HEAT STRESS AND MUSCLE HYPERTROPHY: EFFECTS AND MECHANISMS

Medha Kapoor, Laxmi Prabha Singh

Defence Institute of Physiology and Sciences (DIPAS), Defence Research and Development Organization (DRDO), Timarpur, Lucknow Road, New Delhi

E-mail of Corresponding Author: heatphysiology@gmail.com

ABSTRACT

Maintenance of skeletal muscle integrity is a very important concern for certain occupational workers such as athletes, sportsmen and military personnel. A number of published reports suggest that heat stress in a potent activator of muscle hypertrophy and can be used as a powerful tool to prevent muscle atrophy during conditions of bed rest due to injury or illness as well muscle inactivity due to any other reason. Therefore, application of heat to muscle can be very beneficial not only for soldiers and athletes, but patients during habilitation for gaining muscle mass and force generation. This review attempts to summarize the recent advances in understanding of mechanisms by which heat stress causes skeletal muscle hypertrophy with an emphasis on the signal transduction pathways that play detrimental roles in regulating muscle hypertrophy in response to heat stress.

Keywords: Heat stress, Muscle hypertrophy, mTOR, Satellite cells, Thermotherapy

INTRODUCTION

The skeletal muscle comprises of approximately 50% of total body weight. It is the largest tissue in the human body and controls body functions such as breathing, force generation and movement. Heat stress in one of the prominent stimuli in addition to exercise, overloading, vascular occlusion and vibration for induction of muscle hypertrophy. The enhanced proliferation of muscle specific stem cells, the satellite cells, and their subsequent differentiation into mature muscle cells is one of the major contributors to muscle hypertrophy, apart from protein synthesis [1, 2, 3, 4]. The expression of the developmental (embryonic and neonatal) Myosin Heavy Chain (MHC) isoforms in adult skeletal muscle indicates a state of generation of new muscle fibers. Therefore, at the time of muscle hypertrophy, an enhanced expression of the developmental isoforms is observed [5].There are various signaling pathways regulating protein synthesis for induction of hypertrophy and Akt/mTOR stands out as the most significant and extensively studied pathway amongst all. We attempt to summarize the literature pertaining to effect of heat stress on muscle hypertrophy and understanding of the signal transduction pathways that underlie muscle hypertrophy in response to heat stress.

The role of satellite cells and protein synthesis in muscle hypertrophy

The skeletal muscle specific stem cells called as satellite cells as well as protein synthesis are the major contributors to the skeletal muscle mass. The satellite cells have the potential to proliferate and subsequently fuse and differentiate to form myofibers under conditions of cellular stress. Myogenic precursor cells expressing specific transcriptional factors such as MyoD and Myf5 differentiate into myoblasts, which proliferate and fuse to form myotubes, the immature multinucleated skeletal muscle cells. These develop into mature skeletal muscle cells at the time of embryonic development. The histopathological examination of muscles

following an injury has revealed an increase in the satellite cells numbers and their subsequent differentiation into myotubes and myofibers [5, 6]. Muscles without satellite cells have been found to lack any regenerative potential [7, 8]. An increase in muscle protein content, which results from increased muscle protein synthesis and/or reduction in muscle protein catabolism contributes to skeletal muscle hypertrophy. However, the molecular mechanisms, which mediate the sensing of stimuli for muscle hypertrophy still remain unknown [9].
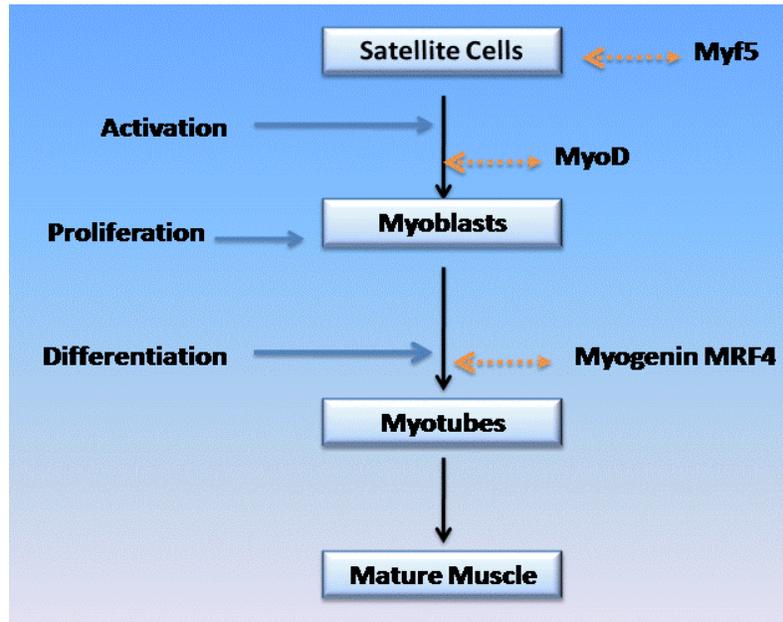

