Heat shock protein 70 immunoexpression in the brown adipose tissue of heat- exposed rats

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The accumulation of heat shock proteins (HSPs) after the exposure of cells or organisms to elevated temperatures it is well established. In rat brown adipose tissue (BAT) cold-induced expression of HSPs has been suggested to facilitate thermogenesis, although the regulation of this response and the mechanism supporting this facilitation has not been established [1]. Norepinephrine (NA), released in response to cold exposure, induces HSP expression in BAT. NA appears to initiate transcription of HSP genes after binding to BAT adrenergic receptors through, as yet, undetermined signal transduction pathways. Thermogenesis results from an increase in activity and synthesis of several metabolic enzymes in BAT of animals exposed to cold challenge and concomitant increase in HSPs may function to facilitate the translocation and activity of the enzymes involved in this process. To our knowledge, there are no studies investigated the effect of heat-exposure on HSP expression in BAT. Herein, we demonstrate that acute exposure of rats to heat (38 °C) results in decreased expression and delocalization of the HSP70 in brown adipose tissue. Also, we revealed the appearance of extra-cellular HSP70 (eHSP70).

Two-month old, male rats of the Wistar strain were used in the experiment. The animals were divided into two groups. Experimental group was exposed to 38 °C for one hour; animals that lived under thermoneutral conditions (22 ± 1 °C) were used as a control group. After decapitation by a guillotine (Harvard Apparatus), the interscapular BAT was quickly removed and routinely fixated and embeded into paraffin blocks. Immunohistochemistry was carried out on $5\mu m$ paraffin sections with avidin-biotin-peroxidase method by using the HSP70 primary antibodies. Negative controls were prepared by omitting the primary antibody. Detection kit used was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Counterstaining was done with hematoxylin. Slides were examined under Leica DMLB (Austria) microscope.

In control rats (Figure 1), immunopositivity is located mainly in endothelial cells but some of brown preadipocytes and adipocyte also contain HSP70. Heat-exposure (Figure 2) induces expression and delocalization of the HSP70 in brown adipose tissue. The extracellular matrix and submembrane area show strong immunopositivity for HSP70, as well as blood vessels.

Our results demonstrate the HSP presence in BAT of control rat in thermoneutral environment suggesting their essential role in tissue maintaining. Recent reports indicate that HSPs are induced in mammalian tissues as part of a homeostatic response to environmental stressors. Acute exposure to heat induces a cascade of physiological changes collectively termed the heat-stress response. Upon heat-exposure HSP expression slightly decreased and

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relocalized to submembrane area. Also, a grate amount of HSP70 is present extracellulary. Although a great deal is known about the function of intra-cellular HSP70 during exposure to acute stressors, little is understood about the potential function of endogenous extra-cellular HSP70 (eHSP70). Endogenous (eHSP70) release may be one unrecognized feature of the acute stress response and may function as an endogenous 'danger signal'. Specifically, acute exposure to heat may lead to release of endogenous eHsp72 into the blood although their exact function remains unclear.

1. W.J.Welch, Physiol. Rev. **72** (1992) p1063.

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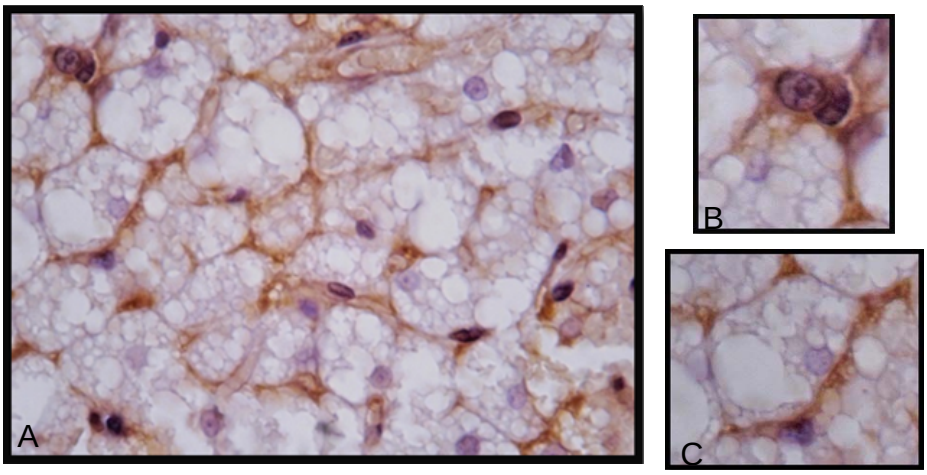

Figure 1. Immunohistochemical staining for HSP70 in brown adipose tissue of control (A-C) rats. Althought immunopositivity is located mainly in endothelial cells but some of brown preadipocytes and adipocyte also contain HSP70. Mag. x100, orig.

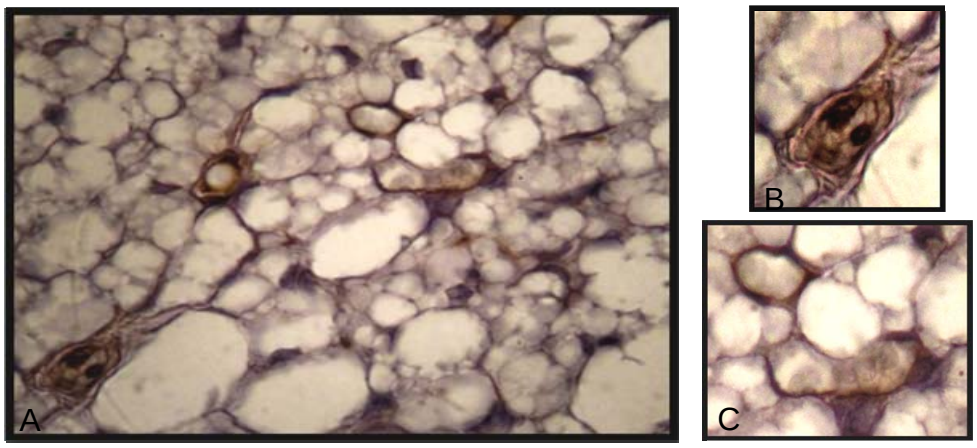

Figure 2. Immunohistochemical staining for HSP70 in brown adipose tissue of heat-exposed (A-C) rats. The extracellular matrix and submembrane area (C) show strong immunopositivity for HSP70, as well as blood vessels (B). Mag. x100, orig.