Heat Shock Proteins in Exercise: A Review

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Abstract

This review has attempted to highlight some of the most recent and original studies involving the stress response. It is clear that, especially regarding *in vivo* human studies, there is a paucity of research regarding the SPR stimulus, it's signalling system, measurement concepts, chronic adaptations, and it's practical use in both the health and elite sporting performance fields. In addition, the differences in exercise intensity, mode duration, subjects, training status etc. contribute to disparity in findings from previous studies.

Key Words: **% Body Fat, Flexibility, Cardiovascular Endurance, VO_2 max**

Introduction

Contemporary exercise physiology research has termed changes in the homeostatic balance within the whole organism as stress. Stress is defined as 'the process by which environmental events threaten or challenge the organism's well being and by which that organism responds to this threat' *(Turner, 1994)*. Stressors of any nature act initially at the cellular level and are manifested as protein damage or impairment, which compromises the function and integrity of the cell. Without intervention, stressors would develop throughout cell lines and eventually affect tissues, organs and perhaps in time, the whole organism. However, a set of highly conserved proteins have been shown to accumulate at such instances and have thus been termed stress proteins (SP). It has been postulated that expression of these proteins convey a response initiated to maintain cellular homeostasis after exposure to stress. The response has been denoted in all types of cells investigated to date, and although the precise function of these proteins remains unclear, their expression in the cell allows it to survive normally lethal stresses.

The most commonly cited SP is the heat shock protein (HSP), so called because the original stressor that elicited the SP response was hyperthermia *(Ritossa, 1962)*. Yet, to date, investigators have been unable to directly ascertain the stimulus and mechanism by which SP are synthesised, and further, their role is asserting stasis. Nonetheless, HSP's have been associated with a number of cytoprotective functions, including the protection of stable proteins *(Dobson and Ellis, 1998)*, chaperoning and folding of nascent polypeptides *(Beckman et al., 1990)*, and degradation of aggregated proteins *(Chiang et al., 1989)*.

A number of conditions, including hyperthermia, ischemia, pH alterations, energy depletion, calcium variations, abnormal protein generation and oxidative phosphorylation uncoupling are known to induce the cellular stress protein response (SPR). The majority of these stressors that are met with the SPR accompany exercise and therefore exercise physiologists have experienced difficulties in drawing firm

conclusions from their findings *in vivo*. A cellular stress protein response to endurance exercise for instance, may be derived from one or more of the conditions outlined above, such as hyperthermia, energy depletion and abnormal protein generation. High-intensity activity is accompanied by pH and calcium alterations and oxidative phosphorylation uncoupling. Therefore the stimulus for the SPR has proven difficult to ascertain, as has the magnitude of the SPR to individual stressors. Gaining an understanding of the way in which the SPR protects cells after exposure will aid researchers in ascertaining how exercise may provide protection to cells and tissues either for health and occupational reasons, or for elite athletes in extreme environments.

Location and Type

Heat shock proteins have a number of different forms and isoforms, which are distinguished by their molecular weight and have enabled researchers to categorise them into families. This, coupled with different sampling methodologies and species examined, makes comparisons between studies very difficult.

The 70-KiloDalton (kDa) family of HSP's are the most highly conserved (Hunt and Morimoto, 1985), and considered to be the most stress inducible of all of the HSPs. Although the precise role of this family remains unknown, it is thought that HSP70 is involved in protein synthesis *(Beckman et al., 1990),* transport *(Chiang et al., 1989),* protection from protein denaturation and aggregation, and restoring the function of damaged proteins subsequent to a stress exposure *(Zietara and Skorkowski, 1995).* The two most extensively studied proteins of the HSP70 family are the cognate (HSC73 = HSC70), and inducible (HSP72 = HSP70) isoforms. The constitutive isoform is only slightly stress-inducible *(Locke, 1997),* whereas HSP70 is highly inducible, both are situated in and around the nucleus, endoplasmic reticulum, cytosol, and mitochondria, although the locations vary depending on the particular protein. During stress episodes, the HSP72 and HSC73 isoforms migrate from the cytoplasm to the nucleus *(Welch and Feramisco, 1984),* presumably to protect protein synthesis processes. In recovery, HSP70 and HSC70 return to the cytoplasm and become associated with proteins *(Welch and Mizzen, 1988),* perhaps to repair aggregated and denatured proteins vital in normal cell functioning to restore cellular homeostasis.

