Eurasian Journal
of Veterinary Sciences
www.eurasianjvetsci.org

RESEARCH ARTICLE

Effect of long term heat stress and dietary restriction on the expression of small heat shock protein (sHSP) genes in rat liver tissue

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Received: 14.01.2016, Accepted: 04.03.2016
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Uzun süreli sıcaklık stresi ve yem kısıtlamasının rat karaciğer doku-sundaki bazı küçük sıcaklık şoku protein (sHSP) genlerinin ekspresyon düzeyleri üzerine etkisi

Eurasian J Vet Sci, 2016, 32, 3, 161-166
DOI: 10.15312/EurasianJVetSci.2016318394

Öz

Amaç: Bu çalışmanın amacı uzun süreli sıcaklık stresi ve yem kısıtlamasının rat karaciğer dokusunda bazı küçük ısı şoku protein (sHSP) genlerinin mRNA düzeyindeki ekspresyonu düzeyleri üzerine etkisinin araştırılmasıdır.

Gereç ve Yöntem: Bu amaçla on haftalık yaştaki toplam 24 Sprague-Dawley rat 4 gruba ayrıldı. Grup I ve Grup II'deki ratlar 22°C'lik ortam sıcaklığında, Grup III ve Grup IV'teki ratlar ise 38°C'lik ortam sıcaklığında tutuldu. Grup I ve III'teki ratlar ad libitum olarak beslendi, Grup II ve IV'teki ratlara ise ad libitum grupların tükettiği yemin %60'ı kadar yem verildi. Uygulama 9 hafta sürdürüldükten sonra karaciğer doku örnekleri alınarak sıvı azot içerisinde dondurulduu ve RNA izolasyonuna kadar muhafaza edildi. Doku örneklerinden total RNA izole edildikten sonra *HspB1, HspB5, HspB6, Hsp10* ve *Hsp11* genlerinin ekspresyon düzeyleri gerçek zamanlı nicel polimeraz zincir reaksiyonu (RT-qPCR) yöntemi ile incelendi.

Bulgular: Sıcaklık stresi *HspB2, HspB8* ve *Hsp70* genlerinin ekspresyonunu önemli ölçüde arttığı, *HspB1, HspB5, HspB6, Hsp10* ve *Hsp11* genlerinin ekspresyonunu ise etkilemediği belirlendi. Yem kısıtlaması *HspB6* geninin expresyonunu arttırırken *HspB1, HspB2, HspB5, HspB8 HspB10, HspB11* ve *Hsp70* genlerinin ekspresyonunu etkilemediği gözlendi. Uygulamalar arasında interaksiyon gözlenmedi.

Öneri: Çalışmanın sonuçları uzun süreli sıcaklık stresinin rat karaciğer dokusundaki sHSP genlerinin ekspresyonlarını değişik düzeylerde etkilediğini, yem kısıtlamasının sHSP genlerinin sıcaklık stresi tarafından etkilenen ekspresyonlarını değştirmediğini göstermiştir.

Anahtar kelimeler: Sıcaklık stresi, yem kısıtlaması, karciğer, rat, sHSP

Abstract

Aim: Investigation of the effects of dietary restriction on expression of certain small heat shock protein (sHSP) genes at mRNA level in liver tissue of rats reared under long-term heat stress.

Material and Method: Sprague-Dawley rats (n=24) 10 weeks of age, were equally divided into four groups. Group I and II were kept at an ambient temperature of 22°C, while Groups III and IV were reared at 38°C. Groups I and III were fed ad libitum, while Groups II and IV were fed 60% of the diet consumed by their ad libitum counterparts. The treatment continued for 9 weeks. At the end of the treatment, liver tissue samples were taken. Total RNA was isolated and mRNA expression level of the *HspB1, HspB2, HspB5, HspB6, HspB8, Hsp10, Hsp11* and *HspA1A* genes were assessed by Real-Time PCR analysis.

Results: Heat stress significantly up regulated mRNA expressions of *HspB2, HspB8 and Hsp70* genes, while it did not change mRNA expressions of *HspB1, HspB5, HspB6, Hsp10* and *Hsp11* genes. Dietary restriction (DR) did not significantly affect the expression of *HspB1, HspB2, HspB8 HspB10, HspB11* and *Hsp70*, while it increased mRNA expression of HspB6 gene. No interaction between treatments was observed.

Conclusion: The results suggested that long term heat stress differentially affected the sHSP genes studied and DR had no affect on the heat stress mediated changes in the expression of sHSP.

