Hyperthermia Induces Functional and Molecular Modifications in Cardiac, Smooth and Skeletal Muscle Cells

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Schools of Nursing & Medicine

Abstract

The practice of using hyperthermia for treating pain, muscle spasms and a myriad of diseases is common to humankind for hundreds of years. More recently, heat therapies such as hyperthermia and thermoablation have shown to be very promising approaches in the treatment of cancer and muscle injuries. Heat is generally thought to stimulate the expression of a group of proteins generally termed Heat-Shock Proteins (HSPs). Importantly, HSPs are also involved in growth and development of muscles; however the exact role they play in muscle hypertrophy and muscle repair has yet to be elucidated. No studies to date have ever tested the effects of hyperthermia on cells models representative of cardiac, smooth and skeletal muscle cells. A critical step towards translation of the knowledge in the basic research laboratory into the clinical practice is the understanding of the biological phenomena at the cellular and molecular levels. Preliminary findings on the effects of mild heat shock (HS) treatment in HL-1 cardiac, AR-75 smooth and C2C12 skeletal muscle cells demonstrated that HS induced a substantial increase in all muscles' cell area (ranging from 10-89%) when compared to controls. C2C12 cells was selected for systematic and detailed studies. First, to biochemically confirm an increase in protein synthesis. It was found an increase of ~8% in total protein content 24h after HS. Second, potential modifications in calcium (Ca) homeostasis regulation was examined by measuring intracellular Ca^{+2}. It was detected a relatively smaller caffeine-induced Ca transients and enhancement of calcium-induced calcium release (CICR) response in C2C12 muscle cells 24h after HS. Next, to search for molecular mechanisms involved with HS-induced hypertrophy and calcium homeostasis modifications, mRNA from C2C12 muscle cells was analyzed at different time points after HS (0, 1, 2, and 24h). It was used an ABI Step One Plus RT² PCR Array System and a custom-built 96 gene array, reporting for the first time that the expression of key heat-shock, hypertrophy/ metabolic, and Ca^{+2} signaling genes were altered after HS. Hsp70 and Hsp72 genes were highly expressed (211-1829 fold change) after HS. Also, Myh7 (MHC-I), Myh6, Srf, Ppp3r1 and Pck1 were up-regulated by 2-6 fold change compared with control cells. Besides, a reduction in the expression of RyR and Trdn genes was observed (2- 3.6 fold change) with an associated increase in the expression of IP3R genes (2-4 fold change). These results indicate that hyperthermia modulates not only heat-shock related and hypertrophy genes, but also genes involved with metabolism, apoptosis repression, calcium homeostasis, and cell homeostasis. Our studies offer an initial exploration of the functional, biochemical and molecular mechanisms that may help explain the beneficially adaptive effects of hyperthermia on muscle function.

Methods

Cell Area Measurements: C2C12 cells were imaged using a Leica DMI 4000 microscope and Lieca Application Suite: Advanced Fluorescence software. Areas of individual cells were determined using the software package to define individual cells as regions of interest, and then areas were calculated in micrometers squared by the software.

Total protein assay: C2C12 cells were collected in cell extraction buffer (Invitrogen) containing a protease inhibitor cocktail (Sigma). Protein was extracted in this buffer solution, and appropriate dilutions of protein samples were prepared in nanopure H_2O. Total protein was measured using a Pierce BCA protein assay kit and finally quantified by optical density at 562 nm using a Bio-TEK KC4 micro-plate reader.

Intracellular Ca measurements: A Photon Technology International (PTI) imaging system was used to measure intracellular Ca homeostasis. Each cell imaged was loaded with Fura-2, and ratiometric analysis was performed using PTI's Felix 32 Photometry system. Both resting calcium and the response to caffeine treatment were measured in these studies.

PCR Array: mRNA was extracted from C2C12 muscle cells at 20min,1h,2h and 24h after heat shock treatment. RT-qPCR was performed using the RT Qiagen Kit. qPCR was run with a custom-built RT 96 well (genes) microplate array (SABiosciences) in a Step One Plus instrument. Data was analyzed using RT² Profiler™ PCR Array Data Analysis (SABiosciences); CT values were normalized to gapdh as a reference gene. Gene expression was determined as up/down regulation of the gene of interest compared to the control.

