



Heat Shock Protein 70 is Upregulated in Blood Leukocytes From Experienced Divers in Response to Repetitive Hyperbaric Stress

by

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2007 UHMS Annual Scientific Meeting

16 June 2007



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Introduction

Heat Shock Proteins (HSPs)



- Intracellular **HSPs** or *stress proteins* are a highly conserved group of proteins present in all cells in all life forms.
- HSPs evolved to cope with the potential for proteins to become unstable or denatured when stressed and thus act as a defense against protein damage.
- **HSP70** is the major inducible stress protein upregulated in blood leukocytes following exposure to a variety of physiological and environmental insults — allowing the cell to survive lethal conditions.
- Human studies show that acute hyperbaric stress can enhance HSP70 expression and animal data suggest that prior induction of HSP70 may be protective against decompression sickness (DCS).
- The effect of repeated hyperbaric stress on HSP70 expression has not been investigated in humans.
- ***This study examined the impact of 3 consecutive days of hyperbaric stress on HSP70 expression in leukocyte subsets.***



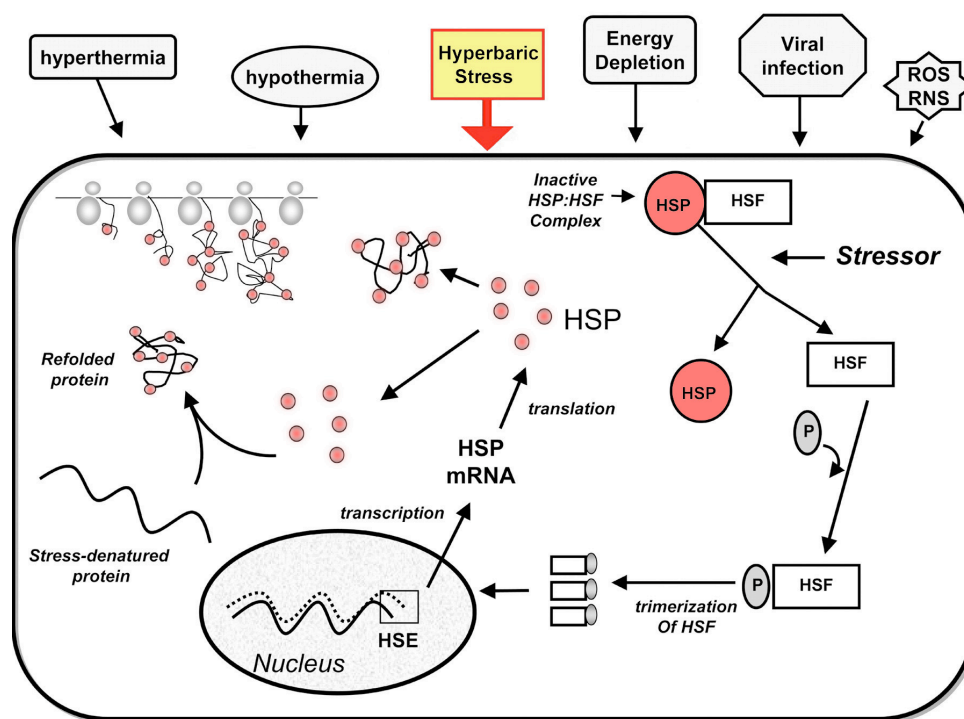
Introduction

Induction of HSP70



HSP70 expression is upregulated in response to various stressors:

- thermal stress
- pressure change
- energy depletion
- ↓ infection
- oxidative stress



Kregel, KC. *J Appl Physiol*, 92: 2177, 2002

➤ ***HSPs are cytoprotective against environmental insults***



Introduction

Cytoprotective Roles of HSP70

Stress-related functions

- stabilization of protein folding
- disaggregation
- refolding
- degradation

House-keeping functions

- de novo folding
- translocation
- assembly and disassembly
- regulation of activity
- regulated degradation