Signalling

Although a number of afferent stimuli have been proposed for HSP synthesis, such as hyperthermia and reactive oxygen species generation, the efferent signal responsible for this stress response is relatively unclear. It has been postulated that the regulation of HSP synthesis is controlled via a feedback system involving heat shock transcription factors (HSF). In unstressed cells, HSF exists as a monomer in the cytoplasm and the nucleus *(Locke, 1998),* perhaps bound to HSP. Following an exposure to physiochemical stress, HSF is converted to a trimetric state and thereby acquires DNA binding activity *(Baler et al., 1993),* a process known as HSF activation. The activation of HSF has indeed been identified within minutes of heat shock *(Locke et al., 1995; Kim et al., 1995),* ATP depletion *(Benjamin et al., 1992),* hypoxia *(Giaccia et al., 1992),* exercise *(Locke et al., 1995)* and changes in pH *(Petronini et al., 1995).* During such stressors HSP

interact with denatured proteins thus allowing HSF to interact with heat shock elements (HSE) on the promoter region of the HSP gene, therefore promoting their transcription *(Fehrenbach and Niess, 1999; Locke and Noble, 1995; Noble and Aubrey, 1999).* When HSP levels subsequently rise, they again bind to HSF, thereby self-regulating their own synthesis *(Noble and Aubrey, 1999).*

The binding activity of HSE was investigated during cold (6º) and heat (38ºC) exposure, and in response to α-adrenergic receptor agonist administration, in brown adipose tissue of adult male mice *(Matz et al., 1995, 1996a).* Both cold and heat exposure resulted in amplified HSE binding, with heat stressed tissue eliciting the greater response *(Matz et al., 1995, 1996a).* Yet, although HSP70 mRNA expression evoked from α-adrenergic receptor agonist treatment was similar if not greater to that induced by heat stress, minimal HSE binding was evident *(Matz et al., 1996a).* These results demonstrated that HSE-binding was not necessarily associated with the induction of HSP70 mRNA from α-adrenergic receptor agonist treatment, suggesting that different transcriptional regulatory pathways exist. Furthermore, HSP70 transcript accumulation was different in magnitude to heat and cold stressors, indicating three different induction mechanisms, these findings were also independent of changes in body temperature *(Matz et al., 1995, 1996a).*

The discovery of attenuated increases in HSP70 expression to cold stress with administration of α-adrenergic receptor antagonists, and amplified HSP70 levels with α-adrenergic receptor agonist treatment in the absence of cold stress, suggests autonomic activity may to some extent mediate HSP70 expression. Specifically, augmented sympathetic outflow through, for instance, noradrenalin, initiate HSP transcription through unknown complex signal transduction pathways *(Matz et al., 1995, 1996a, 1996b).*

This area of SPR has received very little attention in the available literature. Knowledge of the HSP expression efferent signal would enable researchers to determine the stress stimuli. In turn, this information would allow the manipulation of training regimens and acclamatory protocols to derive the optimal benefits.

Muscle Damage

The expression of HSP's after exercise may be interpreted as a marker of muscular damage. *Thompson and colleagues (2001, 2002, and 2003)* completed a series of studies, which aimed to determine the influence of muscle damage on the SPR. In their first study the researchers subjected 8 non-weight trained volunteers to two sets of 25 maximal eccentric contractions *(Thompson et al., 2001).* Muscle biopsies of the biceps brachii (BB) pre and 48 hours post exercise. The HSP70 increased by 1064% 48 hours post exercise, but these were not accompanied by changes in actin protein expression. Thus, the HSP70 response was not a result of bulk or systemic protein adaptations. The magnitude of the HSP70 expression was also large with the type of damaging exercise employed in this study, with respect to that associated with non-damaging endurance activity (e.g. *Liu et al., 1999; Vogt et al., 2001).* The authors concluded that the large calcium and protein alterations associated with damaging repetitive contractions of this nature be responsible for SP synthesis, thus Inferring a possible role of proteolysis for SP's.