Keywords: Heat stress, Dietary restriction, liver, rat, sHSP

Introduction

High ambient temperatures cause heat stress resulting in damages to different organs by inducing apoptosis or necrosis (Kanter et al 2013). Studies have demonstrated that negative effects of heat stress arise due to the impaired functions of tissues or organs and that the liver is the primarily affected organ by heat stress (Hall et al 2000). Increase in body temperatures over physiological levels results in denaturation of cellular proteins which might lead to cell death (Kanter et al 2013). In order to maintain physiological processes under heat stress, expression of numerous genes are stimulated or suppressed, including heat shock protein genes for protecting proteins from denaturation (Zhang et al 2002).

Heat shock proteins (HSPs) are among molecular chaperones which help correct folding of proteins into their three-dimensional forms and by preventing their aggregation. Chaperon function of HSPs is necessary for biological activity in the cell in order to promote degradation of misfolded proteins or to regulate cell growth and cell signaling (Taylor and Benjamin 2005).

Small heat shock proteins (sHSP) are members of a protein family having molecular weight of 15-30 kD and characterized by the presence of a α-crystallin domain (Taylor and Benjamin 2005, Thonel et al 2012). In addition to their chaperone activity sHSPs are also involved in other functions such as support of cellular survival under stress conditions by stabilization of the cytoskeleton or inhibiting apoptosis (Morrow and Tanguay 2012). According to the guidelines of the Human Genome Organization Gene Nomenclature Committee sHSPs include 11 family members named *HspB1* to *HspB11* (Kampinga et al 2009).

Although Hsp70 (*HspA1A*) is not a member of sHSPs, it is expressed in various tissues and its expression is increased in response to different kind of stresses. Therefore an increase in the expression of this protein can be considered as a marker for stress (Leoni et al 2000). Different aproachs have been used in order to alleviate the negative effects of heat stress including modifications in environment, building, breeding, and nutritional practices. Among the nutritional practices, dietary restriction (DR), has been shown to extend life span of different animal species. Several mechanisms relating the effect of caloric restriction on the life span extention have been suggested including, retardation of growth, reduction of body fat, reduction of metabolic rate, attenuation of oxidative damage, alteration of glucose-insulin system, alteration of growth hormone-Insulin like growth factor 1 (IGF-1) axis or hormesis which stands for a beneficial effect resulting from the response of an organism subjected to low intensity stressors (Masoro 2009). Studies have shown that calorically restricted animals had enhanced ability to cope with intense stressors (Hall et al 2000, Abu-Dieyeh 2006).

Short term effects of heat stress on the expression of individual sHSPs or other HSP genes in different tissues has been studied by different research groups (Zhang et al 2002, Huang et al 2007). On the other hand, effects of caloric restriction have been mostly investigated with respect to aging or on aged individuals (Hall et al 2000, Cao et al 2001). In this study we hypothesized that DR would induce heat tolerance by different mechanisms including regulation of the expression of sHSPs. To our knowledge, there is no data on the potential protective role of DR on the expression of sHSPs in the liver of young rats exposed to long-term high temperature stress. Therefore, we conducted this study to determine effect of long-term heat stress on the expression of certain sHSPs at mRNA level in rat liver and whether DR would alter the expression level of these genes in liver tissue of young rats reared under long term heat stress

Materials and Methods

The experimental design of the study has been reported by Aydilek et al (2015). Sprague-Dawley rats, two months of age, were divided into 4 groups. Group I and II were kept at an ambient temperature of 22°C (RT), while Groups III and IV were reared at 38°C (HT). Group I (RT-AL) and Group III (HT-AL) were fed ad libitum, while Group II (RT-DR) and Group IV (HT-DR) were fed 60% of the diet consumed by their ad libitum counterparts. The experiment was continued 9 weeks. At the end of the experiment the animals were sacrificed by cervial luxation following general anesthesia and liver samples were taken and immediately frozen in liquid nitrogen. The samples were stored at -80°C until RNA isolation. The experiments were carried out with the permission of Harran University Animal Experimentation Local Ethics Committee (Approval No: 270-99)

A total of 24 liver samples for RNA isolation were used. Approximately 50 to 100 mg liver tissue was homogenized in 800 µL Tri-ReagentTM and total RNA was extracted according to instructions of the manufacturer. Concentration and the quality of the total RNA was assessed spectorophotometrically by using Nano-Drop ND-100 (Thermo Scientific, Wilmington, DE, USA). In order to remove genomic DNA contamination two µg of total RNA was treated with DNAse I. DNAse I treated total RNA was reverse transcribed using oligo dT primers and random hexamers in equal volume with RevertAidTM First Strand cDNA Synthesis Kit according to protocol of the manufacturer (Fermentas, Vilnius, Lithuania).

