Functions and Availability of HSP72 in Skeletal Muscle

Tomonori OGATA

(Received on October 30, 2020)

ABSTRACT

The cell has a number of defensive systems to promote survival during periods of environmental stress. One of the most highly conserved system of cellular protection involves the expression of a polypeptide family known as heat shock proteins (HSPs). HSPs ubiquitously express in multiple cells and act as molecular chaperones that have cytoprotective functions: 1) to help protein folding in various intracellular compartments, 2) to maintain structural proteins, 3) to translocate proteins across membranes into cellular compartments, 4) to prevent protein aggregation, and 5) to degrade unstable proteins. A 72 kDa stress-inducible HSP72 is one of the most prominent members of the HSP family, and has widely studied in mammalian skeletal muscle. This review focuses mainly on HSP72, which has been well characterized in skeletal muscle and which recent evidence suggests functional availability to skeletal muscle.

Expression of HSP72 in Skeletal Muscle

Multiple HSPs are expressed in skeletal muscle. HSPs have normally been classified according to molecular mass. The most prominent are small HSP (αA- and αB- crystallin and HSP27), HSP40, HSP60, HSP70, HSP90, and HSP110 families. HSPs are expressed either constitutively or can be induced by stress stimulation in skeletal muscle (9, 17). Molecular chaperones such as HSPs are well known to reduce cellular damage (8, 19). HSPs have multiple functions in maintaining intracellular integrity via protection, repair, and even control of signaling for cell death (17, 41). HSP72, a molecular weight 72-kDa and stress-inducible isoform, is one of the most prominent isoforms belonging to the HSP70 family, and has been well studied in mammalian skeletal muscle. Its expression is increased by multiple stressors including thermal stress, oxidative stress, and exercise (9, 22, 33). For example, HSP72 expression is upregulated immediately after thermal stress in the soleus muscle, and within 24 h in the plantaris muscle

(33). Both exhaustive endurance exercise (5) and resistance exercise (43) markedly increase HSP72 expression. On the other hand, decreased mechanical loading, such as cast immobilization and rat tail suspension, leads to the downregulation of HSP72 with increasing susceptibility to damage of skeletal muscle (1, 20, 36). Thus, exercise-related stimulations seem to be necessary to increase and/or sustain HSP72 content in skeletal muscle.

Exercise elicits numerous cellular and molecular stressors, such as heat, substrate depletion, and oxidative stress, appear to behave as inductive stimuli, in isolation or in combination, for increases in HSP72 (34) (6) (15). Although high intensity or endurance exercise could strongly induce HSP72 expression in skeletal muscle, recent study indicates that the development of fatigue of muscle fiber is not necessary for contraction-induced activation of HSP72 transcription in skeletal muscle fiber (42). In addition, the intermittent intracellular Ca^{2+} transient seems to be sufficient to activate HSP72 transcription in muscle fiber even without force generation. Stary and Hogan (2016) observed that both fatigued and nonfatigued muscle with electrical stimulation-related contraction (0.10 Hz and 0.33 Hz) similarly increased HSP72 mRNA. Muscle single fiber treated N-benzyl-p-toluene sulfonamide, which resulted in significant impairment of cross bridge cycling and reduction of the development of muscle fiber tension, also showed sufficient HSP72 mRNA induction by electrical stimulation, as same as untreated control muscle fiber. Thus, excitation-contraction coupling, force generation, fatigue may be not essential for exercise-related HSP72 synthesis in skeletal muscle. The greater expression of HSP72 in slow-twitch fibers than in fast–twitch fibers may strongly depend on frequencies of mechanical stress and intracellular Ca^{2+} transient.

HSP72 and Muscle Protection and Regeneration

The acquisition of muscle tolerance to stressors inducing muscle damage, such as mechanical and oxidative stresses, is related to prevention of cell injury and facilitation of recovery from injury. Heat treatment appears to contribute to cellular protection and facilitation of cellular remodeling after injury in skeletal muscle (37) (44). Prior heat treatment can depress the increments of plasma creatine kinase (CK) and infiltration of mononuclear inflammatory cells in rat skeletal muscle after eccentric running exercise. In addition, pre-heat treated rats present with a greater increase in total protein concentration and neonatal MyHC expression than non-heated rats during recovery after exercise (44).