A subsequent study was undertaken by ten untrained subjects whom performed two sets of 25 maximal voluntary contractions (MVC) on two separate occasions, four weeks apart *(Thompson et al., 2002).* The investigators used the eccentric component of the bicep curl (lowering portion) to induce damage to the BB, from which muscle biopsies were extracted pre and post each exercise session (i.e. B1 & B2). Indicators of muscle damage were apparent after B1, but showed smaller changes after B2; however these changes were not paralleled by HSP expression post exercise. Although the magnitude of the HSP response was the same after both bouts of exercise, the basal level prior to B2 was lower. The authors concluded that the lower basal expression of the cellular responses mediated the attenuation of damage associated with B2.

In their final study, *Thompson and co-workers (2003)* added to their earlier work by supplementing a downhill running protocol to the bicep eccentric loading utilised in their previous research. Muscle biopsies from the vastus lateralis (VL) demonstrated an increase in HSP70, though this was not as pronounced as the HSP70 accumulation derived from the BB. The elevated SPR to both forms of damaging eccentric exercise strengthened the argument for a HSP role for protein remodelling caused by mechanical rather than metabolic stress.

Despite the clarity of Thompson's findings, a number of concerns are apparent. First, both forms of eccentric activity undertaken in their methodologies would have been accompanied by both oxidative phosphorylation uncoupling, and a level of hyperthermia within the muscle. Thus, it is difficult to suggest that the elevations in HSP expression were simply due to mechanical stress alone. Although, there is a strong case for the postulation that the SPR has a role for proteolysis, this process would also be required during, and in recovery from endurance exercise, in which energy depletion, pH alterations and ROS generation would all result in protein unfolding and/or denaturation. Moreover, the results of a decreased basal HSP expression to the second exercise bout is in direct contrast to other studies that investigated stress responses to endurance activities *(Liu et al., 1999; Vogt et al., 2001; Brickman et al., 1996; Ecochard et al., 2000).*

Human Exercise Studies

In terms of studies investigating exercise, his majority of investigations have focused upon the SPR's to different modes of exercise under a number of experimental controls. Invariably these studies have focused upon the responses either during exercise, recovery and/or a second bout to identify possible adaptations.

Liu and colleagues attempted to investigate the influence of training volume *(Liu et al., 1999 & 2000)* and intensity *(Liu et al., 1999 & 2000)* upon the long-term SPR. The experimenters withdrew muscle biopsies from the VL of national level rowers whom were undertaking preparation for international competition. The rowers were prescribed a four-week training program, which progressively increased intensity up to week three, whereas the volume peaked at week two and was reduced thereafter. HSP70 expression increased five-fold and peaked at week three. This was despite a reduced training volume between weeks two and three, suggesting a possible time-delay between HSP70 translation and accumulation. *Liu et al., (1999)* also discovered a reduced creatine kinase (CK) level in the blood with training, inferring that HSP70 may possibly

provide some protection. The major finding of this investigation however was that HSP70 accumulated despite the reduced training volume between weeks two and three, the intensity between these two sampling points did increase, thus implying that training intensity is the predominant stimulus for the SPR.

The subsequent article by *Liu et al., (2000)* supported their earlier findings. In this experiment well trained rowers were divided into two training groups, A and B. Group A performed higher intensity training during phase one, whereas group B performed higher intensity training during the training phase two. Both training intensity and volume were reduced in the final phase. The HSP70 accumulation was related to the intensity of the training i.e. greater in group A during phase one and greater in group B after phase two. Furthermore, HSP70 expression was reduced in both groups as a result of the reduced phase three training load.

These two investigations appear to demonstrate the occurrence of an acute response to the training stimulus. However, as the participants involved in *Liu and co-worker's studies (1999, 2000)* were highly endurance trained, and were therefore frequently exposed to the stressors known to evoke a SPR (i.e. hyperthermia, hypoxia, and pH alterations.). This work did not allow an analysis of the chronic SPR profile, thus it is unclear how long-term exercise regimens adopting a sustained training volume and intensity would adapt the HSP response of whole organism.