Fig 1: Differentiation of satellite cells into mature muscle cells

Induction of muscle hypertrophy via heat stress
The application of thermotherapy to damaged or atrophied muscles is a standard practice to achieve muscle regeneration. Heat application not only leads to the proliferation of satellite cells [3] but also enhances the supply of infiltering phagocytes to the injured muscle and ensuing removal of the damaged cells [10]. Pan et al., 2012 investigated the role of the oxidative stress response in preconditioning heat stimulation in Rat skeletal muscle. In this study, Fluctuating Local Somato Thermal Stimulation (LSTS) between 37-44°C was applied to the left quarter ventral abdomen muscle of male Sprague-Dawley rat for 27 minutes. The concentration of Reactive Oxygen Species (ROS), Nitric Oxide (NO) metabolites, malonaldehyde, dismutase, catalase and glutathione were measured 0, 5,15, 30 and 60 minutes post LSTS with the 0 minute readings

serving as baseline(without LSTS). A significant increase in the concentration of ROS, NO metabolites, malonaldehyde was observed 5 minutes after LSTS. On the other hand the dismutase activity was found to be the least after 5 minutes post LSTS. The catalase and glutathione activity was recorded to be the least after 15 minutes post LSTS. Also it was shown that the inhibition of NO synthesis using NO synthase inhibitor L- N-nitro-L-arginine methyl ester resulted in a decrease in HSP 70 expression [11]. The possible explanation is the partial involvement of NO in HSP 70 production. There are reports highlighting that the ROS can also stimulate other pathways for upregulation of Heat Shock Proteins (HSPs) [12].
Heat stress attenuates skeletal muscle atrophy in hindlimb unweighted rats. Niato et al., 2000 developed a muscle atrophy rat model employing

8 day hind limb unweighting. For the study, 40 rats were divided in a 4 groups with 10 rats in each, namely sedentary control(group 1) heat stress(group 2) hind limb unweighting(group 3) and heat stress(group 4) prior to hind limb unweighting. The second and third groups were subjected to heat stress at 41°C for 60 minutes in a heat chamber. The third and fourth groups were subjected to hindlimb unweighting for 8 days. At the end of the 8th day, soleus muscle was removed and HSP72 and total protein concentration were measured. There was a difference of 32% in the muscle weight loss between group 3 and group 4, with group 3 exhibiting greater loss. Also, group 3 exhibited greater protein loss compared to group 4. The HSP 72 levels were significantly higher in group 2 and 4 compared with control values. These results strongly suggest that thermotherapy can reduce the rate of immobilization and disuse induced muscle atrophy [4]. A study by Booth et al., 1997 suggested that hindlimb unweighting induced atrophy follows a typical course wherein the protein synthesis shows a rapid reduction (peaking 7th day post unloading) whereas the protein degradation takes place at a relatively slower pace (peaking 15th day post unloading) [13]. Protein degradation induced by muscle atrophy is accompanied by oxidative injury in myocytes. This is due to the fact that oxidatively modified proteins are susceptible to proteolytic attack. HSP 72 acts as a chaperon and binds to the oxidatively modified proteins assisting in protein refolding. Hence HSP 72 plays a very crucial role in preventing protein degradation [14].