Recently, direct evidences that HSP72 contributes significantly to provide muscle protection

to exercise stimulation have been reported, supporting the benefits of heat preconditioning (2, 13, 14, 21, 24–26) . First definitive evidence was study of HSP72 transgenic (TG) mice by McArdle et al. (24). They reported that muscles from HSP72 TG mice had less muscle fiber damage and reduced deficits in muscle-specific force compared to wild type (WT) mice following the lengthening muscle contractions. Furthermore, HSP72 also seems to contribute facilitating regeneration following muscle injury as well as muscle protection. Skeletal muscles from HSP72 TG mice were found to reduce the number of necrosed myofibers by cryolesioning and to have enhanced subsequent morphological recovery (26). Pharmacological enhancement of HSP72 by BGP-15 treatment promotes the increment of embryonic MyHC, type I MyHC, and type II MyHC in regenerating muscles and prevents the reduction in tetanic force and fatigue resistance of regenerating soleus muscle after cryolesion-induced muscle damage (30). Moresi et al. directly reported role of HSP70 in regenerating process (27). In their study, HSP72 was overexpressed by plasmid electroporation into muscle 3 days following cryolesioning-induced muscle injury. The cross sectional area of regenerating myofibers positive HSP72 showed significantly larger than control 7 days following muscle injury. Thus, enhancing HSP72 expression post-injury can facilitate the muscle regenerative process, suggesting availability as therapeutic target.

Inversely, lack of HSP70 in skeletal muscle leads impairments of inflammatory response and regeneration after injury. Senf et al. (2013) conducted experiment using WT and HSP70 -/-, *Hspa1a and Hspa1b knockout*, mice which were injured muscles by injection of cardiotoxin, and observed regenerative process at various time points. They observed that the expression of pro-inflammatory cytokines and immune cell infiltration were drastically reduced in injured muscle from HSP70 -/- mice compared with WT mice at early time point (40). This disturbed early inflammatory response in HSP70 -/- mice was associated with impairments in subsequent muscle recovery which were sustained inflammation and smaller fiber size than WT mice at later time points. Because HSP70 could bind and activate macrophages (16) and neurophils (35) which infiltrated damaged muscle and regulate regeneration process, level of HSP70 in skeletal muscle has a significant impact on successful regeneration via inflammatory process after muscle injury.

Collectively, HSP72 has functions to protect skeletal muscle from exercise stimulation and to facilitate recovery from injury. Prolonged exercise training notably induces HSP72 in skeletal muscle, and the expression level is maintained for a long term compared with a single bout of acute exercise (32). Therefore, long-term enhancement of HSP72 by repeated-exercise stimulations may be one of the muscle adaptation for prevention of muscle damage and facilitation of recovery.

HSPs and Sarcopenia

Aging causes a progressive loss of muscle mass and strength, called sarcopenia, independent of any disease process. Aging is associated with an increased susceptibility to contraction-induced muscle damage and disrupted fibers are observed more commonly in older muscle, suggesting less protection from damage than in younger muscle (31). The ability of the cell to induce HSPs following stress stimulation decreases with aging. Aged skeletal muscle in rats fails to increase HSP72 following muscle contractile activity, although increases do occur in younger adult (45–47). The lack of stress response in muscles of older rodents has been proposed as a major factor in the development of age-related functional deficits. The constitutive overexpression of HSP72 improves even normal age-related muscle dysfunction. Lifelong HSP72 overexpression in TG old mice can depress age-related increases in lipid peroxidation, catalase activity, and protein carbonyls (2). In addition, pharmacological enhancement of HSP72 induced by 17-(allylamino)-17-demethoxygeldanamycin treatment facilitates successful recovery of maximum tetanic force generation in aged-skeletal muscle at 28 days following lengthening muscle contraction (13).

