

HIGHLIGHTED TOPIC | *Molecular Adaptations to Exercise, Heat Acclimation, and Thermotolerance*

Heat shock proteins and exercise adaptations. Our knowledge thus far and the road still ahead

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Henstridge DC, Febbraio MA, Hargreaves M. Heat shock proteins and exercise adaptations. Our knowledge thus far and the road still ahead. *J Appl Physiol* 120: 683–691, 2016. First published December 17, 2015; doi:10.1152/jappphysiol.00811.2015.—By its very nature, exercise exerts a challenge to the body's cellular homeostatic mechanisms. This homeostatic challenge affects not only the contracting skeletal muscle but also a number of other organs and results over time in exercise-induced adaptations. Thus it is no surprise that heat shock proteins (HSPs), a group of ancient and highly conserved cytoprotective proteins critical in the maintenance of protein and cellular homeostasis, have been implicated in exercise/activity-induced adaptations. It has become evident that HSPs such as HSP72 are induced or activated with acute exercise or after chronic exercise training regimens. These observations have given scientists an insight into the protective mechanisms of these proteins and provided an opportunity to exploit their protective role to improve health and physical performance. Although our knowledge in this area of physiology has improved dramatically, many questions still remain unanswered. Further understanding of the role of HSPs in exercise physiology may prove beneficial for therapeutic targeting in diseased patient cohorts, exercise prescription for disease prevention, and training strategies for elite athletes.

heat shock proteins; exercise adaptation; exercise training; HSP72; skeletal muscle; oxidative capacity

NUMEROUS STUDIES HAVE IDENTIFIED a large number of genes and/or proteins that are activated in the postexercise state or after a prolonged exercise training period. These exercise responsive genes/proteins are part of a finely tuned system developed to deal with both the acute change to whole body, organ, and cellular homeostasis that occurs with exercise but also as an adaptive response to repetitive exercise bouts. Heat shock proteins (HSPs) are a unique family of proteins classed according to their molecular weight that form part of an integrated response to defend cell integrity and homeostasis including during exercise. The 70-kDa HSP subset (HSP70) is an ancient and highly conserved family of proteins that act as intracellular molecular chaperones to help with intracellular transportation, folding of newly synthesized proteins, and the prevention of unwanted protein aggregation (34). From the HSP70 family, two main isoforms exist: 1) HSC70 (or HSP73)

(HSPA8); a constitutively expressed protein thought to contribute to the majority of its chaperoning activity under resting conditions, and 2) HSP72 (HSPA1A/HSPA1B), the inducible form of the HSP70 family. HSP72 is highly responsive to cellular stress including those associated with exercise and is thought to be primarily responsible for the protective effects of the heat shock response. Expression of HSP72 is largely dictated by its master regulator, the transcription factor heat shock factor 1 (HSF1), which is translocated to the nucleus upon activation and binds to heat shock elements (HSEs) within the nucleus, resulting in the transcription of HSP72 mRNA.

This review provides a commentary on what is known regarding the HSP response to exercise and what still remains to be discovered. Throughout the discussion we will raise questions for consideration in future studies in the field of HSPs and exercise. Although we aim to provide a brief overview of previous research in this area, we encourage readers seeking more information to source the excellent reviews including those by Whitham and Fortes (74) on the release and significance of HSP72 during exercise, Noble and colleagues

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(56) on HSPs in exercise, Morton and colleagues (51) on the human skeletal muscle exercise stress response and Mosley (53), and Kregel (35) on HSPs and thermotolerance.

HSPS AND EXERCISE

Acute Exercise

Before posing any questions for the future we must reflect on the past. Where did it all start? Studies throughout the 1990s and early this century demonstrated that HSP72 gene and protein expression in many organs and in multiple species could be upregulated in response to an acute bout of exercise. These included cardiac tissue (61, 62), the liver (61), brain (72), and most notably skeletal muscle (11, 12, 14, 44, 60, 62). Given that exercise is associated with many cellular stressors including temperature alterations, oxidative stress/free radical formation, glucose/glycogen depletion, hypoxia/ischemia, altered pH, and increased calcium concentration, and that many of these can induce a HSP72 response of their own accord (35), these findings were not surprising. Given the known robust induction of HSP72 in response to hyperthermia, it was postulated that elevations in skeletal muscle temperature during exercise may be the initiating factor in HSP72 activation within this tissue with exercise. However, subsequent studies demonstrated that skeletal muscle temperature elevations to levels comparable with exercise did not result in increased HSP72 response (52), whereas exercising without a subsequent rise in internal temperature could still provide an HSP-inducing effect (62).