Signaling pathways regulating muscle hypertrophy

The response of the skeletal muscles to various physiological stimuli is mediated via the following fundamental steps: Interaction of extracellular stimuli with plasma membrane receptors, activation of intracellular signaling pathways, changes in gene transcription and protein synthesis and ultimately, the muscle remodeling. The IGF-

1/PI3K/Akt signaling pathway has been found to play a key role in muscle hypertrophy. It influences the critical hypertrophic processes such as protein degradation and synthesis. The Akt family comprises of three members: Akt1, Akt2 and Akt3, out of which Akt1 and Akt2 isoforms are predominantly expressed in skeletal muscle. The Akt is activated upon phosphorylation, which is stimulated by the action of growth factors, cytokines and hormones in a PI3K dependent manner. Another very prominent stimulator of this pathway is mechanotransduction, which pertains to the transition from mechanical signal into a biochemical event and is a key regulator of induction of protein synthesis. Thus hypertrophy can be induced independent of IGFI and PI3K via mechanotransduction. The Akt induces two independent pathways which play very critical roles in inducing muscle hypertrophy via regulation of transcription, protein synthesis and degradation: mTOR (mammalian target of rapamycin) and GSK3β (glycogen synthase kinase-3β) pathways. The mTOR upon activation influences protein synthesis via the action of three downstream molecules: p70s6k, 4E-BPI and eEF2. Muscle hypertrophy is also induced via the phosphorylation of GSK-3β, which inactivates GSK3β, which in turn inactivates eIF2B resulting in protein translation. A third mechanism by which Akt regulates skeletal muscle hypertrophy is the inactivation of Forkhead Transcription Factors (FOXO or FKHR), which regulate gene transactivation of the proteolytic system components, constituting the ubiquitin-proteasome system. The FOXO isoforms (1, 2 and 3) are active when located in the nucleus and induce transcription of genes involved in muscle atrophy such as atrogin-I/MAFbx and MuRF, which are the components of ubiquitin proteasome system. Upon phosphorylation mainly by Akt protein, these FOXO proteins migrate to cytosol, and are rendered inactive where they can no longer transcribe the atrophy inducing genes [15].
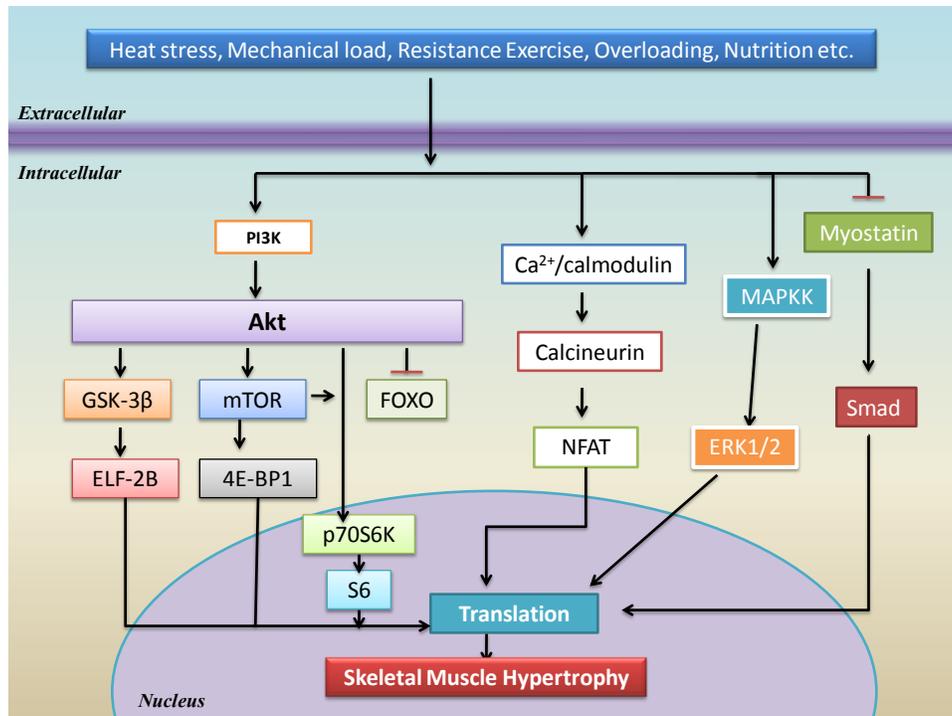
Fig 2: Signaling pathways regulating skeletal muscle hypertrophy