HSP10, localize in mitochondria, also appear to contribute preventing sarcopenia progression. Lifelong overexpression of HSP10 in TG mice clearly can prevent age-related decreases in maximum force generation and fiber cross sectional area in muscle of old wild-type mice (14). In addition, levels of carbonylated mitochondrial proteins in HSP10 TG mice were lower than in wild-type mice. It is well known that mitochondrial dysfunction is associated with progression of the aging process (11) and that oxidative damage to mitochondrial DNA and proteins accumulates over time due to ROS produced by the electron transport chain. Segmental mitochondrial abnormalities containing mitochondrial DNA deletion mutations have been observed in aged skeletal muscle (48). Muscle fibers harboring mitochondrial mutations often display sectional atrophy, splitting, and increased steady-state levels of oxidative nucleic damage (3, 48). Relationship between HSP72 and mitochondria abnormality has been unknown. Because enhancement of HSP72 increases mitochondria number and oxidative capacity in skeletal muscle (12), further investigations are needed to clarify role of HSPs on age-related muscle weakness with mitochondria abnormality.

HSP72 and Disuse atrophy

Mechanical unloading induces skeletal muscle atrophy with reduction of HSP72 expression, but pre-heat treatment can mitigate subsequent unloading-induced muscle atrophy (28). This first evidence has led to investigate relationship between HSP72 and maintenance of muscle mass. HSP72 expression is downregulated in atrophying muscle during hindlimb immobilization in mice (29). On the other hand, HSP72 overexpression restrains progression of disuse-related muscle fiber atrophy (23, 38, 39). Senf et al. (2008) demonstrated that HSP72 overexpression by injection of an HSP72 expression plasmid abolished any increase of total ubiquitinated proteins in muscle after seven days of immobilization (39). In addition, they reported that FOXO3a transcriptional activity was increased by 7-fold following seven days of hindlimb immobilization, but was completely abolished in muscle injected with HSP72. This interaction between HSP72 and FOXO3a is also demonstrated by the inhibition of FOXO3a-dependent transcription of MAFbx by HSP72 (38, 39). Similar to FOXO3a, enhancement of NF-κB transcriptional activity during muscle disuse is completely abolished by HSP72 overexpression (39). Because FOXO signaling is able to account for ~40% of disuse muscle fiber atrophy (38), regulation of ubiquitin proteasome pathway via interaction between HSP72 and FOXO3a may be a main scenario in amelioration of unloading-induced muscle atrophy by pre-heat conditioning.

HSP72 and Regulation of Metabolism

Evidences show that heat treatment blocks the development of insulin resistance in response to a high-fat diet (7, 10). Gupte et al. (2009) showed that high-fat fed rats treated weekly with heat had lower serum insulin and effective glucose clearance after glucose injection compared with non-heat treated high-fat fed rats (10). They observed that heat treatment results in decreased activation of JNK and IKK-β, which are implicated in insulin resistance, and upregulation of HSP72 and HSP25.

In a human study, HSP72 mRNA in muscle is significantly lower in type 2 diabetic patients than in healthy subjects, and HSP72 mRNA content in muscle inversely correlates with the rate of glucose uptake and insulin-stimulated carbohydrate and lipid metabolism (18). Furthermore, obese insulin-resistant humans have lower HSP72 protein levels compared with healthy people (4). Although heat treatment upregulates HSP72, the increase is attenuated by a high-fat diet (4).

The benefit of thermal treatment to insulin resistance have been confirmed by studies of genetic overexpression model of HSP72 (4). TG mice with muscle-specific overexpression of HSP72 have lower levels of fasting glucose and insulin than wild-type mice after high-fat diet. In addition, mice with HSP72 overexpression display notably improved glucose and insulin tolerance compared with wild-type mice when placed on a high-fat diet (4). Upregulation of HSP72 by BGP-15 treatment also shows the same effects as the transgenic model on insulin resistance (4). It has been indicated these effects of HSP72 increment were involved with minimizing inflammation (10) (4) and enhancing metabolic oxidation via mitochondria (12) in skeletal muscle. HSP72 TG mice has a large number of mitochondria and exhibits superior running capacity and increased fatty acid oxidation compared with WT mice (12). Therefore, HSP72 has potentially availability for ameliorations of metabolic condition and enhancement of endurance capacity.