Furthermore it was soon identified that an exercise stimulus (endurance running) was sufficient to result in an increase in the extracellular release of HSP72 into the circulation [extracellular HSP72 (eHSP72)] (71) with a follow up study demonstrating that the magnitude of this phenomenon was duration and intensity dependent (15). Furthermore, data are supportive of a temperature threshold or a “minimum endogenous criteria” set at 38.5°C for induction of eHsp72 into the circulation with exercise (3, 17). This raised the question as to the site of origin of this HSP72 release. Because of its role in muscle contraction, skeletal muscle was at the top of the list as the site of origin. The finding that HSP72 appears in the circulation before any transcriptional change in HSP72 mRNA in the skeletal muscle (71) suggested that this is not the case. However, because there could still be release of existing HSP72 protein, the dissociation of protein and mRNA is not definitive evidence that skeletal muscle is not the site of origin. Experiments using arterial-venous (A-V) difference procedures have demonstrated release of HSP72 during semirecumbent cycling exercise from the hepatosplanchnic viscera (13).

Additional evidence from cycling trials indicated that HSP72 may also be released from the brain/central nervous system (CNS) (37). Although these studies indicate a skeletal muscle independent mechanism, skeletal muscle as a source cannot be entirely excluded. A later study demonstrated a blood handling effect on eHSP72 concentrations using the assay kits that had previously been reported (73). Therefore, a precise answer to the relative contributions of all the different organs to circulating levels of HSP72 is still largely unknown. Other potential sites of origin include but are not limited to epithelial cells (5) and immune cells (30, 36). As was the case with findings in the skeletal muscle, exercising with a thermal

clamp (water immersion trails) resulted in an increase in plasma eHSP72 concentration, suggesting exercise-related stressors other than heat were driving the stimulation of HSP72 release (75).

Future directions. After all these years of research there is still no collective agreement on which organ(s) release HSP72 into the circulation with exercise. Nor is there any known mechanism of action once it is in the circulation. Does the HSP72 initiate a type of metabolic crosstalk with other organs or is it just incidental spillover into the circulation with the stress of exercise? Alternatively, does it have a role to play in the adaptive exercise response or as stress-sensor messenger? To delineate eHSP72's source and role, carefully designed genetic studies in rodents could be performed whereby tagged or labeled HSP72 is overexpressed specifically in an organ-specific manner. For instance, an adeno-associated virus could be used to overexpress HSP72 in skeletal muscle, the animals exercised, and plasma samples taken to identify whether the source of eHSP72 is indeed muscle. The same type of experiment could be conducted using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat) system using gene editing to overexpress HSP72. The same experiments could then be repeated with HSP72 overexpression in various tissues (adipose tissue, liver, immune cells, etc.)

Exercise Training

Adaptations to an exercise training program result from the cumulative effect of transient changes in mRNA and protein expression after each exercise session (22). A heat shock response resulting in the activation of HSP72 can be detected 24 h after aerobic treadmill running (50) or up to 6 days after an exhaustive nondamaging aerobic exercise bout (33). Consequently, it would be expected that exercise training that involves multiple episodes of acute bouts over a longer time frame would result in a generalized increase in HSP72 levels. Indeed, chronic rowing exercise training elicits an increase in the levels of HSP72 in the skeletal muscle (41, 43). This adaptation is not limited to endurance training but is also seen with resistance training protocols. Strength training in previously nontrained individuals for 1-2 mo (18) or 11 wk (58) increases both HSP72 and HSP27 content in skeletal muscle. Although these studies provide evidence of a training effect, the HSP regulation with training is finely tuned with many feedback systems in play to regulate its own expression. An example of this are the reports demonstrating blunted HSP induction in response to an acute bout of exercise in trained individuals (63) and the fact that HSP72 gene and protein expression declines rapidly upon initiation of a rest period after a training protocol (42). This constant fine-tuning of the system most likely is a direct response to the prevailing mechanical and metabolic demands of the musculature.

