increase heat shock protein 72 content. *Ultrasound Med Biol* 27, 1413-1419 (2001)
178. Ingalls C. P, G. L. Warren & R. B. Armstrong: Dissociation of force production from MHC and actin contents in muscles injured by eccentric contractions. *J Muscle Res Cell Motil* 19, 215-224 (1998)
179. Kraniou Y, D. Cameron-Smith, M. Misso, G. Collier & M. Hargreaves: Effects of exercise on GLUT-4 and glycogenin gene expression in human skeletal muscle. *J Appl Physiol* 88, 794-796 (2000)
180. Steinacker J. M & Y. Liu: Stress proteins and applied exercise physiology. In: Exercise and Stress Response - The Role of Stress Proteins. Eds: Locke, M, Noble, E,G, CRC Press, Boca Raton, 211-230 (2002)
181. Febbraio M. A, J. L. Mesa, J. Chung & A. Steenberg: Glucose ingestion attenuates the exercise-induced increase in circulating heat shock protein 72 and heat shock protein 60 in humans. *Cell Stress Chaperones* 9, 390-396 (2004)
182. Whelan S. A & L. E. Hightower: Differential induction of glucose-regulated and heat shock proteins: effects of pH and sulfhydryl-reducing agents on chicken embryo cells. *J Cell Physiol* 125, 251-258 (1985)
183. Poso A. R, S. Eklund-Uusitalo, S. Hyyppa & E. Pirila: Induction of heat shock protein 72 mRNA in skeletal muscle by exercise and training. *Equine Vet J Suppl* 214-218 (2002)
184. Kinnunen S, S. Hyyppa, J. Lappalainen, N. Oksala, M. Venojarvi, C. Nakao, O. Hanninen, C. K. Sen & M. Atalay: Exercise-induced oxidative stress and muscle stress protein responses in trotters. *Eur J Appl Physiol* 93, 496-501 (2005)

185. McArdle A & M. J. Jackson: Exercise, oxidative stress and ageing. *J Anat* 197 Pt 4, 539-541 (2000)
186. McArdle A, A. Vasilaki & M. Jackson: Exercise and skeletal muscle ageing: cellular and molecular mechanisms. *Ageing Res Rev* 1, 79-93 (2002)
187. McDuffee A. T, G. Senisterra, S. Huntley, J. R. Lepock, K. R. Sekhar, M. J. Meredith, M. J. Borrelli, J. D. Morrow & M. L. Freeman: Proteins containing non-native disulfide bonds generated by oxidative stress can act as signals for the induction of the heat shock response. *J Cell Physiol* 171, 143-151 (1997)
188. Servais S, K. Couturier, H. Koubi, J. L. Rouanet, D. Desplanches, M. H. Sornay-Mayet, B. Sempore, J. M. Lavoie & R. Favier: Effect of voluntary exercise on H2O2 release by subsarcolemmal and intermyofibrillar mitochondria. *Free Radic Biol Med* 35, 24-32 (2003)
189. Wallen E. S, G. R. Beuttner & P. L. Moseley: Oxidants differentially regulate the heat shock response. *Int J Hyperthermia* 13, 517-524 (1997)
190. Clarkson P. M & S. P. Sayers: Etiology of exercise-induced muscle damage. *Can J Appl Physiol* 24, 234-248 (1999)
191. Lehmann M, C. Foster, H. H. Dickhutt & U. Gastmann: Autonomic imbalance hypothesis and overtraining syndrome. *Med Sci Sports Exerc* 30, 1140-1145 (1998)
192. Currie R. W, M. Karmazyn, M. Kloc & K. Mailer: Heat-shock response is associated with enhanced postischemic ventricular recovery. *Circ Res* 63, 543-549 (1988)
193. Currie R. W, R. M. Tanguay & J. G. Kingma: Heat-shock response and limitation of tissue necrosis during occlusion/reperfusion in rabbit hearts. *Circulation* 87, 963-971 (1993)