The acute response to exercise induced stress is commonly denoted by an immediate expression of HSP mRNA, which indicates enhanced HSP transcription rates *(Febbraio and Koukoulas, 2000; Ferenbach et al., 2000b),* and a greater HSP expression later in the post exercise (PX) period *(Khassaf et al., 2001; Ferenbach et al., 2000a, 2000b).* *Febbraio and Koukoulas (2000)* exercised five healthy, yet untrained individuals at 63% of peak oxygen uptake (VO_2peak) to fatigue. Muscle biopsies were taken from the VL at rest, 10 minutes, approximately 40-minutes before fatigue (F-40), and at fatigue (F). The HSP72 mRNA did not statistically increase by 10-min, although, significant elevations of 2.2 ± 0.5 and 2.6 ± 0.9 fold were noted at F-40 and F respectively. Therefore HSP72 mRNA increased progressively during acute concentric cycling exercise. Interestingly, blood lactate (BLa) and muscle temperature increased at the 10 min sample period, but these were not paralleled by HSP72 mRNA accumulation. Thus, the authors speculated that heat and Bla were not independently responsible for the SPR. However, it is more likely that a time lag between the stressor and gene transcription exists.

Fehrenbach and colleagues (2000b) also found that HSP transcription in leukocytes was apparent immediately after athletes ran a half-marathon. Furthermore HSP72 expression was elevated above resting levels at both three and 24 hours post exercise *(Ferenbach et al., 2000a, 2000b).* In contrast, however, eleven moderately trained males and females did not display increased HSP70 levels at either 15 or 24 hours after a 60-minute run at 70% VO2max *(Shastry et al., 2002).* It is possible that the contradicting results were caused by the different cellular locations investigated.

A longer time-course of HSP expression was profiled by *Khassaf and co-authors (2001).* Muscle biopsies were taken from the VL at rest, and one, two, three and six days after one-legged cycling at 70% VO_2peak for 45 minutes. The concentric nature of the bout did not elicit

muscular damage, denoted no-increase in CK activity at any of the sampling times over the six days. The results from this study were however, somewhat clouded by a high level of inter-individual variability. Thus HSP70 levels were only statistically increased after six days post exercise, although a steady progression was observed throughout.

The elevated HSP levels six days PX presented by *Khassaf et al. (2001)* were supported by findings from another study *(Vogt et al., 2001).* Here, untrained participants undertook five 30-minute cycling bouts at varying intensities, which were mediated by BLa responses every week for six weeks *(Vogt et al., 2001).* High-intensity (~4-6mmol/l BLa) activity elevated the constitutive HSP70, although muscle biopsies were taken just 24 hours post exercise, which may be indicative of an acute response rather than a cellular adaptation. Nevertheless, the accumulation of constitutive HSP70 has been shown after 4 weeks of intense endurance activity *(Liu et al., 1999).*

Another method of estimating the SPR to chronic exercise regimens involves comparing cellular responses of trained (TR) and untrained (UT) subjects. Only three studies to date have made this comparison *(Ferenbach et al., 2000a, 2000b; Shastry et al., 2002),* and each discovered that basal HSP70 expression was reduced in TR individuals, therefore HSP70 expression is down regulated with endurance training. In addition, *Fehrenbach et al. (2000b)* demonstrated that TR subjects displayed a greater number of HSP70 transcripts with respect to their UT counterparts. Taken together, these studies suggest that endurance activity provides protection by reducing the threshold for HSP70 transcription. The down-regulation in basal HSP70 expression in these studies is likely to be due to the higher oxidant stress threshold encompassed in the TR. It remains to be investigated whether a chronic non-damaging exercise program will derive similar SPRs.

The differences in stress response to acute and chronic stress are depicted in the literature as thermotolerance and acclimatization respectively *(Moseley et al., 1994).* Thermotolerance is the cellular adaptation to an acute stress exposure that allows the organism to survive a subsequent lethal exposure, with the severity of the initial stressor exposure positively related to the magnitude and duration of thermotolerance *(Kregel, 2002).* Contrastingly, acclimatization is the ability to perform greater work in a given stressful environment, for example in the heat due to heat dissipation improvements caused by repeated mild elevations in core temperature *(Moseley, 1997).* It was previously considered that thermotolerance had a short life-span of just several hours, denoted by HSP induction and decay associated with HSP70 induction and degradation *(Moseley, 1997; Kregel, 2002).* Recently, it has been established that thermotolerance may last up to 3-5 days in duration *(Kregel, 2002; Khassaf et al., 2001).* Acclimatization unlike thermotolerance allows an equilibrium to be maintained at a given work-rate in stress-prolonged periods. Unfortunately, as the full time-course of HSP expression following different stressors is still to be elucidated, a clear distinction between acquired thermotolerance and acclimatization remains elusive.