Future directions. From a molecular perspective, the relationship between HSP72, HSEs, and HSF1 dictates protein expression. This pathway is reliant on short nucleotide sequences (HSEs) that act as the binding site for HSF1 in the promoter region of heat shock genes. HSF1 is inactive when bound to HSPs such as HSP70 and HSP90. However, upon stress initiation resulting in damaged and unfolded proteins, HSPs dissociate from HSF1, bind to these proteins, and subsequently HSF1 is free to translocate to the nucleus and bind to

the HSEs, allowing for increased HSP production. Eventually HSP expression reaches an equilibrium point that is sufficient to deal with the quantity of unfolded and damaged proteins, cellular homeostasis is restored, and the available HSPs rebind to HSF1, which in turn shuts off transcription. Ultimately, the underlying mechanism via which exercise training (and subsequent detraining) alters expression is via these specific steps. Most likely it is the metabolic status of the organ, including substrate availability, hormonal signals, oxidative stress, and the unfolded protein response, that impacts on these molecular pathways, but to date these have not been precisely defined. Because there is very limited data on HSF-1 transcriptional activity in response to acute and chronic exercise, future studies could investigate the precise mechanism of action of exercise's impact on HSF1's molecular actions.

HSPS AND TAPERING

An important adaptation that occurs as part of a typical training cycle is the adaptation to a tapering period. Tapering involves the reduction of an athlete's training volume in the lead up to a major event. Although tapering is an area of exercise physiology that has been relatively understudied, many physiological reasons for this improvement in performance upon tapering have been suggested. Alterations to oxidative enzymes and muscle glycogen levels upon short-term taper have been reported (55). Other studies have suggested that tapering largely impacts fast-twitch muscle fibers preferentially over slow-twitch muscle fibers, thereby leading to hypertrophy and subsequently greater power generation (48, 69). HSPs may again have a role in this adaptation. The induction of exercise-induced HSP72 mRNA (*hspa1a*) in whole skeletal muscle tissue homogenates after tapering has been reported to be more robustly elevated compared with samples obtained from the same athletes during a heavy training block (48).

A recent study in competitive runners which analyzed the HSP72 (*hspa1a*) mRNA response to tapering in different skeletal muscle fiber types [myosin heavy chain (MHC I) vs. myosin heavy chain (MHC) IIa fibers] demonstrated a significant elevation in HSP72 in MHC I but not MHC IIa fibers after running in both the heavily trained and tapered states (54). This suggests that the commonly identified finding of an increase in HSP72 levels postendurance exercise is being driven by an increase specifically from the MHC I fibers. This is consistent with the finding of increased HSP72 protein expression in type I compared with type IIa fibers after acute isometric exercise (70) and animal studies that have shown HSP is less constitutively expressed in fast-twitch than slow-twitch muscle (27, 39, 45). There was a trend for the exercise-induced expression levels of HSP72 after a taper to be higher in the type I fibers than in the heavy training block, this did not reach statistical significance (54). Considering this, although MHC I fibers may be providing the majority of the endurance exercise (running)-induced posttaper HSP72 response, a small amount may be contributed by mixed-fiber types to account for the overall increase observed in whole skeletal muscle homogenates. The exact role HSP72 is playing in this exercise-induced posttapering state is speculative, but may involve a protective effect that is paralleled with the cellular stress associated with an all-out peak performance (54).

Future directions. It should also be noted that the story could be quite different for power sports. The HSP72 response likely involves a stress component. In the current study of long-distance endurance running the stress is concentrated on the type I, not type II, fibers, because the type I fibers are doing the majority of the work. In contrast, maximal eccentric exercise causes a type II fiber HSP70 response (59), which is most likely due to the differences in fiber type recruitment, and therefore greater stress exerted on the type II fibers. Consequently, future studies may analyze these findings in different strength or power based exercise activities.

Clearly there are alterations in the HSP pathway during acute exercise, after chronic training, and upon a reduction in training load. However, these responses may be geared toward different physiological stimuli. It is plausible that the early response (to acute exercise) may be targeting the rapid stress responses and the multitude of physiological changes that occur during an exercise stimulus. The longer-term responses may be linked to adaptation to the cumulative bouts and recovery from these bouts (56), whereas an increase in HSP72 induction after tapering may be advantageous in allowing for greater induction of HSP during an event requiring a maximum all-out effort when the physiological stressor that is exercise reaches its peak (refer to Fig. 1).