194. Lepore D. A, J. V. Hurley, A. G. Stewart, W. A. Morrison & R. L. Anderson: Prior heat stress improves survival of ischemic-reperfused skeletal muscle in vivo. *Muscle Nerve* 23, 1847-1855 (2000)
195. Heads R. J, D. S. Latchman & D. M. Yellon: Stable high level expression of a transfected human HSP70 gene protects a heart-derived muscle cell line against thermal stress. *J Mol Cell Cardiol* 26, 695-699 (1994)
196. Cao Y, T. Matsumoto, K. Motomura, A. Ohtsuru, S. Yamashita & M. Kosaka: Impaired induction of heat shock protein implicated in decreased thermotolerance in a temperature-sensitive multinucleated cell line. *Pflügers Arch - Eur J Physiol* 437, 15-20 (1998)
197. Tupling A. R, A. O. Gramolini, T. A. Duhamel, H. Kondo, M. Asahi, S. C. Tsuchiya, M. J. Borrelli, J. R. Lepock, K. Otsu, M. Hori, D. H. MacLennan & H. J. Green: HSP70 binds to the fast-twitch skeletal muscle sarco(endo)plasmic reticulum Ca2+ -ATPase (SERCA1a) and prevents thermal inactivation. *J Biol Chem* 279, 52382-52389 (2004)
198. Baumeister S, N. Ofer, C. Kleist, P. Terne, G. Opelz, M. M. Gebhard, G. Germann & C. Heitmann: Reduction of skeletal muscle injury in composite tissue allotransplantation by heat stress preconditioning. *Plast Reconstr Surg* 114, 1832-1841 (2004)
199. Goto K, M. Honda, T. Kobayashi, K. Uehara, A. Kojima, T. Akema, T. Sugiura, S. Yamada, Y. Ohira & T. Yoshioka: Heat stress facilitates the recovery of atrophied soleus muscle in rat. *Jpn J Physiol* 54, 285-293 (2004)
200. Goto K, R. Okuyama, H. Sugiyama, M. Honda, T. Kobayashi, K. Uehara, T. Akema, T. Sugiura, S. Yamada, Y. Ohira & T. Yoshioka: Effects of heat stress and mechanical stretch on protein expression in cultured skeletal muscle cells. *Pflugers Arch* 447, 247-253 (2003)
201. Duguez S, M. C. Bihan, D. Gouttefangeas, L. Feasson & D. Freyssenet: Myogenic and nonmyogenic cells differentially express proteinases, Hsc/Hsp70, and BAG-1 during skeletal muscle regeneration. *Am J Physiol Endocrinol Metab* 285, E206-E215 (2003)
202. Liu Y, W. Lormes, S. Reißnecker & J. M. Steinacker: Effects of high intensity resistance and low intensity endurance training on myosin heavy chain isoform expression in highly trained rowers. *Int J Sports Med* (2003)
203. Pette D & R. S. Staron: Mammalian skeletal muscle fiber type transition. *Int Rev Cytol* 170, 143-223 (1997)
204. Pette D: Training effects on the contractile apparatus. *Acta Physiol Scand* 162, 367-376 (1998)
205. Pette D & R. S. Staron: Myosin isoforms, muscle fiber types, and transitions. *Microsc Res Tech* 50, 500-509 (2000)
206. Beckmann R. P, L. A. Mizzen & W. J. Welch: Interaction of HSP70 with newly synthesized proteins: Implications for protein folding and assembly. *Science* 248, 850-854 (1990)
207. Locke M, E. G. Noble & B. G. Aktinson: Inducible isoform of HSP70 in constitutively expressed in a muscle fiber type specific pattern. *Am J Physiol* 261, C774-C779 (1991)
208. Neufer P. D, G. A. Ordway, G. A. Hand, J. M. Shelton, J. A. Richardson, I. J. Benjamin & R. S. Williams: Continuous contractile activity induces fiber type specific expression of HSP70 in skeletal muscle. *Am J Physiol* 271, C1828-C1837 (1996)
209. Maglara A. A, A. Vasilaki, M. J. Jackson & A. McArdle: Damage to developing mouse skeletal muscle myotubes in culture: protective effect of heat shock proteins. *J Physiol* 548, 837-846 (2003)