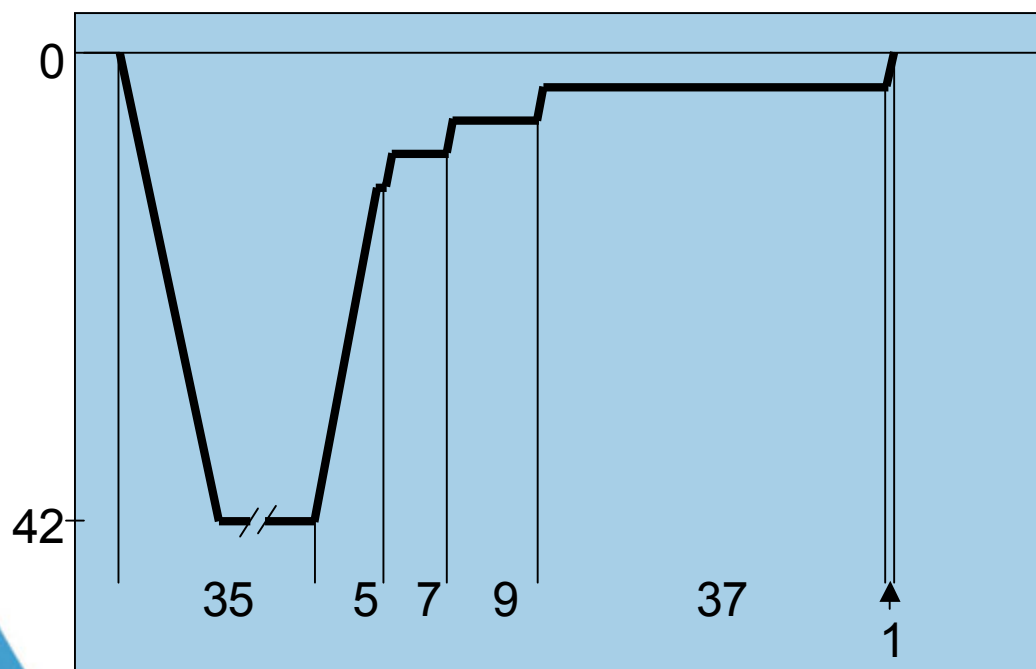
Inhibition of cellular apoptosis



Experimental Design



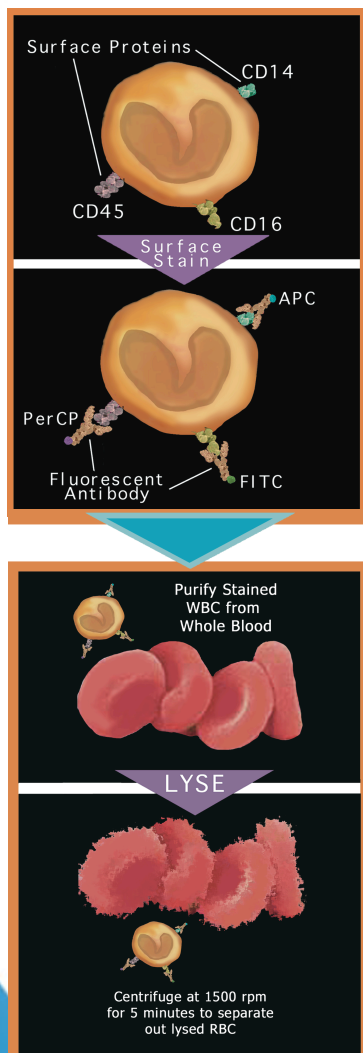
- 8 experienced male divers (aged 21–55 y) underwent 3 dry simulated dives to 42 msw for 35 min in a hyperbaric chamber over a 3-d period
- Venous blood samples (3mL) drawn at rest (24-h *pre-dive*) and immediately after decompression (*post-dive*)





Materials & Methods

Immunofluorescence Staining

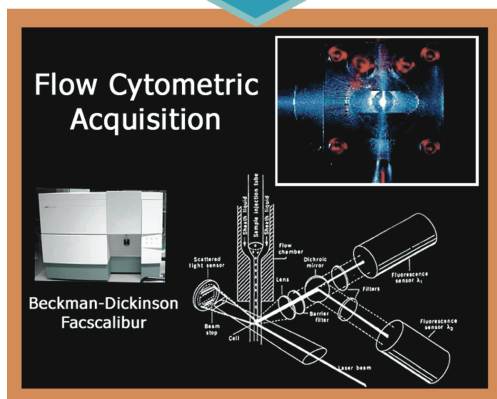
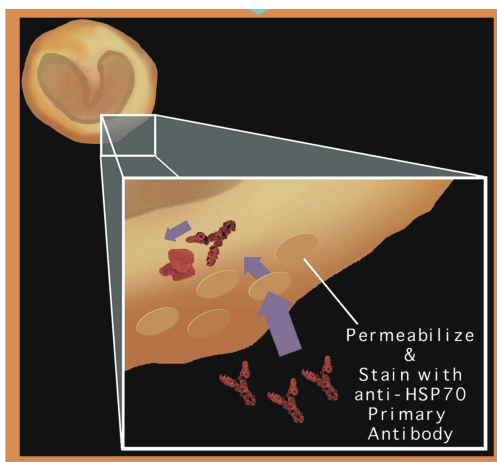