Kakigi et al., 2011 attempted to evaluate the role of mTOR signaling in heat stress related muscle hypertrophy. The study involved 8 human volunteers who performed two sessions of acute resistance exercise separated by three weeks rest period. During the first session, the subjects performed one leg knee extension exercise whereas during the second session, the same exercise was performed using contralateral leg with belly of the *Vastuslateralis* muscle subjected to heat stress with the help of microwave therapy unit. The *Vastuslateralis* muscle biopsies were taken from the non-exercising leg to serve as control samples and from the exercise leg 5 and 60 minutes post exercise. The muscle biopsies were analyzed for the expression levels of myosin heavy chain isoforms, eEF2, 4E-BP1, AMPK, p38, ERK ½, Akt, mTOR, S6K1 and S6. The Akt and mTOR phosphorylation were observed to increase significantly after the second session where exercise was combined with heat stress. There was no significant increase in the Akt and mTOR phosphorylation after first session, where exercise was not coupled with heat stress.4E-BP1

phosphorylation significantly decreased immediately after exercise performed in both the sessions but was found to increase 1-hour post exercise post second session. S6K1 phosphorylation was significantly increased at both time points (5 minutes and 1 hour post exercise) for session 2 compared to session 1. ERK1/2 and p38 phosphorylation also showed significant elevation 1-hour post exercise in session 2 compared to session 1. This provides very clear-cut evidence that heat stress increases the phosphorylation of the mTOR signaling molecules in human skeletal muscle. This in turn results in an increased muscle protein synthesis leading to muscle hypertrophy. However, the study suffered with certain major limitations with lack of a group representing the effect of heat stress alone, independent of exercise on mTOR signaling. Physiological changes such as blood flow were not measured which could have aided in clarification of the mechanism of activation of mTOR signaling molecules [16].

Yoshihara et al., 2013 investigated the role of hyperthermia in stimulating the Akt/mTOR

signaling pathway in skeletal muscles of rats. 42 male Wistar rats were employed for the study and they were divided into 6 groups with 7 rats in each. One group served as control, and the other groups served as thermal stress groups with temperatures 37, 38, 39, 40 and 41 °C. Rats in all the groups fasted over night after which both their legs were emerged in hot water with temperatures as specified for 30 minutes under the influence of anesthesia induced via sodium pentobarbital administration. This was followed by immediate removal of the soleus and plantaris muscle from each leg. Akt and P70S6K phosphorylation was found to be significantly increased at 41 °C in both soleus and plantaris muscle. The increase in Akt and P70S6K phosphorylation was found to increase in a temperature dependent fashion in both muscles indicating that temperature itself may be a fundamental stimulator of Akt/mTOR signaling [17].

HSPs and MHC isoforms: Effect on muscle hypertrophy

Touchberry et al., 2012 investigated the effect of HSPs induction upon heat shock pretreatment on the events of early remodeling and signaling (2 hours and 48 hours) in soleus muscle following a bout of downhill running. The male Wistar rats were divided into 3 groups. Group 1(control) did not receive any exercise or heat treatment, Group II(eccentric exercise group) conducted eccentric exercise and the third group (Heat shock group) was subjected to heat shock treatment 48 hours prior to the exercise. The soleus muscle was removed 2 and 48 hours post exercise and blood was drawn. Muscle damage, regeneration markers and intracellular signaling was evaluated. Muscle damage was analyzed using Creatine Kinase (CK) and mono-nucleated immune infiltration as markers. Muscle regeneration was analyzed by estimating total protein and MHCneo as markers. Akt/ p70S6K and MAPKs intracellular signaling was evaluated. The average CK level was observed to increase in eccentric exercise group 2 and 48 hours post exercise whereas for the heat