210. Vekris A, C. Aurange, C. Moonen, F. Mazurier, H. de-Verneuil, P. Canioni & P. Voisin: Control of transgene expression using local hyperthermia in combination with a heat-sensitive promoter. *J Gene Med* 2, 89-96 (2000)
211. Wagner M, I. Hermanns, F. Bittinger & C. J. Kirkpatrick: Induction of stress proteins in human endothelial cells by heavy metal ions and heat shock. *Am J Physiol* 277, L1026-L1033 (1999)
212. Voss M. R, J. N. Stallone, M. Li, R. N. Cornelussen, P. Knuefermann & A. A. Knowlton: Gender differences in the expression of heat shock proteins: the effect of estrogen. *Am J Physiol Heart Circ Physiol* 285, H687-H692 (2003)
213. Eder K. J, C. M. Leutenegger, B. W. Wilson & I. Werner: Molecular and cellular biomarker responses to pesticide exposure in juvenile chinook salmon (Oncorhynchus tshawytscha). *Mar Environ Res* 58, 809-813 (2004)
214. Zhang Z, J. D. Ferraris, H. L. Brooks, I. Brisc & M. B. Burg: Expression of osmotic stress-related genes in tissues

of normal and hyposmotic rats. *Am J Physiol Renal Physiol* 285, F688-F693 (2003)
215. Fehrenbach E, A. M. Niess, E. Schlotz, F. Passek, H. H. Dickhutt & H. Northoff: Transcriptional and translational regulation of heat shock proteins in leukocytes of endurance runners. *J Appl Physiol* 89, 704-(2000)
216. Walsh R. C, I. Koukoulas, A. Garnham, P. L. Moseley, M. Hargreaves & M. A. Febbraio: Exercise increases serum Hsp72 in humans. *Cell Stress Chaperones* 6, 386-393 (2001)
217. Febbraio M. A, P. Ott, H. B. Nielsen, A. Steensberg, C. Keller, P. Krustrup, N. H. Secher & B. K. Pedersen: Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. *J Physiol* 544, 957-962 (2002)
218. Nishi S, W. Taki, Y. Uemura, T. Higashi, H. Kikuchi, H. Kudoh, M. Satoh & K. Nagata: Ischemic tolerance due to induction of HSP70 in a rat ischemic recirculation model. *Brain Res* 615, 281-288 (1993)
219. Billeter R, H. Weber, H. Lutz, H. Howald, H. M. Eppenberger & E. Jenny: Myosin types in human skeletal muscle fibers. *Histochem* 65, 249-269 (1980)
220. Wada M, N. Hämäläinen & D. Pette: Isomyosin patterns of single type IIB, IID and IIA fibers from rabbit skeletal muscle. *J Muscle Res Cell Motil* 16, 237-242 (1995)
221. Kelly D. A, P. M. Tildus, M. E. Houston & E. G. Noble: Effect of vitamin E deprivation and exercise training on induction of HSP70. *J Appl Physiol* 81, 2379-2385 (1996)
222. Ookawara T, K. Suzuk, S. Haga, S. Ha, K. S. Chung, K. Toshinai, T. Hamaoka, T. Katsumura, T. Takemasa, M. Mizuno, Y. Hitomi, T. Kizaki, K. Suzuki & H. Ohno: Transcription regulation of gene expression in human skeletal muscle in response to endurance training. *Res Commun Mol Pathol Pharmacol* 111, 41-54 (2002)
223. Senisterra G. A, S. A. Huntley, M. Escaravage, K. R. Sekhar, M. L. Freeman, M. Borrelli & J. R. Lepock: Destabilization of the Ca2+-ATPase of sarcoplasmic reticulum by thiol-specific, heat shock inducers results in thermal denaturation at 37 degrees C. *Biochemistry* 36, 11002-11011 (1997)
224. Selvakumar S & P. Geraldine: Heat shock protein induction in the freshwater prawn Macrobrachium malcolmsonii: acclimation-influenced variations in the induction temperatures for Hsp70. *Comp Biochem Physiol A Mol Integr Physiol* 140, 209-215 (2005)