- Whole blood samples for spontaneous and *in vitro* heat shock-induced HSP70 expression were cultured at 37.5°C for 21 h or in a 42°C water bath for 1 h followed by incubation at 37.5°C for 20 h, respectively.
- 100 μ L aliquots were surface stained with monoclonal antibodies against CD14 and CD45 to identify leukocyte subsets:
 - lymphocytes
 - monocytes
 - granulocytes
- Erythrocyte lysis, wash, centrifuge

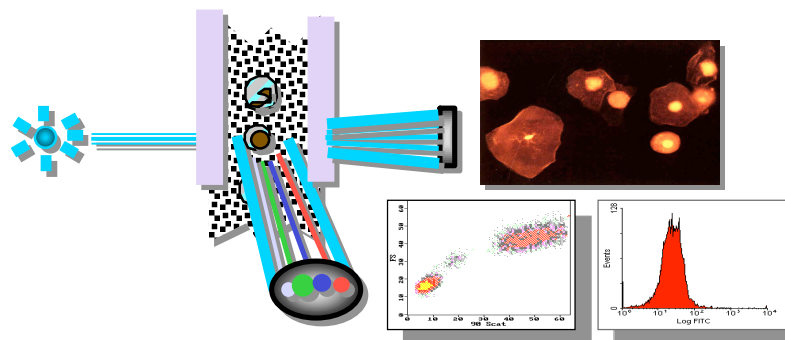


Materials & Methods

Flow Cytometric Analysis



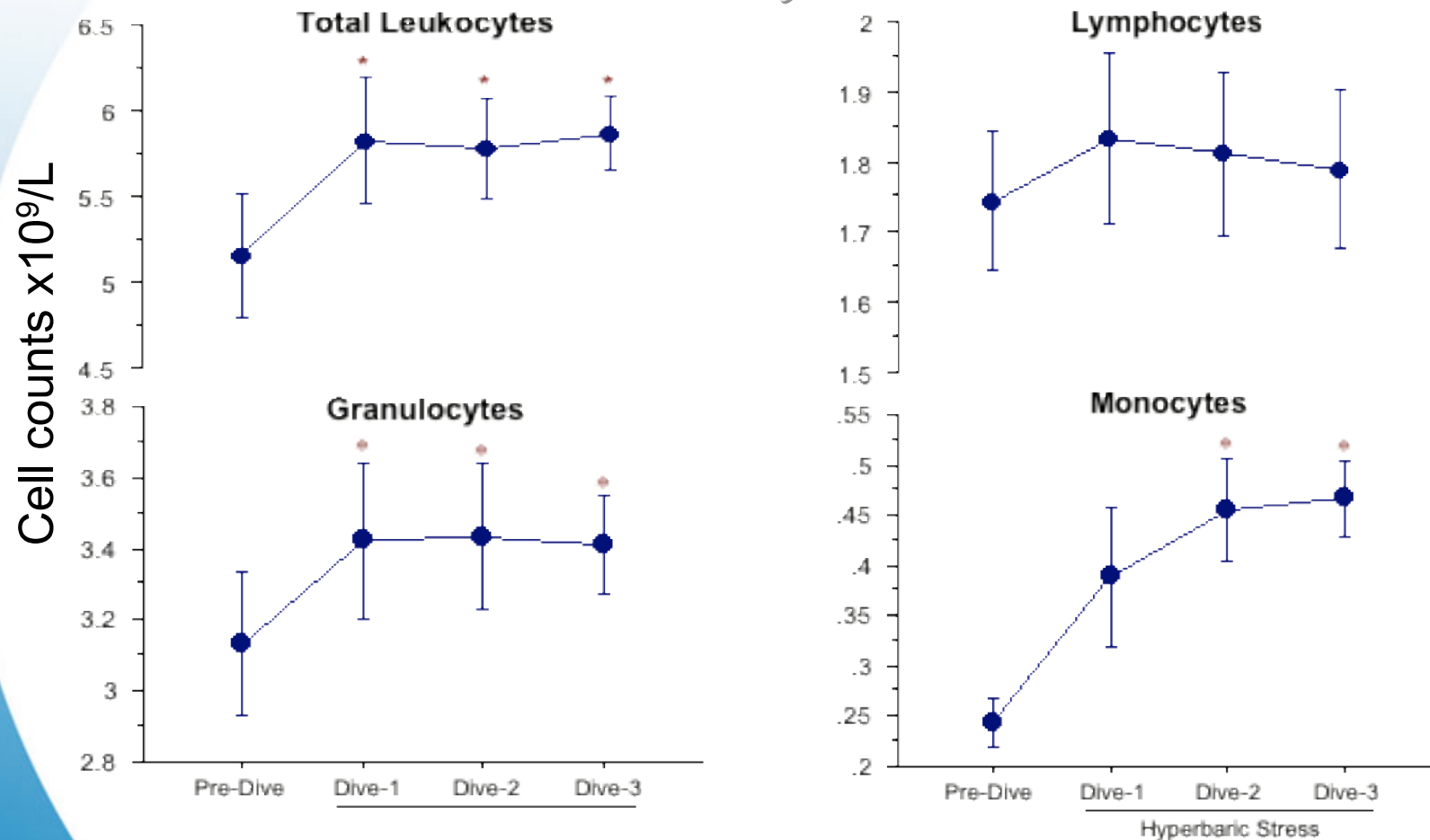
- Fixation and permeabilization of cell membranes for intracellular staining with HSP70 fluorescent antibody
- Multiparameter flow cytometric acquisition and analysis
 - quantify spontaneous and heat-shock induced HSP70 expression
 - data expressed as frequency (%) and median fluorescence intensity (MFI) of HSP70-expressing subset