HSP RESPONSE TO EXERCISE IN OTHER ORGANS

Given the nature of exercise and the contraction of skeletal muscle for locomotion it is not surprising that often researchers take a skeletal muscle-centric approach to characterizing the effects of exercise. However, clearly exercise affects most of the bodily systems, and HSP expression is no different. Behind skeletal muscle the second most studied organ is the heart. Many studies have demonstrated alterations to HSP72 expression in the heart in response to acute exercise or training (21, 46, 47, 57, 61, 62, 64, 68). Long-term habitual exercise training (20 wk of exercise on a voluntary running wheel) also resulted in an increase expression of HSP72 in cardiac muscle of rats (66). Although exercise-induced skeletal muscle HSP72 expression can occur without a concomitant heat rise, it appears that the heart is a different matter. Simulated exercise protocols in isolated and perfused hearts indicated that HSF1 activation and HSP72 gene transcription are linked to elevated heart temperature rather than a direct function of increased cardiac workload (64). In the absence of temperature elevation, cardiac tissue cannot induce a typical heat shock response to exercise, and thus there is a requirement for the heart tissue to increase in temperature (21, 64). The tonically active nature of the cardiac tissue may be the underlying reason for the difference between these two organs in this respect. There does appear to be some sort of common dose-responsive effect of exercise in striated muscle (skeletal and cardiac) on HSP72 induction, with the greater the exercise the greater the induction (4, 63, 65).

Lollo and colleagues (47) demonstrated that alterations to HSP expression in response to exercise were not limited to striated muscle with their study showing that horizontal, uphill, or downhill running not only increased HSP72 levels 6 h after an exercise bout in skeletal muscle (soleus and gastrocnemius) but also in the heart, kidney, and lung. Interestingly for most of the tissues examined (muscle, heart, and kidney), HSP72 was