225. Xi L, D. Tekin, P. Bhargava & R. C. Kukreja: Whole body hyperthermia and preconditioning of the heart: basic concepts, complexity, and potential mechanisms. *Int J Hyperthermia* 17, 439-455 (2001)
226. Chiu J. H, M. T. Tsou, H. H. Tung, C. H. Tai, S. K. Tsai, C. L. Chih, J. G. Lin & C. W. Wu: Preconditioned somatothermal stimulation on median nerve territory increases myocardial heat shock protein 70 and protects rat hearts against ischemia-reperfusion injury. *J Thorac Cardiovasc Surg* 125, 678-685 (2003)
227. Garramone R. R, Jr., R. M. Winters, D. K. Das & P. J. Deckers: Reduction of skeletal muscle injury through stress conditioning using the heat-shock response. *Plast Reconstr Surg* 93, 1242-1247 (1994)
228. Ianuzzo C. D, S. E. Ianuzzo, M. Feild & M. Locke: Cardiomyoplasty: preservation of the latissimus dorsi muscle. *J Card Surg* 10, 104-110 (1995)
229. Luo G, X. Sun, E. Hungness & P. O. Hasselgren: Heat shock protects L6 myotubes from catabolic effects of dexamethasone and prevents downregulation of NF-kappaB. *Am J Physiol Regul Integr Comp Physiol* 281, R1193-R1200 (2001)
230. Suzuki K, R. T. Smolenski, J. Jayakumar, B. Murtuza, N. J. Brand & M. H. Yacoub: Heat shock treatment enhances graft cell survival in skeletal myoblast transplantation to the heart. *Circulation* 102, III216-III221 (2000)
231. Naito H, S. K. Powers, H. A. Demirel, T. Sugiura, S. L. Dodd & J. Aoki: Heat stress attenuates skeletal muscle atrophy in hindlimb-unweighted rats. *J Appl Physiol* 88, 359-363 (2000)
232. Kilgore J. L, T. I. Musch & C. R. Ross: Physical activity, muscle, and the HSP70 response. *Can J Appl Physiol* 23, 245-260 (1998)
233. Hutter M. M, R. E. Sievers, V. Barbosa & C. L. Wolfe: Heat-shock protein induction in rat hearts. A direct correlation between the amount of heat-shock protein induced and the degree of myocardial protection. *Circulation* 89, 355-360 (1994)
234. Koh T. J: Do small heat shock proteins protect skeletal muscle from injury? *Exerc Sport Sci Rev* 30, 117-121 (2002)
235. Mair J, M. Mayr, E. Muller, A. Koller, C. Haid, E. Artner Dworzak, C. Calzolari, C. Larue & B. Puschendorf: Rapid adaptation to eccentric exercise-induced muscle damage. *Int J Sports Med* 16, 352-356 (1995)
236. McArdle A, W. H. Dillmann, R. Mestril, J. A. Faulkner & M. J. Jackson: Overexpression of HSP70 in mouse skeletal muscle protects against muscle damage and age-related muscle dysfunction. *FASEB J* 18, 355-357 (2004)
237. Webster K. A: Serine phosphorylation and suppression of apoptosis by the small heat shock protein alphaB-crystallin. *Circ Res* 92, 130-132 (2003)
238. Kamradt M. C, F. Chen, S. Sam & V. L. Cryns: The small heat shock protein alpha B-crystallin negatively regulates apoptosis during myogenic differentiation by inhibiting caspase-3 activation. *J Biol Chem* 277, 38731-38736 (2002)
239. Roth E, R. Oehler, N. Manhart, R. Exner, B. Wessner, E. Strasser & A. Spittler: Regulative potential of glutamine--relation to glutathione metabolism. *Nutrition* 18, 217-221 (2002)
240. Zhou X & J. R. Thompson: Regulation of protein turnover by glutamine in heat-shocked skeletal myotubes. *Biochim Biophys Acta* 1357, 234-242 (1997)