1. Adams GR, Caiozzo VJ, and Baldwin KM. Skeletal muscle unweighting: spaceflight and ground-based models. *J Appl Physiol* 95: 2185–2201, 2003.
2. Broome CS, Kayani AC, Palomero J, Dillmann WH, Mestril R, Jackson MJ, and McArdle A. Effect of lifelong overexpression of HSP70 in skeletal muscle on age-related oxidative stress and adaptation after nondamaging contractile activity. *Faseb J* 20: 1549–1551, 2006.
3. Bua EA, McKiernan SH, Wanagat J, McKenzie D, and Aiken JM. Mitochondrial abnormalities are more frequent in muscles undergoing sarcopenia. *J Appl Physiol* 92: 2617–2624, 2002.
4. Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, Lancaster GI, Southgate RJ, Bruce CR, Duffy SJ, Horvath I, Mestril R, Watt MJ, Hooper PL, Kingwell BA, Vigh L, Hevener A, and Febbraio MA. HSP72 protects against obesity-induced insulin resistance. *Proceedings of the National Academy of Sciences of the United States of America* 105: 1739–1744, 2008.
5. Febbraio MA, and Koukoulas I. HSP72 gene expression progressively increases in human skeletal muscle during prolonged, exhaustive exercise. *J Appl Physiol* 89: 1055–1060, 2000.
6. Febbraio MA, Steensberg A, Walsh R, Koukoulas I, van Hall G, Saltin B, and Pedersen BK. Reduced glycogen availability is associated with an elevation in HSP72 in contracting human skeletal muscle. *The Journal of physiology* 538: 911–917, 2002.
7. Geiger PC, and Gupte AA. Heat shock proteins are important mediators of skeletal muscle insulin sensitivity. *Exercise and sport sciences reviews* 39: 34–42, 2011.
8. Glover JR, and Lindquist S. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* 94: 73–82, 1998.
9. Gonzalez B, Hernando R, and Manso R. Stress proteins of 70 kDa in chronically exercised skeletal muscle. *Pflugers Archiv: European journal of physiology* 440: 42–49, 2000.
10. Gupte AA, Bomhoff GL, Swerdlow RH, and Geiger PC. Heat treatment improves glucose tolerance and prevents skeletal muscle insulin resistance in rats fed a high-fat diet. *Diabetes* 58: 567–578, 2009.
11. Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc* 20: 145–147, 1972.
12. Henstridge DC, Bruce CR, Drew BG, Tory K, Kolonics A, Estevez E, Chung J, Watson N, Gardner T, Lee-Young RS, Connor T, Watt MJ, Carpenter K, Hargreaves M, McGee SL, Hevener AL, and Febbraio MA. Activating HSP72 in rodent skeletal muscle increases mitochondrial number and oxidative