Results

Leukocyte Counts

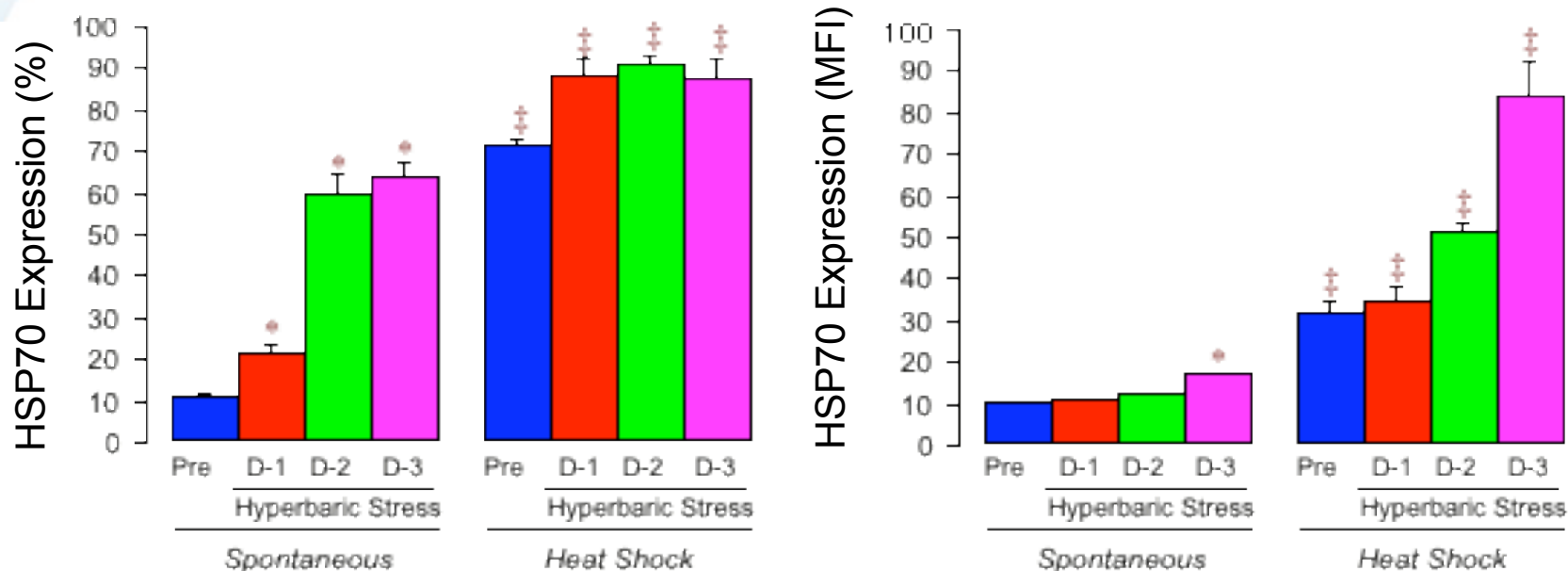


➤ ***Hyperbaric stress elicited a significant leukocytosis, with marked increases in monocytes and neutrophils, but no change in lymphocyte counts***