241. Ito H, T. Shimojo, H. Fujisaki, M. Tamamori, S. Ishiyama, S. Adachi, S. Abe, F. Marumo & M. Hiroe: Thermal preconditioning protects rat cardiac muscle cells from doxorubicin-induced apoptosis. *Life Sci* 64, 755-761 (1999)
242. Matouschek A: Protein unfolding--an important process in vivo? *Curr Opin Struct Biol* 13, 98-109 (2003)
243. Gorza L & M. Vitadello: Reduced amount of the glucose-regulated protein GRP94 in skeletal myoblasts results in loss of fusion competence. *FASEB J* 14, 461-475 (2000)
244. Antonio J & W. J. Gonyea: Skeletal muscle fiber hyperplasia. *Med Sci Sports Exerc* 25, 1333-1345 (1993)
245. Uehara K, K. Goto, T. Kobayashi, A. Kojima, T. Akema, T. Sugiura, S. Yamada, Y. Ohira, T. Yoshioka &

H. Aoki: Heat-stress enhances proliferative potential in rat soleus muscle. *Jpn J Physiol* 54, 263-271 (2004)
246. Yamashita-Goto K, Y. Ohira, R. Okuyama, H. Sugiyama, M. Honda, T. Sugiura, S. Yamada, T. Akema & T. Yoshioka: Heat stress facilitates stretch-induced hypertrophy of cultured muscle cells. *J Gravit Physiol* 9, 145-146 (2002)
247. Horsley V, K. M. Jansen, S. T. MiLls & G. K. Pavlath: IL-4 acts as a myoblast recruitment factor during mammalian growth. *Cell* 113, 483-494 (2003)
248. Prokopchuk O, Y. Liu, K. Wirth, D. Schmidtbleicher & J. M. Steinacker: Response of IL-4 and IL-4r of human skeletal muscle to strength training. *Med Sci Sports Exerc* 37, S241-(2005)
249. Kalem V, S. Grözinger, C. Hrabak, S. Reißnecker, Y. Liu & J. M. Steinacker: Die Auswirkungen von Kraftausdauertraining auf die HSP70 Induktion im menschlichen Skelettmuskel bei chronischer Herzinsuffizienz. *Dtsch Z Sportmed* 54, S46-(2003)
250. Kontos M. C, J. B. Shipley & R. C. Kukreja: Heat stress improves functional recovery and induces synthesis of 27- and 70-kDa heat shock proteins without preserving sarcoplasmic reticulum function in the ischemic rat heart. *J Mol Cell Cardiol* 28, 1885-1894 (1996)
251. Nosek T. M, M. A. Brotto, D. A. Essig, R. Mestril, R. C. Conover, W. H. Dillmann & R. C. Kolbeck: Functional properties of skeletal muscle from transgenic animals with upregulated heat shock protein 70. *Physiol Genomics* 4, 25-33 (2000)
252. Elefant F & K. B. Palter: Tissue-specific expression of dominant negative mutant Drosophila HSC70 causes developmental defects and lethality. *Mol Biol Cell* 10, 2101-2117 (1999)
253. Hernando R & R. Manso: Muscle fibre stress in response to exercise: synthesis, accumulation and isoform transitions of 70-kDa heat-shock proteins. *Eur J Biochem* 243, 460-467 (1997)
254. Scott M. A, M. Locke & L. T. Buck: Tissue-specific expression of inducible and constitutive Hsp70 isoforms in the western painted turtle. *J Exp Biol* 206, 303-311 (2003)
255. Ogata T, Y. Oishi, R. R. Roy & H. Ohmori: Endogenous expression and developmental changes of HSP72 in rat skeletal muscles. *J Appl Physiol* 95, 1279-1286 (2003)
256. Ogata T, Y. Oishi, R. R. Roy & H. Ohmori: Effects of T3 treatment on HSP72 and calcineurin content of functionally overloaded rat plantaris muscle. *Biochem Biophys Res Commun* 331, 1317-1323 (2005)
257. Oishi Y, A. Ishihara, R. J. Talmadge, Y. Ohira, K. Taniguchi, H. Matsumoto, R. R. Roy & V. R. Edgerton: Expression of heat shock protein 72 in atrophied rat skeletal muscles. *Acta Physiol Scand* 172, 123-130 (2001)