The genes examined and the primers sequences used for real time PCR were shown in Table 1. Primer sequences have been reported by Kirbach and Golenhofen (2011). The reaction mixture was prepared in 20 µL of final volume consisting of 10 µL 2X SYBR Green Master Mix (Fermentas, Vilnius, Lithuania), 5 pmol of each primer, 1 µL cDNA added with ddH2O. Thermal conditions of PCR consisted of an initial de-

Table 1. Primers used for amplification of target regions of the genes.

Gene	Primer Sequence		Product Length (bp)
HspB1 (Hsp25)	Frw.	CGGCAACTCAGCAGCGGTGTCT	160
	Rev.	CATGTTCATCCTGCCTTTCTTCGTG	
HspB2 (MKBP)	Frw.	CCGAGTACGAATTTGCCAACCC	191
	Rev.	AAATGCCTGGAACTTGCCTTCACT	
HspB5 (αB-crystallin)	Frw.	CTTCTACCTTCGGCCACCCTC	164
	Rev.	GCACCTCAATCACGTCTCCC	
HspB6 (Hsp20)	Frw.	CCATGTGGAGGTCCATGCTCG	195
	Rev.	GCAGGTGGTGACGGAAGTGAG	
HspB8 (Hsp22)	Frw.	CCGGAAGAACTGATGGTAAAGAC	166
	Rev.	CCTCTGGAGAAAGTGAGGCAAATAC	
HspB10 (ODF1)	Frw.	AACGTCTGCGGCTTTGAACCT	195
	Rev.	ACTGCCGAGCCCGTAGGAGTAGGTC	
HspB11 (Hsp16.2)	Frw.	ATTATGGCACGAGATGGCTACG	128
	Rev.	TGTAATCACTAAGGAAGACTTGAGACTG	
HspA1A (Hsp70i)	Frw.	GGTCATCTCCTGGCTGGACTCTAACACG	212
	Rev.	GCCAGAAAAGCCTCTAATCCACCTCC	
Reference gene			
GAPDH	Frw.	CCTGGAGAAACCTGCCAAGTAT	141
	Rev.	AAGGTGGAGGAATGGGAGTTG	

naturation at 95°C for 10 min followed by 40 cycles of denaturation, annealing and amplification at 95°C for 30 s, 60°C for 1 min and 72°C for 30 s, respectively, on a Real-Time PCR System (Applied Bioscience Stepone plus, Foster City, CA). A melting curve analysis was performed by measuring fluorescence signal at every 1-degree increments between 55°C and 95°C. For each cDNA sample PCR was performed as duplicate

Amplification efficiencies of the target genes and internal control (GAPDH) were assessed by amplification of cDNA samples which were serially diluted. After the amplification efficiencies of the target and reference genes were assessed to be nearly the same, data normalization was performed according to Livak and Schmittengen (2001) via method, where $\Delta C_T = C_T$, *target* - C_T, *reference* (where C_T, *target* and C_T, *reference* are threshold cycles for target and reference genes amplifications, respectively). A mixed model was employed in the analysis of normalized gene expression data. In the model fitting procedure, temperature and diet were fitted as fixed effect and technical replicates as random effect. All statistical analyses were carried out by using Genstat Release 7 (Payne 2003).

Results

Effect of heat stress and dietary restriction on the relative expression levels of the sHSP genes in terms of normalized Ct values were shown in Table 2. Heat stress significantly up regulated mRNA expressions of *HspB2* ($P<0.05$), *HspB8* ($P<0.01$) and *Hsp70* ($P<0.001$), while it did not change mRNA expressions of *HspB1, HspB5, HspB6, HspB10* and *HspB11*. Dietary restriction did not significantly affect the expression of *HspB1, HspB2, HspB8 HspB10, HspB11* and *Hsp70* while it only increased mRNA expression of HspB6 gene ($P<0.01$).