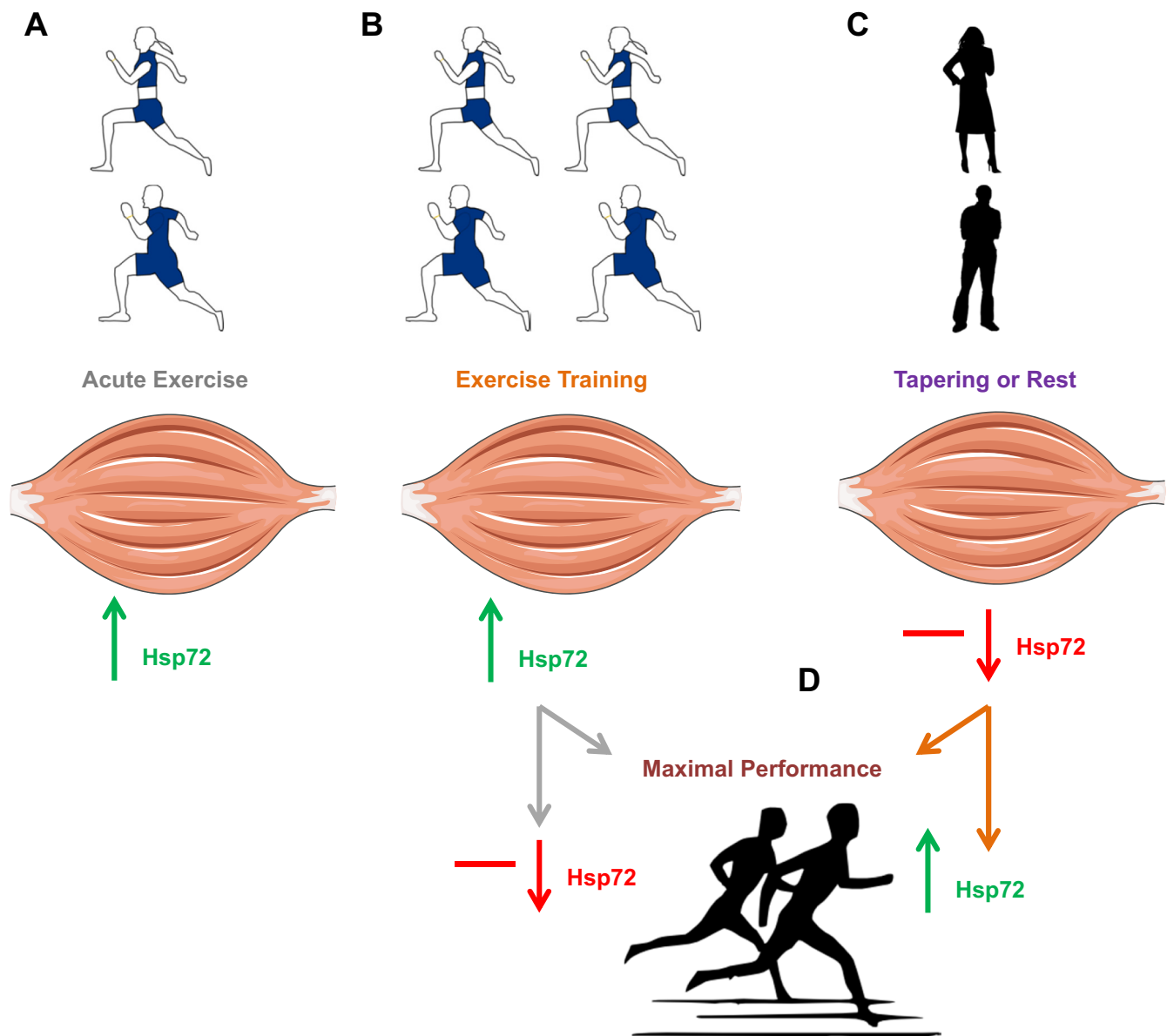


Fig. 1. Schematic representation of the effects of an exercise training cycle on skeletal muscle heat shock protein (HSP) 72 expression levels. *A*: acute exercise or a single bout increases skeletal muscle HSP72 expression. *B*: an exercise training program will also increase HSP72 levels. The response to a single exercise bout is likely a response to deal with the acute disruption to cellular homeostasis during exercise, whereas the longer term training response is likely an adaptation to the training load. *C*: a decrease in training load with tapering does not appear to alter HSP72 levels, whereas resting or returning to a pretrained state will decrease HSP72. *D*: a maximal performance during a heavy training period (indicated by gray arrows) results in a blunting of the usual acute HSP72 response to exercise, whereas a maximal performance after a taper (indicated by orange arrows) allows for the induction of HSP72 once again. This potentially acts in buffering the muscle against the heavy homeostatic disturbance that is seen with an all-out effort and is beneficial for performance.

most strongly elevated after the uphill running protocol (47), and when comparing all of the organs, the kidneys had the greatest relative increase in expression (47). Exercise training has also resulted in an increase in HSP72 expression in the liver with a corresponding increase in HSF1 (4). The precise role of this exercise-induced HSP72 expression in the lung, kidney, and liver has not been determined. The known organs whereby exercise increases HSP72 expression is illustrated in Fig. 2.

Another organ that is responsive to exercise in a number of different physiological ways is the brain. Indeed aerobic exercise is efficacious as a treatment in individuals with depression (10). HSP expression is also altered in whole brains of exer-

cise-trained mice. Treadmill training 5 days a wk for 8 wk increased HSP72 and HSP90 levels as well as HSP60 mRNA levels (without a corresponding change in protein levels) (38). An important factor in the exercise-induced expression of HSP72 in the brain may be the precise location in the brain in which it is activated. A prior period of habitual exercise leads to higher induction of HSP72 expression in specific brain areas in response to an acute exercise bout or stressor. Physically active rats responded with a greater induction of HSP72 in response to stress than did sedentary rats. This included elevated HSP72 in dorsal vagal complex, frontal cortex, hippocampus, and the pituitary (6). Recently, an area of the brain that plays an important role in the regulation of voluntary