Thus far the attempt to determine the specific stimulus for the SPR associated with exercise has proved difficult, and human *in vivo* investigations are rare due to their highly invasive nature. *Vogt and co-*

workers (2001), attempted to manipulate the oxidative stress during exercise by using different exercise intensities and simulating various levels of oxygen availability. After a six-week training intervention the authors discovered that their previously untrained participants increased their basal HSP70 mRNA levels after high intensity bouts under conditions of both normoxia (146.5%) and simulated hypoxia (137.7%) at 3850m above sea level. Low intensity training below the lactate threshold did not evoke a HSP70 mRNA expression regardless of the O2 availability. These results led the authors to conclude that metabolic stress caused by the high-intensity activity, rather than simulated hypoxia, is the primary influence upon HSP70 transcription. *Vogt et al. (2001)* suggest that the accumulation of lactate and pH alterations may be the primary stimuli for HSP expression from exercise. However, this study design was not able to control the effects of heat gain during exercise. The high-intensity exercise would have generated a greater local heat load and perhaps consequently elevated HSP transcription. Moreover, the biopsies were taken just 24 hours post exercise, and the acute response may also have been responsible for elevated constitutive HSP70.

Animal Exercise Studies

In vivo animal studies have enabled researchers to control erroneous variables and have administered more invasive but accurate measurement techniques. Furthermore, higher sampling frequencies can be employed with animal studies, and therefore investigations of this nature have provided more research regarding the time-course profile of HSP levels and their adaptation to a chronic exercise regime. Human studies have inferred that exercise training may dampen the volume of HSP70 expression in the muscle or blood cells, but this stimulus elevates the HSP70 mRNA levels. This has been postulated to be the result of a lowered threshold for HSP production at the onset of stress *(Fehrenbach et al., 2000b; Maloyan et al., 1999).* However, the basal levels and acute response to exercise have still to be determined from a chronic training study, as to date such inferences have been made from TR Vs UT cross-sectional comparisons. Animal studies have been able to investigate these responses. *Ecochard and co-workers (2000)* found that after just two weeks of training, male rat basal HSP72 levels were elevated, yet these levels were not further increased after eight weeks of chronic exercise. *Brickman et al (1996)* found that only after 63 days of training, was the basal HSP content amplified. In addition, the acute response to exercise demonstrated an augmented HSP level in the TR compared with UT controls. Interestingly, repeated electrically stimulated muscle contractions evoked a peak HSP70 response at 18-24 hours PX *(McArdle et al., 2001).* Despite the wide variation in the time-to-peak HSP response derived from animal studies, these responses are markedly quicker than those displayed by human subjects *(e.g. Khassaf et al., 2001).* Yet, many human studies have utilised sampling frequencies that may have been insensitive to determine the peak SPR.

Reasons for the large variation in the time profile of the HSP response include the nature, intensity and duration of the stress imposed. *Demirel et al., (1999)* ran female rats at 70-75% VO2max for either 30, 60 or 90 minutes per day for 10 weeks. After the final exercise bout, 24 hours later, HSP72 concentrations were identified from the adrenal glands. All

durations of exercise elicited increased HSP72 levels compared to a sedentary control group. Moreover, HSP72 levels elevated with increasing daily exercise duration, demonstrating a role for training volume in the SPR, directly contrasting with the results from human studies *(Liu et al., 1999, 2000).*

