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

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Hyperthermia is used for the treatment of a number of diseases, including muscle injuries, inflammations, tendinitis, and osteoarticular disorder. More recently, hyperthermia has been used as an adjuvant in cancer treatment. Only two studies have shown that hyperthermia leads to hypertrophy in *in-vitro* models of cardiac and skeletal muscle cells. Functional, biochemical and molecular mechanisms of hyperthermia-induced hypertrophy in muscles remain largely undiscovered. We investigated the effects of mild heat shock (HS) on C2C12 skeletal, HL-1 cardiac and AR-75 smooth muscle cells. Mild HS (20 min 43ºC) induced increases in the cell area in all muscle cells tested. C2C12 cells are a well-accepted model of skeletal muscle fibers, and were selected for complementary studies. First, to biochemically confirm an increase in protein synthesis we measured and found an increase of ~6% in total protein content 24 hrs after HS. Second, we examined potential modifications in calcium (Ca) homeostasis regulation by measuring intracellular Ca. We detected a lower resting level of intracellular Ca and smaller and longer caffeine-induced Ca transients in C2C12 muscle cells 24 hrs 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 24 hrs). We used an ABI Step One Plus RT^2 PCR Array System and a custom-built 96 gene array. We report 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.. Furthermore, 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 signaling, 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. Our studies shall also prove useful for the refinement of a specific device (EM-Stim) to be employed for the treatment of muscle and bone diseases (See poster by Hatem et al). Importantly, our studies have potential translational applications. By learning how to more precisely use hyperthermia to control specific genes that can improve or treat muscle injuries, musculoskeletal, and cardiovascular diseases, the ensuing benefits shall be unmistakable. Our short and long-term goals are: i) optimize our protocols; ii) test HS in animal models; iii) manipulate expression of promising genes of interest in vitro and in in-vivo animal models; iv) initiate clinical studies to fully translate from the bench to the bed-side. (***Support***: Missouri Life Sciences Research Board and The Center in Excellence for Mineralized Tissue Research-CEMT)