capacity and decreases insulin resistance. *Diabetes* 63: 1881–1894, 2014.
13. Kayani AC, Close GL, Broome CS, Jackson MJ, and McArdle A. Enhanced recovery from contraction-induced damage in skeletal muscles of old mice following treatment with the heat shock protein inducer 17-(allylamino)-17-demethoxygeldanamycin. *Rejuvenation Res* 11: 1021–1030, 2008.
14. Kayani AC, Close GL, Dillmann WH, Mestril R, Jackson MJ, and McArdle A. Overexpression of HSP10 in skeletal muscle of transgenic mice prevents the age-related fall in maximum tetanic force generation and muscle Cross-Sectional Area. *Am J Physiol Regul Integr Comp Physiol* 299: R268–276, 2010.
15. Khassaf M, Child RB, McArdle A, Brodie DA, Esanu C, and Jackson MJ. Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise. *Journal of applied physiology (Bethesda, Md: 1985)* 90: 1031–1035, 2001.
16. Kovalchin JT, Wang R, Wagh MS, Azoulay J, Sanders M, and Chandawarkar RY. In vivo delivery of heat shock protein 70 accelerates wound healing by up-regulating macrophage-mediated phagocytosis. *Wound repair and regeneration: official publication of the Wound Healing Society [and] the European Tissue Repair Society* 14: 129–137, 2006.
17. Kregel KC. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J Appl Physiol* 92: 2177–2186, 2002.
18. Kurucz I, Morva A, Vaag A, Eriksson KF, Huang X, Groop L, and Koranyi L. 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.
19. Latchman DS. Heat shock proteins and cardiac protection. *Cardiovasc Res* 51: 637–646, 2001.
20. Lawler JM, Song W, and Kwak HB. Differential response of heat shock proteins to hindlimb unloading and reloading in the soleus. *Muscle & nerve* 33: 200–207, 2006.
21. Liu CC, Lin CH, Lin CY, Lee CC, Lin MT, and Wen HC. Transgenic overexpression of heat shock protein 72 in mouse muscle protects against exhaustive exercise-induced skeletal muscle damage. *Journal of the Formosan Medical Association = Taiwan yi zhi* 112: 24–30, 2013.
22. Locke M, Noble EG, and Atkinson BG. Inducible isoform of HSP70 is constitutively expressed in a muscle fiber type specific pattern. *The American journal of physiology* 261: C774–779, 1991.
23. Lomonosova YN, Shenkman BS, and Nemirovskaya TL. Attenuation of unloading-induced rat soleus atrophy with the heat-shock protein inducer 17-(allylamino)-17-demethoxygeldanamycin. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology* 26: 4295–4301, 2012.
24. McArdle A, Dillmann WH, Mestril R, Faulkner JA, and Jackson MJ. Overexpression of HSP70 in mouse skeletal muscle protects against muscle damage and age-related muscle dysfunction. *Faseb J* 18: 355–357, 2004.
25. Miyabara EH, Martin JL, Griffin TM, Moriscot AS, and Mestril R. Overexpression of inducible 70-kDa heat shock protein in mouse attenuates skeletal muscle damage induced by cryolesioning. *American journal of physiology Cell physiology* 290: C1128–1138, 2006.
26. Miyabara EH, Nascimento TL, Rodrigues DC, Moriscot AS, Davila WF, AitMou Y, deTombe PP, and Mestril R. Overexpression of inducible 70-kDa heat shock protein in mouse improves structural and functional recovery of skeletal muscles from atrophy. *Pflugers Archiv: European journal of physiology* 463: 733–741, 2012.
27. Moresi V, Garcia-Alvarez G, Pristera A, Rizzuto E, Albertini MC, Rocchi M, Marazzi G, Sassoon D, Adamo S, and Coletti D. Modulation of caspase activity regulates skeletal muscle regeneration and function in response to vasopressin and tumor necrosis factor. *PLoS One* 4: e5570, 2009.
28. Naito H, Powers SK, Demirel HA, Sugiura T, Dodd SL, and Aoki J. Heat stress attenuates skeletal