Another factor, which may have equal relevance, is the site from which the HSP has been extracted. Even if muscle samples were utilised universally, the variation in muscle fibre typology may profoundly influence the SPR manifested. Although this hypothesis is yet to be justified in human subjects, a number of animal studies have extracted biopsies from different muscles. Acute stress exposures to electrical muscle stimulation and hot water immersion of the rodent hindlimb, produced a more rapid response in the soleus muscle (< 2 hours), which is known to have a larger proportion of type I muscle fibres *(Oishi et al., 2002; McArdle et al., 2001).* These slow muscles also demonstrated a larger basal level of HSP72 and thus an innate ability to synthesise HSP72 *rapidly (Oishi et al., 2002; McArdle et al., 2001; Kelly et al., 1996; Ecochard et al., 2000).* Muscles composed predominantly of type II fibres such as the plantaris and the extensor digitorum longus (EDL) produced an elevated HSP72 expression which peaked at 48 and 24 hours post exercise respectively *(Oishi et al., 2002; McArdle et al., 2001).* The muscle specific chronic SPR has shown that the greatest increases in basal HSP expression have transpired in faster muscles *(Kelly et al., 1996),* such that no differences between the muscle types were denoted.

The cause of the elevated HSP expression after training in predominantly type I muscle has been attributed to myosin heavy chain production and protein resynthesis. However, recent studies have negated this postulation, by discovering that HSP72 was only correlated to myosin heavy chain in sedentary populations *(Kelly et al., 1996),* and consequently muscle fibre typology did not change with chronic stress exposure *(Ecochard et al., 2000).* The latter study manipulated the oxidative stress caused during chronic exercise training by occluding the iliac artery in male rats *(Ecochard et al., 2000).* HSP72 accumulation was greater in the ischemic hindlimb by 40% when superimposed on exercise training; however, this adaptation was not apparent in UT and one-week TR rats. Furthermore, the SPR was not associated with mitochondrial oxidative capacity of the occluded limb, as although cytochrome oxidase and citrate synthase activities increased with training, these changes were prevented by arterial insufficiency.

Kelly and co-investigators (1996) also exposed rats to an elevated oxidative stress by restricting vitamin E supplementation. Vitamin E is the primary intramembrane antioxidant and membrane stabiliser, thus depletion disrupts organelle membrane function. In addition, vitamin E depletes oxyradicals and disrupts the chain reaction of phospholipids peroxidation initiated by reactive radicals *(Halliwell & Gutteridge, 1989).* Taken together these processes were hypothesised to enhance the oxidative stress experienced by exercising muscles and thus evoke a HSP response. The results of this study indicated that the HSP expression was not increased in vitamin E deprived rats with aerobic exercise training when compared to the exercised control group (vitamin E supplemented). The authors speculated that their methodology was not sufficient to generate oxidative stress.

The role of hyperthermia in the HSP response, independently of exercise, has not been investigated in great detail. Muscle biopsies from rat hindlimbs immersed in 42˚C water for one hour, manifested an immediate HSP72 response, and these amplified levels were sustained for four hours in the soleus muscle before returning to basal levels *(Oishi et al., 2002)*. In contrast, HSP72 concentration in the plantaris was unaffected until 12-hours post heat exposure, yet the HSP72 accumulation remained higher than basal levels at 24, 36, 48 and 60 hours post exposure. Again, these differences were attributed to higher protein turnover in slow muscle, coupled with their innate ability to synthesise HSP72 more rapidly. The magnitude of the HSP response in both muscle types was lower than that produced after exercise stress, inferring that oxidative stress may be the primary stimulus for the SPR. *Salo et al., (1991)* discovered that HSP70 transcripts and the protein itself were increased subsequent to both exercise to exhaustion and *in vitro* heat shock (~42˚C) of isolated tissues for 2 hours. Induction was greater in active muscle and liver tissue samples after exercise in comparison to an acute heat exposure.

Nonetheless, to date, studies have not measured local temperature of the cell extraction site, either during or post exercise, thus the role of hyperthermia should not be underestimated. Indeed, *Walters (1998)* found that although HSP70 increased similarly with both active and passive heat stress in male rats, HSP70 in various regions of the brain was correlated with hypothalamic temperature. In addition, active heating in moderate ambient temperatures (~24˚C) did not evoke a central HSP70 response. More conclusively, exercise *per se* did not increase HSP70 in the brain without hyperthermia. Yet again though, the SPR was investigated from a different tissue, in this case from the brain, therefore making comparisons between studies extremely complex.