HS induces cell area growth in cardiac, smooth and skeletal muscle cells

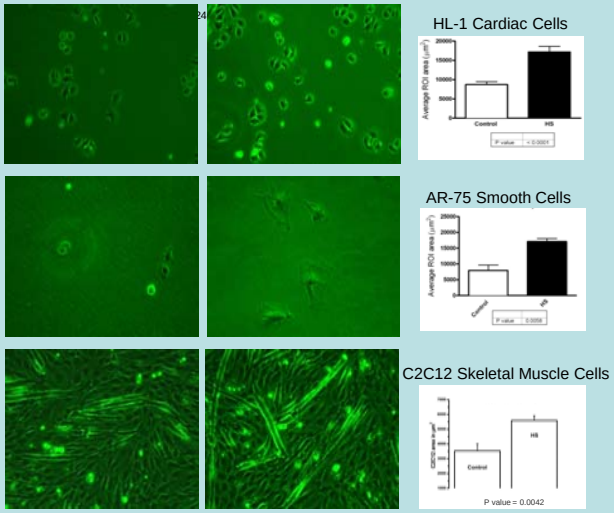


Fig. 1. HS effectively induces hypertrophy as measured by cell area in HL-1 cardiac, AR-75 smooth and C2C12 muscle cells. Images of muscle cells at 24h post HS illustrate that cells respond to HS by increasing their relative cell areas. Images are representative of hundreds of cells

HS increases protein synthesis and modifications in intracellular calcium

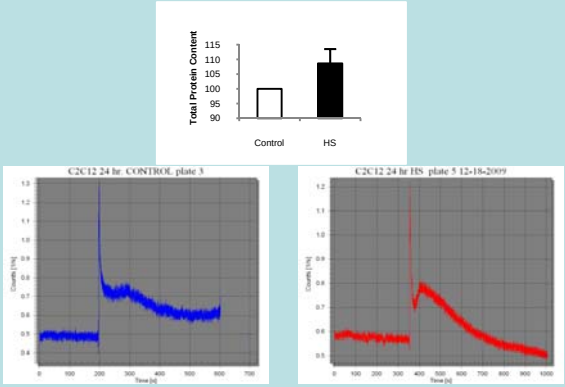


Fig. 2. HS Alters Protein Synthesis and Ca Homeostasis. Top panel shows that HS induced a 8% increase in total protein content. Two bottom panels: Cells were loaded with Fura-2 and challenged with 10 mM caffeine. While the peak response is smaller in HS treated cells, calcium re-uptake and extrusion mechanisms are improved as demonstrated by a faster and more efficient clearance of calcium and establishment of a lower level of calcium after exposure to caffeine. In HS treated cells, a noticeable secondary peak after caffeine exposure is present, suggesting enhancement of CICR.

Molecular Pathways Activated by HS

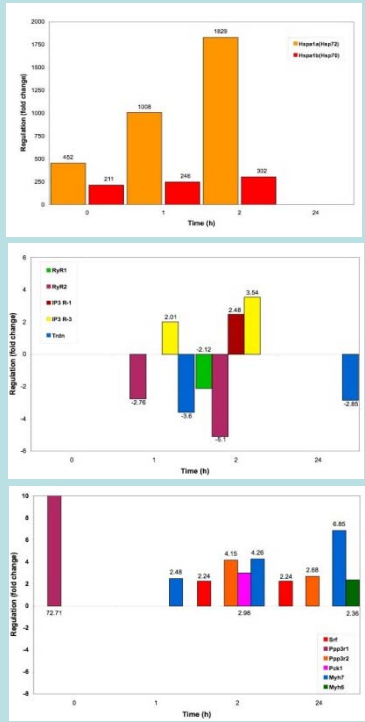
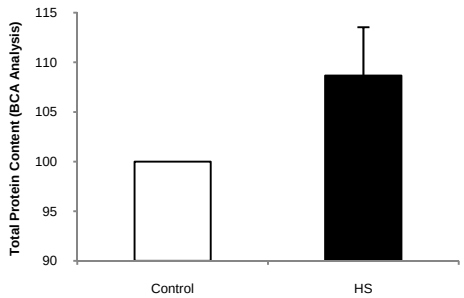
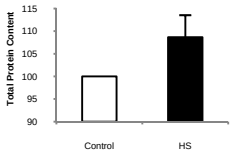


Fig. 3. Novel genes regulated by hyperthermia. (*Top Panel*) shows drastic increase in HS genes, (middle panel) shows three group of key genes related to intracellular calcium homeostasis, and (bottom panel) shows expression changes in metabolic, hypertrophy-related and apoptosis repression genes.

Conclusions

For the first time, our group has demonstrated that hyperthermia treatment is able to produce hypertrophy in all muscle cell types: cardiac, smooth and skeletal muscle cells. Using C2C12 muscle cells as a model, we found that protein content increases by 8% after HS. In addition, intracellular calcium homeostasis was modified. It was also discovered key genes modulated by HS. The reduction in RyR expression along with the increase in IP3R expression provides a solid explanation for the changes in calcium homeostasis. It is also evident that molecular adaptations to HS go beyond Heat-Shock related genes, since metabolic and cell homeostasis genes were also altered by HS. Our studies offer new insights into the mechanisms/pathways that might help explain the beneficial effects of HS on animal and human health. By exploring these mechanisms and better learning on how to use HS to control these mechanisms, new therapeutic interventions for treatment of muscle disorders and other diseases may develop.


Total Protein Content (BCA Analysis)
115
110
105
100
95
90
Control
HS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