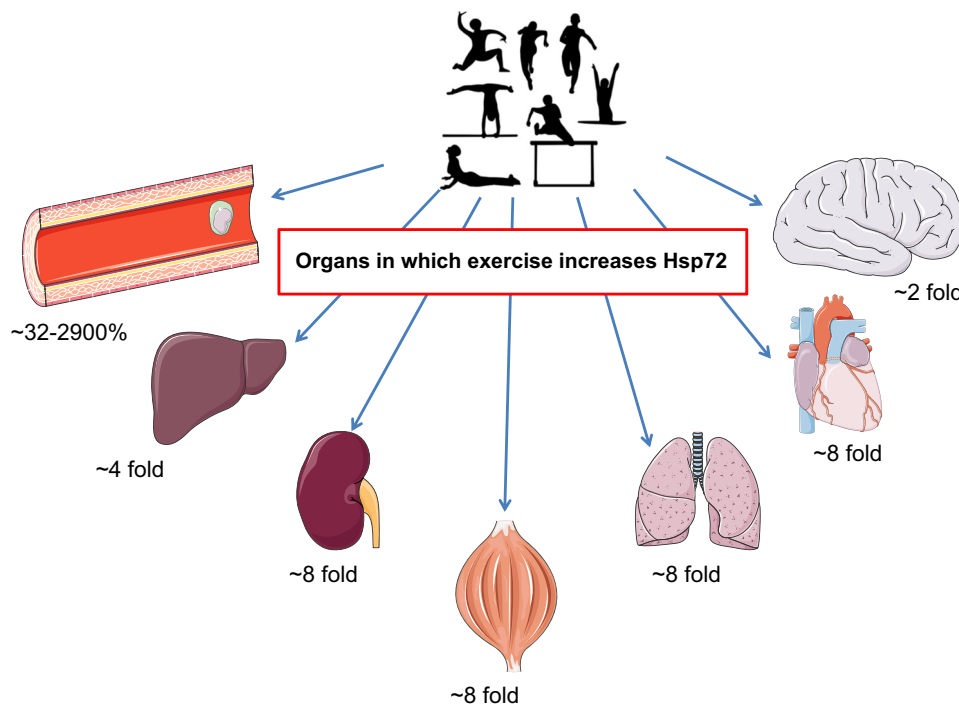


Fig. 2. Impact of exercise on the HSP72 response in various organs. Studies in humans or rodents (or both species) have demonstrated that multiple forms and types of exercise (aerobic based or resistance based) increases HSP72 expression in the circulation (extracellular and intracellular), liver, kidney, skeletal muscle, lungs, heart, and brain. It is likely other tissues also experience the same response. [Fold increase data for heart, skeletal muscle, lung, and kidney from Lollo et al. (47); data for liver taken from Atalay et al. (4); data for brain taken from Lappalainen et al. (38); percentage increase data for extracellular HSP72 taken from Whitham and Fortes (74).]

activity, was identified. This area, the dorsal medial habenula (dMhb), was identified via genetic deletion of neurons in this area of the brain and resulted in a decrease in exercise motivation (29).

Future directions. It is an intriguing thought as to what the levels of HSPs are in the dorsal medial habenula and whether these levels correlate with any indices of sedentary behavior or physical activity. It has also been postulated that increased levels of eHSP72 in the plasma during exercise may participate in fatigue sensation by the CNS. In this way eHSP72 would be acting as a messenger from the peripheral tissues to the brain to provide feedback on the level of fatigue (23); however, this hypothesis remains to be verified. Additionally it is clear that exercise activates HSP72 in tissues such as the lung, liver, and kidney. Studies investigating the reasons behind HSP72 induction in these tissues would be interesting. Although the stress response of contraction in the skeletal muscle could be the initiating factor for skeletal muscle induction, the same cannot be said for these alternative tissues. Therefore, could there be organ-specific activators of exercise-induced HSP72 expression?

HSPS AND THE UNFOLDED PROTEIN RESPONSE

The endoplasmic reticulum (ER) controls the synthesis, folding, assembly, and transport of proteins. Newly synthesized proteins translocate into the ER lumen, where they fold into proper conformations with the aid of molecular chaperones (77). When cellular perturbations impact on the execution of this protein folding process, unfolded or misfolded proteins accumulate and cause a situation known as ER stress. Many diseases have now been linked with varying degrees of ER stress including metabolic disease such as type 2 diabetes. The activation of the unfolded protein response (UPR) is an intracellular signal transduction pathway that communicates information on the status of protein folding in the ER to the nucleus

and cytosol (28). During times of ER stress, the UPR is activated to restore ER homeostasis and plays a critical role in adapting to the stress to ensure the ER functionality and flexibility.

Skeletal muscle houses an extensive network of specialized ER called the sarcoplasmic reticulum (SR) that regulates the calcium ion concentration in the cytoplasm. A number of physiological stressors associated with exercise have been demonstrated to activate a UPR response (1, 31, 77). These include such stressors as low glucose levels, hypoxia, and disrupted calcium homeostasis. Additionally, it has been demonstrated that the UPR is activated in skeletal muscle by exercise itself and that this UPR activation may form part of an adaptive skeletal muscle response to exercise training (77). This suggests a number of things. First, that mechanical stress exerted by muscular contraction or metabolic, hormonal, or substrate changes in the muscle that are altered with exercise can activate the UPR (77). It also suggests that exercise and the activation of a physiological UPR leads to adaptations that can protect against further future stress (77), much the way that HSP induction also operates.