Studies have not yet been able to delineate the effects of hyperthermia and exercise on the heat shock response. *Skidmore et al., (1995)* however, attempted to achieve this by exposing male adult rats to passive and active heating protocols in different ambient temperatures (36-42 Vs ~14˚C). Although, passive heating increased HSP70 expression in soleus, gastrocnemius and left ventricle tissues, HSP70 increases were greater in the active heating trials and exercise in the cold. These findings suggest that factors associated with exercise have a greater effect on the SPR than the independent influence of heat. However, sufficient intensity of heat shock (≥ 41 ºC) independently stimulates a stress response *(Skidmore et al., 1995; Salo et al., 1991; Oishi et al., 2002; Flanagan et al., 1995)*. *Flanagan et al., (1995)* studied the effects of heating rates of male rats *in vivo*. The rodents were exposed to low (0.045˚C/min) and high (0.166˚C/min) heating rates until colonic temperature reached 42˚C, at which point they were cooled passively in ambient temperatures of 22-24˚C for four hours before tissue samples were withdrawn. Both heating protocols induced HSP72 expression in the small intestine, liver and kidney, with the greatest response denoted in the liver tissue samples, where HSP72 increased 21-fold compared to baseline concentrations. Despite experiencing a lower thermal load (time colonic temperature was maintained ≥ 40.4˚C), the animals exposed to the higher heating rate showed greater concentrations of HSP72. The results presented by *Flanagan et al., (1995)* infer that the rate of heating is very

important in respect to HSP72 synthesis. However, these results should be interpreted with caution, as heat induced vasoconstriction may result in ischemia of the gut and liver, which subsequently may amplify the stress experienced *(Kregel et al., 1988; Kregel and Gisolfi, 1989).*

Very little work has been undertaken concerning heat exposure *per se* in human subjects. *Fehrenbach et al., (2000b)* exposed resting human leukocytes to an *in vitro* acute heat shock (42˚C) for two hours. The heat stimulus was sufficient to induce a HSP70 mRNA increase, showing higher increases in trained individuals compared to their sedentary counterparts *(Fehrenbach et al., 2000b).* Unfortunately, the time-course and the magnitude of the HSP70 protein expression were not examined here. *Schneider et al., (2002)* reported that one hour of heat shock at 42.5˚C induced HSP70 accumulation in peripheral blood mononuclear cells three hours post exposure. The up-regulation of HSP70 was sustained until 12 hours, but declined after 24 hours of incubation at control temperatures (37˚C).

Maloyan et al., (1999), performed the most comprehensive study undertaken to date to determine the HSP response to chronic and acute stress. The authors exposed 3-wk-old rats to short (1-2 days) or long-term (30 days) periods of heat acclimation at 34˚C. The acute response was measured immediately after a two hour exposure to either a 41 or 43˚C environment in both acclimated (AC) and control groups. After 30 days of heat acclimation, peak HSP72 mRNA level was attained earlier after an acute heat shock, and the resting stock of inducible HSP72 was increased with respect to the control group. Whereas the short-term heat acclimated rats did not manifest altered HSP levels. The acute response to heat strain trials indicated that HSP72 accumulation was faster with augmentation of the stress and that the time-to-peak HSP response was four-times quicker in AC than in the control group (1 Vs 4 hours respectively). These results supported the notion of an altered threshold for the mobilisation of HSP's with acclimation. However, *Moloyan et al. (1999)* used direct heat exposure as their stressor, whereas other studies have used exercise to generate a SPR in both *animals (e.g. Brickman et al., 1996; Echochard et al., 2000; McArdle et al., 2001; Samelman and Always, 1996; Kelly et al., 1996)* and humans *(Liu et al., 1999, 2000; Khassaf et al., 2001; Ferenbach et al., 2000a, 2000b; Febbraio and Koukoulas, 2000; Shastry et al., 2002; Vogt et al., 2001).* Moreover, *Maloyan et al. (1999)* extracted a sample from cardiac tissue, other studies have utilised blood or muscle samples.

