

Heat shock induced changes of adipokines gene expression in 3T3-L1 adipocytes

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ABSTRACT: To study the effects of heat shock on adipokines gene expression 3T3-L1 adipocytes were used. Heat shock differently affected gene expression of leptin, adiponectin and acylation stimulating protein (ASP): exposure of cells to temperature higher than 39°C caused upregulation of leptin and downregulation of adiponectin and ASP genes. The present study provides the first evidence about the effects of heat shock on adipokines gene expression. Changes in gene expression of the three adipokines may help to explain the alteration of lipid metabolism and liver functionality occurring in animals exposed to hot conditions.

Key words: 3T3-L1 adipocytes, Heat shock, Adipokines

INTRODUCTION - Adipose is an active secretory tissue. It sends out and responds to signals that modulate appetite, energy expenditure, insulin sensitivity, endocrine and reproductive systems, bone metabolism, inflammation and immunity. Adipocytes communicate with other systems including the central nervous system, by secreting hormones, so-called adipokines, in either an endocrine or a paracrine manner. Recent studies suggest that in addition to leptin, two other adipocyte hormones, adiponectin and acylation stimulating protein (ASP), are implicated in the regulation of energy balance and carbohydrate/lipid metabolism (Trayhurn, 2005). Leptin, a 16 kDa cytokine-like protein, is an essential signal from adipocytes to the hypothalamus in the control of food intake, energy balance, reproduction and immunity (Kershaw and Flier, 2004). Leptin affects energy homeostasis by decreasing food intake, upregulating fatty acid oxidation, and downregulating lipogenesis in peripheral tissues and in white adipose tissue (WAT) acting throughout a paracrine mechanism (Trayhurn, 2005; Lafontan and Viguerie, 2006). Adiponectin is a 30kDa protein secreted exclusively by adipocytes. A number of roles have been attributed to adiponectin, and these include modulation of insulin sensitivity, anti-atherogenic and an anti-inflammatory action, activation of glucose utilization by muscle, induction of muscle and hepatic fatty acid oxidation, and decrease of hepatic glucose synthesis (Lafontan and Viguerie, 2006). ASP is a 8.9kDa protein produced from complement factor C via an interaction requiring factor B and adipsin (factor D). ASP acts in WAT where it stimulates glucose uptake, triglyceride synthesis and storage (Havel, 2004). Dysregulation of gene expression of these adipokines in humans is crucial to the pathogenesis of multi-system disease related to metabolic disorders.

Several studies have shown in cattle that heat stress can lead to modification of important physiological and metabolic functions. In particular it was reported that high temperatures are responsible for modifications of lipid metabolism and liver functionality (Ronchi *et al.*, 1999).

To the best of our knowledge, there is no information available concerning the possible relationship between heat stress and adipokines gene expression. Therefore, the objective of the present study was to investigate the possible effects of heat shock on gene expression of leptin, adiponectin and ASP adipokines in 3T3-L1 adipocytes.

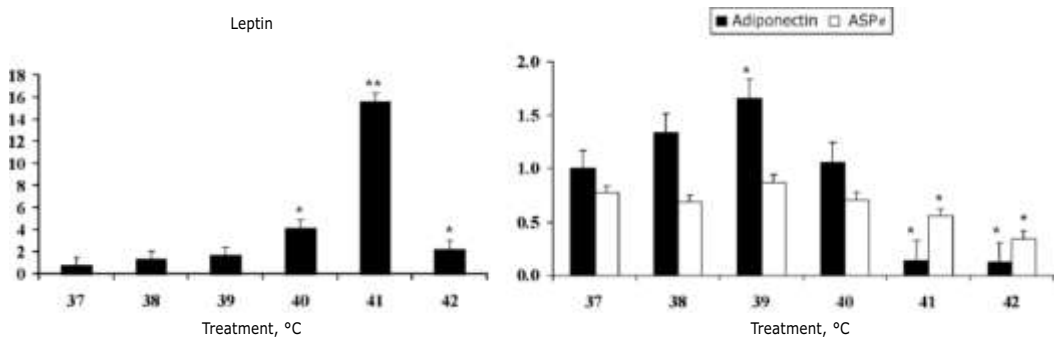
MATERIAL AND METHODS - Mouse 3T3-L1 fibroblasts were obtained from European collection of cell culture (Sigma-Aldrich, MI, Italy). Cells were grown at 37°C in 5% CO₂ in DMEM containing 25mM Hepes, 8mg/L D-biotin, 100 U/ml penicillin, and 100?g/ml streptomycin (medium A) with 10% FBS. After postconfluence cells were

