

**GLUTATHIONE STATUS: A TRIGGER IN THE HEAT SHOCK  
PROTEIN RESPONSE IN FISH?**

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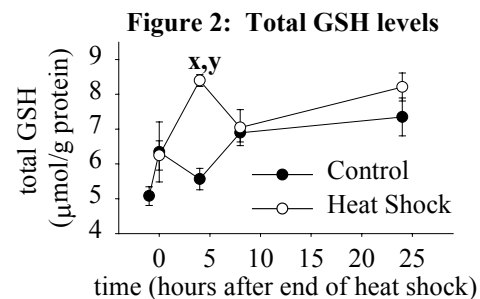
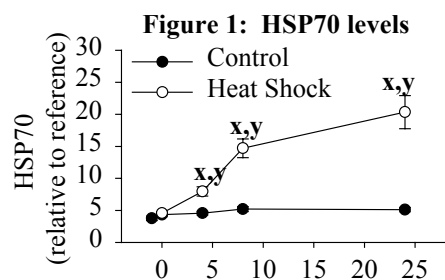
**EXTENDED ABSTRACT ONLY- DO NOT CITE**

Heat shock protein 70 (HSP70) is a ubiquitous intracellular molecule involved in the proper folding of proteins. HSP70 is upregulated in response to a wide variety of stressors to assist in the refolding of proteins damaged during stress. There has been much speculation on what may be the trigger for HSP70 upregulation during stress. One hypothesis is that decreased levels of cellular glutathione (GSH) may be involved in HSP70 regulation. GSH is a ubiquitous molecule responsible for the reduction of various oxidants, particularly lipid peroxides, through oxidation of GSH to oxidized glutathione (GSSG). Both GSH levels and GSH/GSSG ratios decrease during various stressors. Studies in mammalian systems have shown that a heat shock, a classical inducer of HSP70 upregulation, can also decrease GSH and GSH/GSSG, and increase lipid peroxidation (Lord-Fontaine and Averill-Bates, 2002; Ando et al., 1994; Ohtsuka et al., 1994). Although there has been an increase in knowledge about the role of HSP70 in fish, little is known about possible correlations between GSH and HSP70 in these organisms.

To determine if there is a correlation between GSH status and the HSP70 response in fish, we examined the HSP70 and GSH levels in cultured rainbow trout (*Oncorhynchus mykiss*) hepatocytes after a 30°C, one hour heat shock. Hepatocytes were isolated from a 300g rainbow trout and cultured in 6-well tissue culture plates at  $1.5 \times 10^6$  cells per well in 2mL L-15 media. The

hepatocytes were incubated at 15°C for 72 hours before experimentation, with a media change at 48 hours. Hepatocytes were sampled and then divided into two groups, a heat shock group and a control group. The heat shock group was placed in a 30°C incubator for 1 hour and then returned to 15°C, while the control group was moved concurrently with the heat shock group, but otherwise remained at 15°C. Hepatocytes were sampled at 0, 4, 8 and 24 hours post stress, and six wells were sampled per treatment and time period. Data were analyzed by two-way ANOVA followed by SNK test. All values are given in mean±SEM.

While HSP70 in the control group remained constant, HSP70 in the heat shock group increased significantly with time ( $p < 0.001$ , Fig. 1). HSP70 levels were higher in the heat shock than the control group at times 4, 8 and 24 hours post stressor ( $p < 0.001$ ). Total GSH in the control group did not change significantly with time ( $p > 0.05$ ). However, in the heat shock group total GSH showed a significant increase at 4 hours, not only in time ( $p = 0.021$ ) but also between groups ( $p < 0.001$ , Fig.2). There were no significant differences between control and heat shock groups in GSSG or GSH/GSSG levels at any time.



x indicates a significant difference from time 0 within treatment and y indicates a significant difference between heat shock and control groups within time

Unlike previous mammalian studies, rainbow trout hepatocytes showed a transient increase, rather than a decrease, in GSH levels after exposure to a heat shock at the times measured. This indicates that, in rainbow trout, a decrease in GSH or GSH/GSSG may not be involved in HSP70 upregulation after a heat shock. In a previous study, Harris et al. (1991) found that GSH levels increased one hour after a 30 minute heat shock in cultured rat embryos. However, Kurganova et al. (1999) found that pea plants exposed to a one hour heat shock

had decreased GSH content and increased lipid peroxidation during the first 15 minutes, but 30-60 minutes into the heat shock had increased GSH and GSH/GSSG, as well as decreased lipid peroxide intermediates. The results of these studies as well as our own suggest that a 30-60 minute heat shock may upregulate GSH, and possibly confer a transient protection against lipid peroxidation. However, a transient decrease in GSH may also occur during the first 30 minutes of a heat shock. As GSH levels during the heat shock were not measured in this experiment, we cannot rule out the possibility that a transient decrease in GSH levels is involved in HSP70 regulation during a heat shock in cultured rainbow trout hepatocytes.

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