REPEATED HEAT STRESS SENSITIZES (not protects) C2C12 MYOTUBES AGAINST SUBSEQUENT LPS EXPOSURE

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PURPOSE: This study investigated the hypothesis that “preconditioning” hyperthermia affords cytoprotection against subsequent LPS stimulation in C2C12 myotubes.

METHODS: C2C12 myotubes were incubated for 2hr at 43ºC for 6d (HEAT) or maintained at 37ºC (CONTROL). Following 24 hours, myotubes were stimulated with LPS (500ng/ml) for 2hr, following which protein markers of the heat shock response (HSR), Myotube activation and lipid/glycogen storage capacity were examined via Western Blot. RESULTS: As expected, the HSR was strongly activated by HEAT (HSP23 (+38% p<0.01), HSP60 (+32% p<0.01), HSP70 (+68% p<0.01)). Unexpectedly, HEAT exhibited a heightened inflammatory response (p-IKKa/b (+81% p<0.01), p-NFκB (+283% p<0.01)). Intermediate enzymes of lipid (p-ACC (+33% p<0.01)) and glycogen (p-GSKa/b (+36% p<0.01)) biosynthesis were also down-regulated, with elevated p-AMPK (+80% p<0.01) suggesting an energetic deficit. Apoptosis activators Caspase 8 (+53% p<0.04) and FOXO1 (+74% p<0.02) were up-regulated, as was p-JNK (+41% p<0.03). Through follow-up analysis we determined these undesirable responses were linked to up-regulation of TLR4 (+24% p<0.03) and MyD88 (+30% p<0.01), as well as p-NIK (+199% p<0.02) but not IRAK-1 (p<0.46).

CONCLUSION: Despite a robust activation of the HSR, repeated thermal stress imparts an exaggerated pro-inflammatory and pro-apoptotic response to LPS stimulation in C2C12 myotubes. This may be due to elevated TLR4 signaling capacity. We speculate that reduced glycogen storage in HEAT may have contributed (p=0.46).

INTRODUCTION

• The pathogenesis of exertional heatstroke (EHS) is primarily driven by the translocation of lipopolysaccharide (LPS) from the gastrointestinal tract into circulation under exercise/heat stress conditions.

• Subsequent to interaction with its pattern recognition receptor (toll like receptor 4: TLR4), LPS initiates a NFκB-mediated pro-inflammatory cascade that contributes to further core temperature elevation, disseminated intravascular coagulation, and multiple organ failure (2). LPS also compromises the metabolic capacity of skeletal muscle; shifting substrate metabolism away from lipid and towards glucose utilization and affecting the overall mitochondrial efficiency in general (3).

• In contrast to the deleterious consequences listed above, repeated exposure to low-grade thermal stress (40ºC) for short durations (3hr) has been shown to promote favorable effects in skeletal muscle. In vivo (C2C12 myotube) experiments suggest that such exposure stimulates a conversion from fast to slow twitch fiber type metabolism (4), in concert with an increased capacity for oxidative metabolism (5) as well as improvements in both antioxidant and anti-inflammatory responses via induction of the cypotective heat shock response (HSR) (6).

• Through the combination of these effects, it appears that prior low grade thermal stress may improve skeletal muscle oxidative metabolism and macronutrient storage capacities in concert with a reduction in inflammation. While this would be expected to protect muscle against subsequent LPS exposure (i.e. thermal preconditioning); to our knowledge this hypothesis has not been experimentally tested.

METHODS

C2C12 myotubes were incubated for 2hr at 43ºC for 6d (HEAT) or maintained at 37ºC (CONTROL). Following 24 hours, myotubes were stimulated with LPS (500ng/mL) for 2hr, then collected. From these samples the protein content of mediators of the HSR, markers along the pro-inflammatory NFκB cascade, and multiple enzymes that are known to control glycogen and lipid biosynthesis were examined via Western Blot.

RESULTS

• Repeated thermal exposure imparts an exaggerated pro-inflammatory and pro-apoptotic response to subsequent large dose LPS stimulation in C2C12 myotubes.

• Elevated HSP content alone does not afford adequate protection against the increased cellular responsivity to LPS that is imparted by elevations in the TLR4-mediated signaling cascade.

• Myotube capacity to tolerate cellular stress was likely hampered by an energetic deficit, which may have been imparted by reductions in glycogen storage capacity.

• Elevated p-NIK contributed to an upregulation of Caspase 8-mediated apoptosis, reducing myotube competition for macronutrient resources.

• These experiments indicate that repeated thermal challenge of C2C12 myotubes does not confer metabolic or cytoprotective benefits against subsequent LPS stimulation. This may have broader implications in the skeletal muscle of humans during exertional heat stress.

REFERENCES


