Heat stress-induced changes in skeletal muscle: Heat shock proteins and cell signaling transduction

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Abstract  Many researchers have been interested in the effects of heat stress on skeletal muscle. Recently, it has been demonstrated that heat stress on skeletal muscle results in an increase in muscle protein mass and subsequent muscle hypertrophy, and attenuation of muscle atrophy. Although the cellular mechanism remains unclear, it is thought that heat shock proteins (HSPs), which are highly conserved proteins induced by heat stress, play a major role in these phenomena. However, new insights suggest that heat stress-induced muscle hypertrophy and the prevention of muscle atrophy may be regulated not only by elevated HSP expression but also by multiple signaling pathways associated with protein synthesis and breakdown. Additionally, heat stress seems to cause various changes in other muscle functions. Although further studies are required to reveal the molecular biological mechanisms involved in the heat stress-induced changes in skeletal muscle, heat stress may be a useful tool for increasing muscle mass, attenuating disuse skeletal muscle atrophy, facilitating an early recovery from muscle damage, and improving glucose metabolism. This paper reviews studies of these effects of heat stress on skeletal muscle.

Keywords: HSPs, hypertrophy, disuse atrophy, muscle damage, glucose metabolism

Introduction

Local heat has been used clinically as therapy for sports-related muscle injuries. As muscle atrophy and hypertrophy are also affected by heat stress, many researchers have attempted to clarify the mechanisms of the effects of heat stress on skeletal muscle. The induction of heat shock proteins (HSPs) and changes in cell signaling transduction related to hypertrophy and atrophy have been well studied as the key to solving the problems. This paper reviews studies of the effects of heat stress on skeletal muscle.

Basic role of HSPs in skeletal muscle

Organisms have various mechanisms for defending against stresses. At the cellular level, proteins are synthesized rapidly in response to stress to maintain homeostasis. These proteins are called heat shock proteins or HSPs because elevated temperature (or heat shock; +3–5°C) was one of the first stressors applied. HSPs can be divided into several groups based on their molecular mass, and distinct families of HSPs have been identified. Of particular interest is the inducible form of the 70-kDa family of HSPs (HSP72). HSP72 facilitates protein folding and is important in the repair of damaged proteins. The expression of HSP72 is controlled by intracellular heat shock transcription factor (HSF), and the production of HSP72 in cells is dependent on both the intensity (temperature) and duration of the stressor.

HSPs play important physiological roles in both normal and stressed cells, and elevated cellular levels of HSP72 protect against potentially lethal stresses such as hyperthermia, oxidative stress, ischemia, metabolic inhibition, and exercise. In skeletal muscles when protein synthesis is enhanced because of muscle hypertrophy, HSP72 assists in the assembly of myofibrillar proteins.

Methods used to elevate muscle temperature

There are many methods of causing heat stress in vivo and the method of producing heat stress should be selected carefully depending on the research purpose. For example, a thermal blanket, which is used to apply whole-body heat stress to animals, set at 45°C can increase the rat rectal temperature to around 41°C. Another method often used to heat the whole body of small animals is a heat chamber set at 41–42°C. This heat stress can increase the colonic temperature of animals to 41°C at 30 min after initiating heating, and then maintain the colonic temperature at 41–42°C. Both whole-body heating methods can induce HSPs in skeletal muscle. Local heat stress such as warm water immersion can be applied to
both animals and humans. Immersing the lower body in water at 42°C increases the muscle temperature of animals to 41~42°C9). Unlike animal studies, trying to increase the muscle temperature in human subjects above 40°C by using water immersion is difficult and creates ethical problems. Surprisingly, Morton et al.10) reported that one-leg immersion in warm water maintained at approximately 45°C for 1 h increases the muscle temperature to 39.5°C in humans. Other methods, such as microwaves11,12) or ultrasound13,14), are often used in human studies to increase the muscle temperature. The authors confirmed that 2450-MHz microwave systems increased the muscle temperature to around 41°C at 20 min after initiating heating15) and induced HSPs16) in the human vastus lateralis muscle. A few comparative studies have examined the use of heating methods on living tissue such as muscle and reported that microwaves are more effective than ultrasound17).

Heat stress, muscle hypertrophy, and the muscle-strengthening effect

Skeletal muscle hypertrophy is characterized by an increase in muscle protein mass. Adaptive mechanisms are thought to increase the net protein balance through either increased protein synthesis or reduced protein degradation in skeletal muscle, leading to muscle protein accretion. Therefore, increased protein synthesis is essential for muscle hypertrophy, and it has been proposed that heat stress might be a potent stimulus for increased protein synthesis and muscle mass. Uehara et al.18) also demonstrated that a bout of heat exposure resulted in an increase in the mass of the rat soleus muscle 7 days after heat exposure. Consequently, heat stress seems to promote muscle protein synthesis and muscle hypertrophy.