differentiated with 0.5mM 3-isobutyl-1-methyl-xanthine, 1µM dexamethasone, and 10µg/ml insulin for 3 days. Then, cells were incubated with 5µg/ml insulin for 3 days. Between 8-12 days after the induction, the cells were starved for 12h with medium A containing 0.5% BSA before each experiment, in which 3T3-L1 adipocytes were incubated at different temperatures (37, 38, 39, 40, 41 and 42°C). For each temperature samples (in quadruplicate) were collected after 2, 4, 8, 16 and 24h of exposure. Gene expression of leptin, adiponectin and ASP was measured by quantitative real-time reverse trascription-polymerase chain reaction (RT-PCR). Total RNA was extracted from 3T3-L1 adipocytes using QIAzol Lysis reagent (Qiagen, Chatsworth, CA, USA) according to Bernabucci *et al.* (2004). Total RNA (1?g) was reverse transcribed with ImProm-II reverse trascription system (Promega, Madison, WI). Aliquots of cDNA were subjected to real time-PCR using a Quantitect SYBR Green kit (Qiagen, Chatsworth, CA) and the following mouse primer pairs: adiponectin (3' TATTGATGGGTCTGCCGAGG; 5' GCAATGCCGCTGCAAACGTGTT); leptin (3' AAGGCCGTTCTCTTCACCTACGAC; 5' CCATACACCTGGAGCCAGACTTGG); ASP (3' TGTGCCGTTGCTGAAGAG; 5' TGAACCTGCGTTGCTGCCCT). 18s ribosomal RNA QuantiTect primer assay (Qiagen, Chatsworth, CA) was used as internal control. PCR assays were conducted with LightCycler 3.5 amplification system (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. Expression of each mRNA was estimated after adjustment with the expression of 18s rRNA. Cell viability was determined by XTT Cell Proliferation kit II (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. Data for all variables measured were analyzed as repeated measures using the GLM procedure of SAS (SAS Institute, 1999). The model included fixed effects: temperature (37, 38, 39, 40, 41, and 42°C), time of exposure (2, 4, 8, 16 and 24h) and the error term. Since time of exposure was not significant for all variables it was not considered in the final model. Least squares means were separated with the PDIFF procedure of SAS (SAS Institute, 1999). Significance was declared at P<0.05.

RESULTS AND CONCLUSIONS - Heat shock affected in a different way the three adipokines considered: leptin was upregulated; in contrast adiponectin and ASP were downregulated when temperature increased (Figure 1). Leptin mRNA showed (P<0.01, P<0.001) higher levels when adipocytes were exposed to 40, 41 and 42°C compared with control temperature (37°C). The highest levels of leptin mRNA were registered at 41°C. Compared with control temperature adiponectin gene expression showed higher levels at 39°C, and lower (P<0.01) levels at 41 and 42°C. Gene expression of ASP was lower (P<0.01) at 41 and 42°C compared with 37°C. Exposure to different temperatures did not affect cell viability (data not shown). Results obtained in the present study provide the first evidence about effect of heat on adipokines mRNA, and demonstrate a direct influence of heat shock on adipokines gene expression in 3T3-L1 adipocytes.

Changes of adipokines mRNA levels imposed by heat shock are likely comparable with those observed in patients suffering from some pathological conditions. In particular, in humans and laboratory animals it was reported that hyper-leptinemia and hypo-adiponectinemia are associated with obesity and its concomitant disorders, atherosclerosis, diabetes type 2 and metabolic syndrome (Lafontan and Viguerie, 2006). Present findings encourage further studies to verify whether the heat shock induced impairment of adipokines biology may be a cofactor for aggravation of the clinical status observed in patients suffering from the above mentioned metabolic diseases and exposed to heat or heat waves (Braga *et al.*, 2002).

Figure 1. Effect of temperature on adipokines gene expression (mRNA/18S rRNA) by 3T3-L1 adipose cells. * = P<0.01, ** = P<0.001 compared with control temperature: 37°C. # Acylation Stimulating Protein complement component C3.



Leptin, adiponectin and ASP are directly involved in the regulation of glucose and lipid metabolism in WAT and peripheral tissues such as muscle and liver in humans and laboratory animals (Havel, 2004; Lafontan and Viguerie, 2006). In particular adiponectin levels are predictive of steatosis and are inversely related to hepatic fat content (Lafontan and Viguerie, 2006). In farm animals information are available for leptin (Chilliard *et al.*, 2005) about its changes and biological role, whereas no data are available in literature for adiponectin and ASP. Studies carried out in dairy cattle have pointed out that the exposure to hot environments affects metabolic status, causing alteration of lipid metabolism, liver functionality and increasing hepatic lipid accumulation (Ronchi *et al.*, 1999). Those authors reported a possible direct effect of high temperature on metabolic status, but the mechanisms involved are not known yet. Considering changes of metabolic status and on the basis of findings of the present study it would be interesting to investigate changes and biological role of adipokines in farm animals exposed to hot environment.

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