Results

Monocytic HSP70

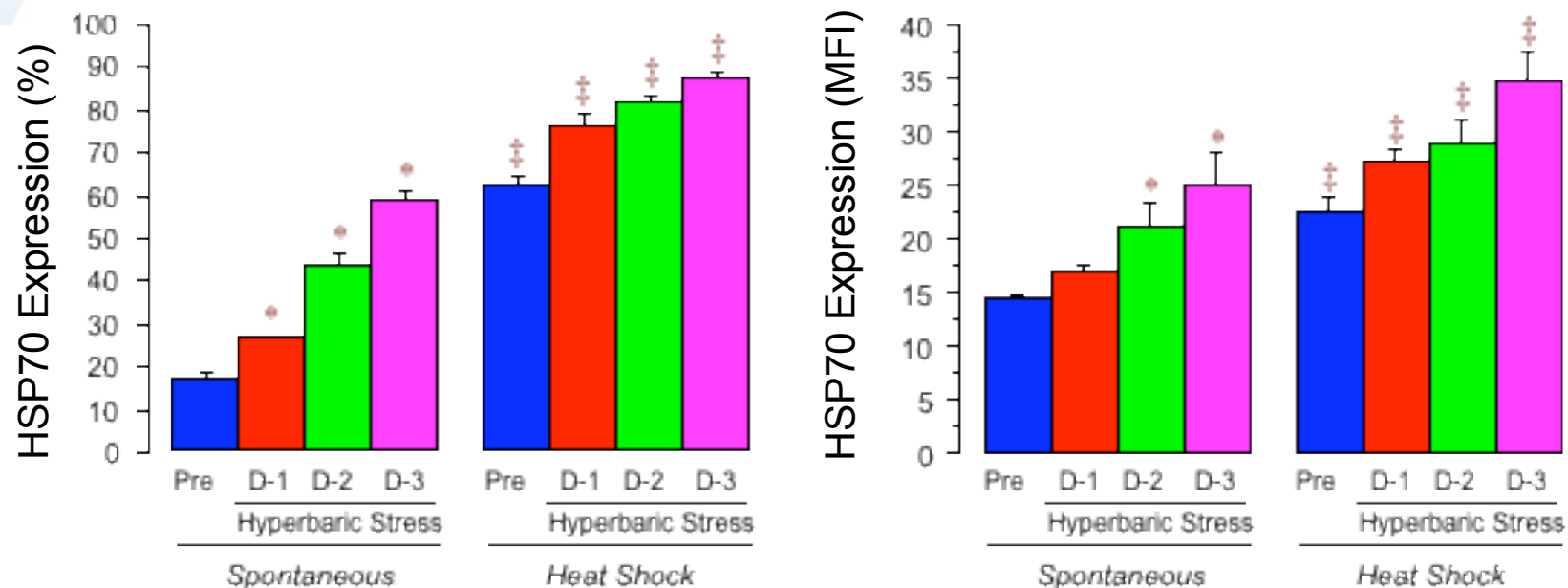


- ***Relative to Pre-Dive levels, spontaneous and heat shock-induced monocytic HSP70 expression (% & MFI) was incrementally upregulated over 3 consecutive days of hyperbaric stress.***



Results

Neutrophilic HSP70



- ***Spontaneous and heat shock-induced neutrophil HSP70 expression (% & MFI) was incrementally upregulated with hyperbaric stress.***



Conclusions



- This study demonstrates that both spontaneous and heat-shock induced intracellular *HSP70 expression is progressively upregulated in circulating mononuclear phagocytes*, but not lymphocytes, of humans exposed to repetitive episodes of hyperbaric stress (compression–decompression).
- These results suggest that HSP70 induction is a cellular adaptation to recurrent diving, which *may contribute to diving acclimatization and a reduced susceptibility to DCS* in individuals undergoing chronic hyperbaric stress.



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Questions?
Comments