258. Morimoto R. I: Heat shock: the role of transient inducible responses in cell damage, transformation, and differentiation. *J Biol Chem* 267, 295-301 (1991)
259. Morimoto R. I: Cells in stress: transcriptional activation of heat shock genes. *Science* 259, 1409-1410 (1993)
260. Arnaud C, M. Joyeux, C. Garrel, D. Godin-Ribuot, P. Demenge & C. Ribuot: Free-radical production triggered by hyperthermia contributes to heat stress-induced cardioprotection in isolated rat hearts. *Br J Pharmacol* 135, 1776-1782 (2002)
261. Bellmann K, A. Wenz, J. Radons, V. Burkart, R. Kleemann & H. Kolb: Heat shock induces resistance in rat pancreatic islet cells against nitric oxide, oxygen radicals and streptozotocin toxicity in vitro. *J Clin Invest* 95, 2840-2845 (1995)
262. Febbraio M. A, A. Steensberg, R. Walsh, I. Koukoulas, H. G. van, B. Saltin & B. K. Pedersen: Reduced glycogen availability is associated with an elevation in HSP72 in contracting human skeletal muscle. *J Physiol* 538, 911-917 (2002)
263. Flaherty K. M, D. B. McKay, W. Kabsch & K. C. Holmes: Similarity of the three-dimensional structures of actin and the ATPase fragment of a 70-kDa heat shock cognate protein. *Proc Natl Acad Sci U S A* 88, 5041-5045 (1991)
264. Koranyi L, E. Hegedus, E. Peterfal & I. Kurucz: The role of hsp60 and hsp70 kDa heat shock protein families in different types of diabetes mellitus. *Orv Hetil* 145, 467-472 (2004)
265. Lazzari G, C. Wrenzycki, D. Herrmann, R. Duchi, T. Kruip, H. Niemann & C. Galli: Cellular and molecular deviations in bovine in vitro-produced embryos are related to the large offspring syndrome. *Biol Reprod* 67, 767-775 (2002)
266. Walgren J. L, T. S. Vincent, K. L. Schey & M. G. Buse: High glucose and insulin promote O-GlcNAc modification of proteins, including alpha-tubulin. *Am J Physiol Endocrinol Metab* 284, E424-E434 (2003)
267. Ehrhardt M. R, J. L. Urbauer & A. J. Wand: The energetics and dynamics of molecular recognition by calmodulin. *Biochemistry* 34, 2731-2738 (1995)
268. Penso J & R. Beitner: Clotrimazole decreases glycolysis and the viability of lung carcinoma and colon adenocarcinoma cells. *Eur J Pharmacol* 451, 227-235 (2002)
269. Penso J & R. Beitner: Detachment of glycolytic enzymes from cytoskeleton of Lewis lung carcinoma and colon adenocarcinoma cells induced by clotrimazole and its correlation to cell viability and morphology. *Mol Genet Metab* 76, 181-188 (2002)
270. Zietara M. S & E. F. Skorkowski: Thermostability of lactate dehydrogenase LDH-A4 isoenzyme: effect of heat shock protein DnaK on the enzyme activity. *Int J Biochem Cell Biol* 27, 1169-1174 (1995)
271. Moon B, N. Duddy, L. Ragolia & N. Begum: Stimulation of glycogen synthesis by heat shock in L6 skeletal-muscle cells: regulatory role of site-specific phosphorylation of glycogen-associated protein phosphatase 1. *Biochem J* 371, 857-866 (2003)

Key Words: Hsp70, Molecular Chaperone, Stress Protein, Skeletal Muscle, Exercise, Heat Shock Protein, HSP, Review

Send correspondence to: Dr. Yuefei Liu, Sektion Sport- und Rehabilitationsmedizin, Abt. Innere Medizin II, Universitätsklinikum Ulm, Steinhövelstr. 9, D-89070 Ulm, Germany, Tel.: 49-731-50026963, Fax.: 49-731-50026686, E-mail:yuefei.liu@medizin.uni-ulm.de

http://www.bioscience.org/current/vol11.htm