The time-to-peak HSP response from an acute stress exposure has varied. Rats exposed to a similar heat (42˚C) to that administered by *Maloyan et al (1999)* displayed a more prolonged response, peaking at 48 hours post exercise *(Oishi et al., 2002),* although wet heating rather than dry was used in the more recent study, which is known to transfer a greater thermal load at a quicker rate *(Armstrong, 2000).* Also, the HSP response was identified within a muscle sample in the Oishi et al. (2002) study, which is more likely to be affected by a thermal stress than the more centrally located cardiac tissue extracted by *Maloyan and colleagues (1999).*

Implications

Although the specific function of HSP's have not yet fully been comprehended, it is clear that their expression confers some form of protective response to stress. Therefore, a greater

understanding of the SPR's stimuli and signal would enable coaches and athletes to design appropriate and effective training schedules. If HSP's are expressed in this manner, their content may also provide a marker of the stress experienced in both physical and occupational tasks. The altered threshold for HSP accumulation may also provide us with new information regarding acclimation, helping scientists to devise optimal exposure protocols. Finally, if HSP's are produced in response to heat shock, then their content may also provide an index of the heat strain exposed during a particular activity.

Future Directions

The literature reviewed in this article is diverse, in terms of its sample population, site, frequency and methodology. Many of these articles require further support work to collaborate their findings. The main areas for further research centre around the stimulus for the HSP response. Various human and animal studies have attempted to manipulate one of the many factors thought to induce a SPR, such as hyperthermia *(Mizzen and Welch, 1988; Li et al., 1995), ischemia (Emami et al., 1991; Gray et al., 1999),* pH alterations *(Weitzel et al., 1985; Gapen and Moseley, 1995),* energy depletion *(Febbraio and Koukoulas, 2000; Febbraio et al., 2004),* calcium accumulation *(Kiang et al., 1994)* and abnormal protein generation *(Chiang et al., 1989).* However, many of these studies have utilised exercise as the primary stressor, yet it is known that many of the factors listed above are associated with exercise. The few investigations that have attempted to isolate a specific stimulus have lacked the appropriate controls, and there are still more factors that remain to be investigated.

One of the major fields of study within the discipline of stress response is the time-profile of the response and it's adaptation in the acute response from chronic stress exposure References. Past research detailed earlier has studied the acute response over either-short or long sampling periods, which are often insensitive to the peak and time-to peak HSP accumulation. However, the variation of sample populations such as human, animal, trained and untrained; and different sampling sites (muscle, liver, brain, leukocytes etc.) makes firm conclusions extremely difficult. To date, there is no study that has investigated the acute response to a stressor after chronic exposure in sufficient detail. The few studies that have, did not control various factors associated with exercise, moreover, chronic exposure to heat, hypoxia and high intensity activity are still to be analysed.

One area of interest is the role of hyperthermia in the HSP response. This area has been under-researched in the human population. Many have reported the SPR, yet no studies have simultaneously monitored the local temperature at the sampling site i.e. muscle, blood, cardiac and cerebral temperature, thus associations between temperature and the HSP response have been indirect thus far. It may be possible to control the local temperature of muscles by water immersion or by wearing a water perfused suit, such strategies would allow a subject to exercise at a given intensity without affecting the local body temperature. An investigation of this nature would aid researchers to determine the magnitudes of the stress response to various stimuli. Similarly, active versus passive heating protocols have yet to be utilised in human subjects. This procedure may enable investigators to delineate the effects of heat and exercise on the HSP70 response.

The signalling system for HSP transcription is also still to be elucidated. It is possible that the rate of reduced parasympathetic withdrawal is associated with the SPR *(Maloyan et al., 1999)*. Certainly this area requires further research, and with the contemporary non-invasive measurement of cardiac autonomic activity via heart rate variability, such research is feasible.

One major area for future research involves the measurement site of the SPR. The available literature has so far sampled from muscle, blood, cardiac tissue and cerebral regions. Although, the various areas will derived different results to different stressors, these sample site variations have yet to be quantified. Can HSP expression from the blood accurately measure the stress response from a daily training regimen? Does sampling form the blood accurately monitor the acute response? If yes, then daily blood letting will allow optimal training strategies to be implemented and overtraining symptoms can be avoided.

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