Discussion

Exposure of rats to heat at 42°C for a short time (15-30 minutes) dramatically has been shown to increase the expression of *HspB1* in liver both at mRNA and protein level up to 16 hours after heat treatment (Zhang et al 2002, Huang et al 2007). In contrast to these studies, heat stress applied in the present study did not affect the mRNA level of *HspB1* in liver. This might be due to the degree and the length of the heat stress applied in the present study. We applied a heat treatment at 38°C for 9 weeks. Therfore this level of heat stress might be insufficient for upregulation of mRNA synthesis of *HspB1*. On the other hand the mRNA synthesis of HspB1 might have decreased to its normal level after an acute increase. Vallanat et al (2010) have reported that no trascriptional change has been observed for *HspB1* after 4 hours of a heat treatment at 42°C for 40 minutes in mice liver.

Although, in accordance with literature (Quraishe et al 2008,

Table 2. Effect of temperature and diet on the normalized expression levels of sHSP genes.

	Temperature				Diet			
Genes	RT	HT	SE	p	AL	DR	SE	p
HspB1	0.1135	0.1029	0.01010	n.s.	0.1068	0.1096	0.01022	n.s.
HspB2	0.000163	0.000308	0.0000385	*	0.000202	0.000269	0.0000431	n.s.
HspB5	0.97746	0.866852	0.160901	n.s.	0.848291	0.99602	0.160901	n.s.
HspB6	0.006633	0.006908	0.0005600	n.s.	0.005688	0.007854	0.0004569	**
HspB8	0.03712	0.04767	0.002596	**	0.04431	0.04048	0.002990	n.s.
HspB10	0.07689	0.02181	0.02032	n.s.	0.03427	0.06442	0.02147	n.s.
HspB11	0.01234	0.01511	0.001105	n.s.	0.01425	0.01425	0.001171	n.s.
Hsp70	0.001096	0.003163	0.0003760	***	0.002499	0.001759	0.0004754	n.s.

*(P<0.05); ** (P<0.01), ***(P<0.001), n.s.: Not siginificant, RT: Room Temperature (22°C), HT: High Temperature (38°C), AL: Ad Libitum, DR: Dietary Restriction, SE: Pooled Standard Error.

Kirbach and Golenhofen 2011), RNA expression of *HspB2* was found to be very low, heat stress applied in the present study significantly increased mRNA expression of *HspB2* in rat liver. One heat-shock element (HSE) is present in the upstream enhancer region of *HspB2*. However this HSE primarily affect expression of HspB5 which is located near *HspB2* gene (Doerwald et al 2004). Although dietary restriction did not significantly affect (P>0.05) the expression of *HspB2*, the highest expression of *HspB2* was observed in DR group under heat stress. In the intergenic region between *HspB5* and *HspB2* a αBE1 element is present which has been reported to interact with glucocorticoid receptor and positively affect *HspB2* promoter (Swamynathan et al 2007). Caloric restriction has been shown to increase unbound serum corticosteroid level (Sabatino et al 1991).

Long term heat treatment in the present study did not affect the expression of *HspB5* and *HspB6* genes. In accordance with the results of the present study, Bhusari et al (2008) have shown by using a microarray technique that only 12 genes were upregulated while seven genes were down regulated in liver of mice exposed to a chronic heat stress (34 °C for two weeks). The mRNA expression of *HspB8* was significantly upregulated by heat stress (P<0.01). Similarly, Chowdary et al (2004) also reported the presence of two putative HSEs 1000 bases upstream of translation start site of *HspB8* gene.

Heat stress applied in the present study did not significantly alter the expression of *HspB10* encoding outer dense fiber protein (ODF1), which plays important roles in flagellar integrity of sperm (Fontaine et al 2003). Although its expression was not detected in liver of rats in an earlier study (Kirbach and Golenhofen 2011) its expression in other tissues such as eye and muscle in mice has been reported (Quraishe et al 2008). Long term heat stress did not alter the expression of HspB11 gene in liver tissue of rats. To our knowledge no study has been published with respect to the effect of heat stress or DR on expression of *HspB11* (Hsp16.2) in rat liver.

Dietary restriction applied in the present study did not change the mRNA expression of the genes studied except for *HspB6*. These findings seem to contradict with those reported by other researchers. Studies of Heydari et al (1993) demonstrated that the induction of *HspA1A* mRNA levels by heat shock at 42.5°C for 30 min was significantly higher in hepatocytes of calori-restricted old rats than that from old rats fed ad libitum. Furthermore in contrast to our findings DR increased the heat induced *HspA1A* mRNA level in hepatocytes isolated from 4-6 weeks old rats. Hall et al (2000) have shown that whole body exposure of rats to 41°C for 30 min by periodically resuming heating for two days resulted in a significant increase of *HspA1A* in hepatocytes while caloric restriction reduced *HspA1A* accumulation in hepatocytes.