muscle atrophy in hindlimb-unweighted rats. *Journal of applied physiology (Bethesda, Md: 1985)* 88: 359–363, 2000.
29. Naito H, Powers SK, Demirel HA, Sugiura T, Dodd SL, and Aoki J. Heat stress attenuates skeletal muscle atrophy in hindlimb-unweighted rats. *J Appl Physiol* 88: 359–363, 2000.
30. Nascimento TL, Silva MT, and Miyabara EH. BGP-15 improves contractile function of regenerating soleus muscle. *Journal of muscle research and cell motility* 2018.
31. Ogata T, Machida S, Oishi Y, Higuchi M, and Muraoka I. Differential cell death regulation between adult-unloaded and aged rat soleus muscle. *Mech Ageing Dev* 130: 328–336, 2009.
32. Ogata T, Oishi Y, Higashida K, Higuchi M, and Muraoka I. Prolonged exercise training induces long-term enhancement of HSP70 expression in rat plantaris muscle. *American journal of physiology Regulatory, integrative and comparative physiology* 296: R1557–1563, 2009.
33. Oishi Y, Taniguchi K, Matsumoto H, Ishihara A, Ohira Y, and Roy RR. Muscle type-specific response of HSP60, HSP72, and HSC73 during recovery after elevation of muscle temperature. *J Appl Physiol* 92: 1097–1103, 2002.
34. Oishi Y, Taniguchi K, Matsumoto H, Ishihara A, Ohira Y, and Roy RR. Muscle type-specific response of HSP60, HSP72, and HSC73 during recovery after elevation of muscle temperature. *Journal of applied physiology (Bethesda, Md: 1985)* 92: 1097–1103, 2002.
35. Ortega E, Hinchado MD, Martin-Cordero L, and Asea A. The effect of stress-inducible extracellular Hsp72 on human neutrophil chemotaxis: a role during acute intense exercise. *Stress* 12: 240–249, 2009.
36. Selsby JT, Rother S, Tsuda S, Pracash O, Quindry J, and Dodd SL. Intermittent hyperthermia enhances skeletal muscle regrowth and attenuates oxidative damage following reloading. *J Appl Physiol* 102: 1702–1707, 2007.
37. Selsby JT, Rother S, Tsuda S, Pracash O, Quindry J, and Dodd SL. Intermittent hyperthermia enhances skeletal muscle regrowth and attenuates oxidative damage following reloading. *Journal of applied physiology (Bethesda, Md: 1985)* 102: 1702–1707, 2007.
38. Senf SM, Dodd SL, and Judge AR. FOXO signaling is required for disuse muscle atrophy and is directly regulated by Hsp70. *American journal of physiology Cell physiology* 298: C38–45, 2010.
39. Senf SM, Dodd SL, McClung JM, and Judge AR. Hsp70 overexpression inhibits NF-kappaB and Foxo3a transcriptional activities and prevents skeletal muscle atrophy. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology* 22: 3836–3845, 2008.
40. Senf SM, Howard TM, Ahn B, Ferreira LF, and Judge AR. Loss of the inducible Hsp70 delays the inflammatory response to skeletal muscle injury and severely impairs muscle regeneration. *PloS one* 8: e62687, 2013.
41. Sreedhar AS, and Csermely P. Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: a comprehensive review. *Pharmacol Ther* 101: 227–257, 2004.
42. Stary CM, and Hogan MC. Cytosolic calcium transients are a determinant of contraction-induced HSP72 transcription in single skeletal muscle fibers. *Journal of applied physiology (Bethesda, Md : 1985)* 120: 1260–1266, 2016.
43. Thompson HS, Scordilis SP, Clarkson PM, and Lohrer WA. A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. *Acta Physiol Scand* 171: 187–193, 2001.
44. Touchberry CD, Gupte AA, Bomhoff GL, Graham ZA, Geiger PC, and Gallagher PM. Acute heat stress prior to downhill running may enhance skeletal muscle remodeling. *Cell Stress Chaperones* 17: 693–705, 2012.
45. Vasilaki A, Iwanejko LM, McArdle F, Broome CS, Jackson MJ, and McArdle A. Skeletal muscles of

aged male mice fail to adapt following contractile activity. *Biochem Soc Trans* 31: 455–456, 2003.
46. Vasilaki A, Jackson MJ, and McArdle A. Attenuated HSP70 response in skeletal muscle of aged rats following contractile activity. *Muscle & nerve* 25: 902–905, 2002.
47. Vasilaki A, McArdle F, Iwanejko LM, and McArdle A. Adaptive responses of mouse skeletal muscle to contractile activity: The effect of age. *Mech Ageing Dev* 127: 830–839, 2006.
48. Wanagat J, Cao Z, Pathare P, and Aiken JM. Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fiber splitting, and oxidative damage in sarcopenia. *Faseb J* 15: 322–332, 2001.