The activation of the UPR during exercise indicates that the accumulation of misfolded and unfolded proteins is a part of the exercise-induced disruption to cellular homeostasis. Given that one of the major roles of HSPs is to assist in the folding of misfolded or unfolded proteins and to decrease protein aggregates, it suggests that the two pathways may be associated and, indeed, the HSP70 family does have links to the UPR pathway. Immunoglobulin binding protein BiP (also referred to as glucose-regulated protein 78 or Grp78) is a HSP70 family molecular chaperone located within the ER. BiP is implicated in the synthesis and export of protein in the ER and plays a major role in the UPR. BiP mRNA and protein levels are increased in skeletal muscle after acute exercise (treadmill running) when mice are naive to training or after a 4-wk training program

(77). Because BiP is a component of the adaptive response to physiological ER stress, this implicates HSP proteins in the UPR adaptive response to exercise in skeletal muscle. Although BiP is an ER-specific HSP70 family member, cytosolic HSP72 may also be playing its own role in the UPR process. Indeed, evidence for a physical connection between cytosolic chaperones and the ER stress response has been described (20). Inositol-requiring transmembrane kinase/endonuclease-1 (IRE1 α) is a highly conserved UPR stress sensor. Regulation of the UPR upon ER stress insult is associated with the formation of a stable protein complex between HSP72 and the cytosolic domain of IRE1 α , ultimately resulting in the inhibition of ER stress-induced apoptosis (20). Although this complex is yet to be studied in relation to exercise-induced UPR adaptation, the concept is in line with HSP72's known cellular protective attributes.

Future directions. The UPR is an attractive target for therapeutics because of the number of studies linking the UPR to various disease models. It will be interesting to observe what happens to HSP72 expression with the use of these developing drugs and the impact if any on exercise capacity or performance.

EXERCISE TRAINING IN COMBINATION WITH HEAT

An exposure to a stressor initiates a cellular response that includes the induction of HSPs and results in a type of preconditioning of individual cells, organs, or indeed the whole organism. This preconditioning results in future stressors not having the same impact as the initial event. Consequently preconditioning or priming (heat-shock priming) can be used to "train" the internal environment to be able to deal with future stressors. Exercise is one such preconditioning stress and heat exposure is another. Combining training with a hot environment could potentially be advantageous for performance, providing a protective thermotolerance effect to any subsequent heat stress during exercise. Indeed combining exercise and heat exposure can result in a greater Hsp expression than either treatment alone (62).

A recent study has put a new twist on this thinking. The paper by Tamura and colleagues (67) suggested that whole body heat stress after training additively enhances endurance training-induced mitochondrial adaptations in skeletal muscle. The rationale for this study came from previous findings in both *in vitro* and *in vivo* models. Heat stress treatment (enough to induce HSP72 expression) increased mitochondrial biogenesis in the skeletal muscle cell line C2C12 myoblasts (40). Furthermore, mice overexpressing HSP72 in skeletal muscle have increased markers of mitochondria function (7, 24), whereas mice lacking skeletal muscle HSP72 present with retention of enlarged, dysmorphic mitochondria that is paralleled with reduced muscle respiratory capacity, lipid accumulation, and muscle insulin resistance (9). Because an increase in skeletal muscle mitochondrial capacity is an important aspect of endurance training, the authors asked whether heat treatment after exercise could produce a synergistic HSP72 induction response. There were, indeed, additive effects observed such as for increased citrate synthase activity (marker of oxidative capacity) and an increase in protein expression of NDUFB8 and ATP5A (markers of mitochondrial content) (67).

Future directions. The authors suggest heat stress could be translated to a clinical setting to act as an effective postexercise treatment to enhance mitochondrial adaptations in skeletal muscle. This could be used for individuals who have difficulty in participating in sufficient exercise training such as the elderly, injured athletes, and patients (67). Clearly, there would be certain considerations if heat were to be used to enhance training-induced mitochondrial adaptations in skeletal muscle. If used in athletes, hydration would be imperative to allow for recovery between sessions. Patient groups would also need close monitoring, particularly the elderly who are more susceptible to the heat. If this type of protocol was going to be examined it may be worth considering limb-specific heating (i.e., lower limb only) to reduce the chances of any adverse events that could be caused by whole body hyperthermia.