However the results of our study are not directly comparable with those of the authors mentioned above, since we applied a long term heat stress at 38°C and dietary restriction for 9 weeks. Leoni et al (2000) have reported that multiple exposures to 39°C (30 min sessions for 4 days) triggered expression of *HspA1A* in liver and the induction of this protein depends both on intensity and duration of the heat stress applied. They obtained maximal induction of hepatic *HspA1A* when the animals were exposed to a single heat shock at 43.5°C. Animals previously treated at 39°C before a shock at 43.5°C, showed an attenuation of the induction of hepatic *Hsp72* and starvation for 48 hours did not cause *HSP72* induction. In accordance with the results of the present study Cao et al (2001) have found by using microarray method that short term caloric restriction decreased while long term caloric restriction did not affect the mRNA level of *HspB1 (Hsp25)* in the liver of old mice.

Animals reduce feed consumption under high ambient temperatures in order to reduce heat production resulting from digestion of feeds. Reduction of feed consumption can ameliorate negative effect of heat stress (Abu-Dieyeh 2006). Thus the effect of heat stress on the expression of the sHSP genes

in heat stressed AL group might be reduced by decreased feed consumption. However except for HspB6, no significant differences between Group III (HT-AL) and Group IV (HT-DR) reared under high ambient temperatures were detected. These results suggest that dietary restriction did not affect the expression of the sHSP genes, except for HspB6, under the experimental conditions of the present study.

Expression of *HspB6* gene was significantly up-regulated by DR both under room and high ambient temperatures in the present study ($P<0.01$). Implications of this finding need to be elucidated. However phosphorylation of *HspB6* protein (P20) has been reported to be associated with the action of insulin and over expression of this protein suppress insulin-stimulated glucose uptake thus suggesting a direct role of this protein in the regulation of glucose metabolism (Wang et al 2001). Therefore expression of *HspB6* might be enhanced in order to maintain blood glucose level by supressing the glucose uptake by liver. Phosphorylation of *HspB6* has been associated with vasorelaxation by modulating cytoskeletal or contractile elements in smooth muscle cells (Tessier et al 2003). Therefore up-regulation of *HspB6* gene in liver tissue of DR rats might be involved in regulation of blood supply into the liver. Further studies such as in situ hybridization are required in order to assess which cell types contributed to the up-regulation of *HspB6* gene in liver tissue of DR rats.

Transcriptional response to heat stress is mainly controlled by transcription factors (TF) defined as heat-shock factors (HSF). The HSFs are involved in the regulation of HSPs by binding to the heat-shock element (HSE) consisted of five repeats of the NGAAN sequences located at the promoter region of the target HSP gene. Three HSFs have been identified in mammals which were named as HSF1, HSF2 and HSF4. HSF1 has been reported to be involved in acute response to heat (Sonna et al 2002). The function of HSF1 can be modulated by HSF2 through formation of heterodimers and this effect has been reported to be more appearent under mild heat stress like febrile range temperatures (Shinkawa et al 2011). However expression of sHSP genes can also be regulated by interaction of HSFs with other TFs or through HSF-independent pathways (Thonel et al 2012). Therefore different expression patterns of the sHSP genes observed in this study might be due to involvement of different transcriptional factors affecting different regulatory elements.

An increase in the mRNA expression does not necessarily result in an increase of protein synthesis. On the other hand amount of a specific protein can change without a change in the mRNA level of this protein (Chen et al 2005). Furthermore post translational modifications such as phosphorylation are also necessary for the activity of sHSPs (Wang et al 2001, Chen 2005). Therefore further studies will be neceesary in order to elucidate the effect of heat stress and DR on the expression of sHSPs at protein level in rat liver.

Conclusion

The results of this study indicated that expression of the sHSP genes and *HspA1A* gene in rat liver was differentially affected by long term heat stress, while DR altered only expression of the *HspB6* gene by increasing the mRNA level of this gene. The results suggested that DR did not alter the long term effect of heat stress on the RNA expression of sHSP genes. Since acute effects of heat stress and DR on the expression of the sHSP genes included in this study was not investigated, the results might reflect a stituation after the metabolism in liver of the rats reached a stable condition as a result of adaptation process.

Acknowledgement

This study was finacially supported by Harran University Scientific Research Council (Grant No: 12035). We also thank Dicle University Research Centre of Health Sciences (DUSAM) for permitting the use of Real-Time PCR system.

References

Abu-Dieyeh ZHM, 2006. Effect of chronic heat stress and long-term feed restriction on broiler performance. Int J Poultry Sci, 5, 185-190.