stress group CK level was not significantly elevated 2 Hours post exercise compared to control and was significantly less compared to eccentric exercise group and returned to baseline level 48 hours post exercise. Quantitative analysis of mono-nucleated immune infiltration revealed an increased number of immune cells infiltering the muscle in both eccentric exercise and heat stress groups but the number of immune cells in heat stress group was significantly less compared to eccentric exercise group. Protein content was found to be elevated in heat stress group 48 hours post exercise compared to control and eccentric exercise group. MHCneo content was found to be significantly elevated 2 hours and 48 hours post exercise compared to the control and eccentric exercise groups. No significant difference was observed in activation of Akt/p70S6K and MAPKs intracellular signaling 48 hours post exercise in heat stress group compared to control or eccentric exercise group suggesting that Akt/p70S6K may not be crucial for heat shock induced muscle regeneration. These observations are contradictory to the observations made by Kakeki et al., 2011. The possible mechanism for the increase in muscle protein concentration and MHCneo content has been postulated to occur by the recruitment of satellite cells [18].

Frier et al., 2007 conducted a study with an aim to determine the extent to which HSPs accumulation resulting from a single heat stress may influence the early events associated with skeletal muscle hypertrophy. To achieve this, 24 hours prior to overloading of 1 plantaris muscle by surgical removal of the gastrocnemius muscle, Sprague-Dawley rats were subjected to heat stress at 42 °C for 15 minutes. HSP 25, HSP 72, MHC 1, muscle mass, total muscle protein contents were measured for both control (contralateral plantaris muscle) and heat stressed and/or overloaded plantaris muscle over the course of 7 days after gastrocnemius muscle was removed. For animals subjected to overloading without heat stress,

muscle mass, total muscle protein, MHC I, HSP 25, HSP 72 were found to increase significantly upon overloading. In the heat stress and overloading group, the expression level of HSP72 was significantly higher than that of the overloading group without heat stress and muscle mass, total muscle protein and MHC I were comparatively diminished suggesting the inhibitory role of heat induced HSP expression to these parameters, which is contrary to many published reports included in this review [19].

CONCLUSION

A vast body of literature establishes heat stress as a potent inducer of muscle hypertrophy. It is also known that satellite cells' proliferation and subsequent differentiation into mature muscle cells coupled with protein synthesis results in muscle hypertrophy. Various signaling pathways such as Akt/mTOR have been found to play critical roles in inducing muscle hypertrophy but the mechanisms of activation of these pathways are still incompletely understood. The understanding of the molecular phenomenon underlying the activation of various signal transduction pathways in response to heat stress is crucial for design of measures to combat and treat the conditions of muscle atrophy.

Future perspectives

Although a significant body of literature exists on signaling pathways that regulate muscle hypertrophy in response to heat stress, the precise understanding of the mechanisms of activation of these pathways is still lacking. Therefore, further research initiatives are required to understand the exact mechanism by which heat stress activates key signal transduction pathways associated with muscle hypertrophy. The precise understanding of the regulation of muscle hypertrophy via heat stress at the level of signal transduction involved will facilitate the design of modalities to combat conditions of muscle atrophy. Application of heat stress to induce hypertrophy will serve as a very economical, practical and easy to use alternative

compared to the existing mechanical stress application to alleviate muscle atrophy observed under the conditions of injury, illness, unloading and immobilization.

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Abbreviations

Myosin Heavy Chain (MHC)
Fluctuating Local Somato Thermal Stimulation (LSTS)
Reactive Oxygen Species (ROS)
Nitric Oxide (NO)
Heat Shock Proteins (HSPs)
Creatine Kinase (CK)