HSPS AND PERFORMANCE/EXERCISE CAPACITY

Although exercise training increases HSP72 levels, it also alters many other physiological parameters and as such it is hard to definitively say that an increase in HSP72 levels is directly linked to an increase in performance. One way to try and decipher this is to use genetically modified rodents to investigate whether HSP72 overexpression leads to an increase in exercise performance. Our laboratory has demonstrated that overexpression of HSP72 in a transgenic mouse model (HSP72 Tg mice have increase in expression in the skeletal muscle, heart and brain) is protective against obesity-induced insulin resistance (7, 24–26). Interestingly, these animals also have an improved exercise tolerance that is associated with increases in markers of oxidative capacity in the skeletal muscle (24). Because these animals have the overexpression of the transgene from birth, it is possible that the increased levels of HSP72 predisposes the mice to improved exercise performance or alternatively increases habitual activity, which in turn initiates a training response. Although the finding that the HSP72 Tg mice have increased oxidative markers in the skeletal muscle would suggest that the increase in performance is due to a skeletal muscle phenotype, it cannot be excluded that overexpression of the transgene in another organ such as the brain is driving the increased performance. As mentioned earlier the part of the brain that drives habitual activity has been identified (29) and it is likely that brain plays a major role of maximal exercise performance particularly in relation to perception of effort.

HSP72 ACTIVATORS

If HSP induction is a key component of exercise adaptation and could contribute to improvements in athletic performance, then a key question arises as to whether pharmaceuticals could activate HSP and produce a "training-like" response. Hydroxylamine derivatives such as the compound BGP-15 have been demonstrated to activate HSP72 in skeletal muscle (7, 16). BGP-15 inhibits the early-phase acetylation of HSF1, prolonging the duration of it binding to HSEs activating HSP72 (19). Furthermore, an antiulcer drug acyclic polyisoprenoid derivative geranylgeranylacetone (GGA) increases HSF1 and HSP72 in liver of diabetic monkeys (32) and mice (2). Glutamine is another substance shown to induce a HSP72 in a number of organs and settings (76), whereas whey protein hydrolysate enhances the exercise-induced heat shock protein (HSP72)

response in rats (8). An alternative approach to produce an HSP72 response is to inhibit HSP90. Upon HSP90 inhibition, HSF1 dissociates from HSP90, moves to the nucleus, and initiates an HSP72 response. KU-32 is one such HSP90 inhibitor that has been shown to act via HSP72 and has been shown to improve mitochondrial biogenesis (49). The effect most of these HSP72 activators or coactivators have on athletic performance is unknown and studies are warranted to determine their impact on exercise capacity.

In addition to enhancement of exercise performance, HSP activators may have clinical utility in acting as a partial replacement for exercise. In this sense, one could hijack the exercise-induced heat shock response for patients that cannot otherwise exercise. Clearly, exercise is an effective therapy for many conditions where an individual's metabolism is impaired, including in obesity, insulin resistance, and Type 2 diabetes. However, many people, especially those who are frail and/or elderly or those recovering from serious accident or injury, cannot exercise and of those that can exercise, rates of compliance are low. Therefore, finding therapeutics that target pathways regulated by exercise such as the HSP response and/or finding the best modes of exercise training to give a maximal HSP response in those that can exercise is of interest.

Future directions. Recent data from transgenic mouse models suggest that skeletal muscle HSP72 overexpression may lead to improved exercise capacity and mitochondrial biogenesis (26). Given that HSP72 coactivators such as BGP-15 also increase mitochondrial density (26), this begs the question: are HSP72 mimetics performance enhancing? Although heat stress and thermotolerance may be seen as legal ergogenic aids, would this extend to HSP72 activating compounds? If future studies prove this to be the case this would need to be considered by the relevant doping-control authorities. Furthermore, although there are now plenty of studies on the induction of HSP72 with exercise, there is a distinct lack of explanation to the mechanisms leading to the degradation or removal of HSP72 after exercise. Would delaying degradation actually be a more effective target than inducing more HSPs?

LOOKING FORWARD

We now know that the induction of HSPs with exercise differs with the class of HSPs, the organ examined, with different species or sexes, and with different types of exercise performed. Although the level of understanding in relation to the exercise-induced heat shock protein response has increased dramatically within the last two decades, many questions still remain. This review has suggested some of the potential avenues of future research in the field of exercise-induced HSP biology. The answering of these lingering questions will give us a better understanding of the biology of the HSP response and its potential application.

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DISCLOSURES

M.A.F. is Chief Scientific Officer of N-Gene Research Laboratories Ltd. D.C.H. and M.H. have no conflict.

AUTHOR CONTRIBUTIONS

Author contributions: D.C.H. and M.H. prepared figures; D.C.H., M.A.F., and M.H. drafted manuscript; D.C.H., M.A.F., and M.H. edited and revised manuscript; D.C.H., M.A.F., and M.H. approved final version of manuscript.

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