A potential mechanism for heat-stress-related muscle hypertrophy seems to be the effect of HSPs, particularly the inducible form of HSP72 induced by heat stress. As mentioned above, HSPs play an important role in chaperoning nascent peptides during translation4), and the increased HSP72 due to heat stress may facilitate muscle protein synthesis, thereby increasing skeletal muscle mass. However, Frier and Locke20) reported that heat stress before mechanical overloading did not result in hypertrophy in the rat plantaris muscle, despite increased HSP72 expression. On the other hand, Goto et al.21) found that heat stress at 38°C (45 and 60 min) increased rat soleus muscle mass; and they also found that heat stress for 10 weeks (4 days/week, 8 h) increased force development and the cross-sectional area of the vastus lateralis in humans without inducing HSP72 expression21). Thus, it is difficult to explain heat stress-related muscle hypertrophy through only the induction of HSPs. Other mechanisms may be responsible for heat stress-related muscle hypertrophy.

Heat stress and cell signaling transduction related to muscle hypertrophy

Recent studies have demonstrated that the mammalian target of rapamycin (mTOR) signaling pathway has a key role in stimulating translation initiation, which is a
Heat stress and skeletal muscle

Heat stress and skeletal muscle affect the mTOR signaling pathway in skeletal muscle. For example, Akt and 70S6K phosphorylation were shown to be temperature sensitive over a wide range of temperatures in vitro. Future studies need to clarify the optimal conditions and threshold of heat stress that activate the mTOR signaling pathway in skeletal muscle. Additionally, protein synthesis related to other cell signaling pathways should be studied. For example, calcineurin is a phosphatase modulated by Ca²⁺/Calmodulin that produces signal transduction involved in muscle hypertrophy. Activated calcineurin dephosphorylation of the nuclear factor of activated T cells (NFAT) leads to its transfer into the nucleus and promotes the transcription of genes related to muscle hypertrophy. Kobayashi et al. found calcineurin expression with the increased mass of the rat soleus muscle 7 days after heat exposure. One possible explanation of this phenomenon is the increment in the intracellular Ca²⁺ level caused by heat exposure, leading to the calcineurin signaling pathway.

Heat stress boosts the anabolic effect of strength training

Recently, Goto et al. demonstrated that the combination of low-intensity resistance exercise and heat stress caused an increase in muscle size compared to exercise alone in humans. Therefore, resistance exercise combined with heat stress has the potential to enhance resistance-exercise-induced muscle hypertrophy. However, the
Components of the mTOR pathway are upregulated rapidly in rat and human skeletal muscle following mechanical stimuli such as resistance exercise. Kumar et al. showed that p70S6K phosphorylation was related to the rate of muscle protein synthesis after resistance exercise in human skeletal muscle. Furthermore, studies of rodent and human skeletal muscle have shown that p70S6K phosphorylation after resistance exercise is positively correlated with the degree of skeletal muscle hypertrophy after long-term resistance training. These results indicate that the activation of mTOR and its downstream targets (p70S6K and 4E-BP1) after resistance exercise is critically important in regulating skeletal muscle mass. Based on in vitro observations that heat stress increases the phosphorylation of Akt in rat ventricular myocytes and of p70S6K in fibroblasts, the authors tested a hypothesis that resistance exercise during heat stress could activate the mTOR signaling pathway with an additive effect. As a result, it was observed that resistance exercise with heat stress increased the phosphorylation of Akt, mTOR, S6, and 4E-BP1 in the vastus lateralis muscle of young subjects more than exercise alone. Thus, it was speculated that heat treatment activates the key anabolic regulator molecule Akt, which starts a reaction cascade in the human muscle cells.

**Heat stress and the prevention of muscle atrophy**

A prolonged period of skeletal muscle inactivity results in a loss of muscle mass and strength. Therefore, it is important to develop countermeasures to prevent the loss of muscle mass and strength caused by disuse atrophy. Heat stress has attracted attention as an effective measure to counter disuse muscle atrophy. The authors previously demonstrated that one bout of whole-body heat stress (41°C for 60 min) attenuated rat soleus muscle atrophy induced by 8 days of hindlimb unloading. It has been thought that HSPs play a key role in the facilitation of protein translation, as molecular chaperones, and in the prevention of proteolytic activity by binding denatured proteins. Some investigators have also reported that heat stress prevents hindlimb unloading- or immobilization-induced muscle atrophy and facilitates the recovery of atrophied muscle. Although the cellular mechanism responsible for the heat stress-induced pre-

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**Fig. 3** Effects of resistance exercise with heat stress on the phosphorylation of Akt, mTOR, S6 and 4E-BP1 in human skeletal muscle before (pre), immediately after (post), and 1 h after (+1 h) resistance exercise. All values are expressed relative to the pre values for each condition. The data are expressed as mean ± SEM (n = 8). *P < 0.05 versus pre; †P < 0.05 versus post; †P < 0.05 versus resistance exercise. (From Kakigi et al.)

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vention of disuse muscle atrophy remains unclear, HSPs clearly suppress myonuclear apoptosis, or programmed cell death, which is a potential candidate pathway for inactivity-induced muscle atrophy. Some studies have demonstrated that overexpression of HSPs inhibits apoptosis and prevents caspase activation in different cellular models. In general, these effects are thought to be because HSPs inhibit cytochrome c release and the distribution of apoptosome formation by blocking their interaction with cytochrome c, apoptotic protease activating factor 1, and caspase-3. Moreover, HSPs influence caspase-independent apoptosis by interacting with apoptogenic factors, such as apoptosis-inducing factor (AIF) and endonuclease G (EndoG). These data suggest that HSPs could suppress muscle atrophy mediated by caspase-dependent or -independent apoptosis.

Proteolytic responses to heat stress in unloaded muscle

Although the rate of protein synthesis declines rapidly following unloading, disuse muscle atrophy occurs mainly via an increase in the rate of protein degradation. Heat stress could also protect muscle cells from oxidative stress and inhibit protease. Selsby et al. reported that intermittent heat stress reduced oxidative stress in the immobilized soleus muscle. Furthermore, they indicated that heat stress also attenuated the reloading-induced oxidative damage to the muscle cells. A more recent study reported that one bout of whole-body heat stress suppressed the activation of calpain, which is a Ca2+-dependent cysteine protease, induced by 6 days of immobilization in the soleus muscles of adult rats. These data indicate that heat stress attenuates inactivity-induced muscle atrophy by suppressing the acceleration of proteolysis. However, there is limited information on the effect of heat stress on proteolytic responses, and further studies are required to clarify the mechanism of the heat stress-induced prevention of muscle atrophy.

Effects of heat stress on preventing muscle damage and the regeneration process

Unaccustomed exercise induces muscle damage and soreness. This phenomenon is known as delayed-onset muscle soreness (DOMS). It has been proposed that heat preconditioning can prevent the reduction in muscle strength and range of motion associated with DOMS in humans. The authors observed that heat preconditioning 1 day before eccentric exercise of the upper arm prevented the decrease in muscle strength and range of motion compared with the non-heated upper limb. The suggested mechanism is that the induction of HSPs by heat stress protects cells from eccentric-exercise-induced muscle damage. Furthermore, in rat skeletal muscle, several studies showed that heat stress attenuates muscle damage and contributes to promoting recovery. For example, Kojima et al. showed that a bout of heat stress before or after muscle damage promoted the recovery of the muscle protein content. Oishi et al. also observed that heat-stressed soleus muscle had an increased muscle mass and cross-sectional area 2 weeks after muscle damage. Both studies showed that heat stress activated satellite cells and induced HSP72 expression. Therefore, heat stress may help the recovery of muscle regeneration via activated satellite cells and HSP72 expression.

Glucose metabolism in skeletal muscle

Skeletal muscle is a therapeutic target tissue for preventing and improving type 2 diabetes. Hooper subjected patients with type 2 diabetes to “hot tub therapy” over 3 weeks and found that this modestly improved their fasting blood glucose and glycosylated hemoglobin levels. Subsequently, studies have examined heat treatment as a therapeutic strategy for type 2 diabetes. A part of the mechanism underlying these improvements is thought to be associated with HSP induction in skeletal muscle with heat stress. The activation of stress kinases such as c-Jun N-terminal kinase (JNK) and inhibitor of κB kinase (IKK) is associated with the development of insulin resistance in skeletal muscle. These stress kinases phosphorylate insulin receptor substrate-1 (IRS-1) on serine residues and impair the downstream insulin-dependent signal pathway. HSPs seem to suppress these stress kinases, resulting in improved insulin sensitivity and glucose tolerance. However, Kurucz et al. demonstrated that reduced skeletal muscle HSP72 expression correlates with the degree of insulin resistance in individuals with type 2 diabetes. Therefore, it is important for type 2 diabetics to increase HSPs induction via extraneous stimuli, such as exercise and hyperthermia.

Chung et al. reported that elevating HSP72 protein by heat treatment, muscle-specific transgenic overexpression, or pharmacological means could protect mice from diet- or obesity-induced glucose intolerance and insulin resistance. Moreover, this protection was tightly associated with the prevention of JNK phosphorylation. Additionally, Gupte et al. suggested that weekly heat treatment for 12 weeks decreased JNK phosphorylation and IKKβ activation, and improved insulin signaling and glucose uptake in skeletal muscle in insulin-resistant rats fed a high-fat diet. Furthermore, they reported that acute heat treatment also improved the insulin-stimulated glucose uptake in age-related insulin-resistant skeletal muscle in vivo and in vitro. Combined, these studies demonstrate the possibility of the beneficial effects of heat stress in preventing and improving type 2 diabetes.
References