Aydilek N, Varisli O, Kocyigit A, Taskin A, Kaya MS, 2015. Effect of dietary restriction on sperm characteristic and oxidative status on testicular tissue in young rats exposed to long-term heat stress. Andrologia, 47, 1055-1061

Bhusari S, Hearne LB, Spiers DE, Lamberson WR, Antoniou E, 2008. Transcriptional profiling of mouse liver in response to chronic heat stress. J Therm Biol, 33, 157-167.

Cao SX, Dhahbi JM, Mote PL, Spindler SR, 2001. Genomic profiling of short-and long-term caloric restriction effects in the liver of aging mice. PNAS, 98, 10630-10635.

Chen X, Zhang HY, Pavlish K, Benoit JN, 2005. Effects of chronic portal hypertension on small heat-shock proteins in mesenteric arteries. Am J Physiol-Gastr L, 288, 616-620.

Chowdary T, Raman B, Ramakrishna T, Rao C, 2004. Mammalian Hsp22 is a heat-inducible small heat-shock protein with chaperone-like activity. Biochem J, 381, 379-387.

Doerwald L, van Rheede T, Dirks RP, Madsen O, Rexwinkel R, van Genesen ST, Lubsen NH, 2004. Sequence and functional conservation of the intergenic region between the head-to-head genes encoding the small heat shock proteins αB-crystallin and HspB2 in the mammalian lineage. J Mol Evol, 59, 674-686.

Fontaine JM, Rest JS, Welsh MJ, Benndorf R, 2003. The sperm outer dense fiber protein is the 10th member of the superfamily of mammalian small stress proteins. Cell Stress Chaperon, 8, 62.

Hall DM, Oberley TD, Moseley PM, Buettner GR, Oberley LW, Weindruch R, Kregel KC, 2000. Caloric restriction improves thermotolerance and reduces hyperthermia-induced cel-

lular damage in old rats. FASEB J, 14, 78-86.

Heydari AR, Wu B, Takahashi R, Strong R, Richardson A, 1993. Expression of heat shock protein 70 is altered by age and diet at the level of transcription. Mol Cell Biol, 13, 2909-2918.

Huang L, Min JN, Masters S, Mivechi NF, Moskophidis D, 2007. Insights into function and regulation of small heat shock protein 25 (HSPB1) in a mouse model with targeted gene disruption. Genesis, 45, 487-501.

Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Hightower LE, 2009. Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperon, 14, 105-111.

Kanter M, Aktas C, Erboga M, 2013. Heat stress decreases testicular germ cell proliferation and increases apoptosis in short term: an immunohistochemical and ultrastructural study. Toxicol Ind Health, 29, 99-113.

Kirbach BB, Golenhofen N, 2011. Differential expression and induction of small heat shock proteins in rat brain and cultured hippocampal neurons. J Neurosci Res 89, 162-175.

Leoni S, Brambilla D, Risuleo G, DeFeo G, Scarsella G, 2000. Effect of different whole body hyperthermic sessions on the heat shock response in mice liver and brain. Mol Cell Biochem, 204, 41-47.

Livak KJ, Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods, 25, 402-408.

Masoro EJ 2009. Caloric restriction-induced life extension of rats and mice: a critique of proposed mechanisms. BBA-Gen Subjects, 1790, 1040-1048.

Morrow G, Tanguay RM, 2012. Small heat shock protein expression and functions during development. Int J Biochem Cell Biol, 44, 1613– 1621

Payne RW 2003. The guide to GenStat release 7.1. Lawes Agricultural Trust.

Quraishe S, Asuni A, Boelens WC, O'Connor V, Wyttenbach A, 2008. Expression of the small heat shock protein family in the mouse CNS: Differential anatomical and biochemical compartmentalization. Neuroscience, 153, 483-491.

Sabatino F, Masoro EJ, McMahan CA, Kuhn RW, 1991. Assessment of the role of the glucocorticoid system in aging processes and in the action of food restriction. J Gerontol, 46, 171-179.

Shinkawa T, Tan K, Fujimoto M, Hayashida N, Yamamoto K, Takaki E, Nakai A 2011. Heat shock factor 2 is required for maintaining proteostasis against febrile-range thermal stress and polyglutamine aggregation. Mol Biol Cell, 22, 3571-3583.

Sonna LA, Fujita J, Gaffin SL, Lilly CM, 2002. Invited review: Effects of heat and cold stress on mammalian gene expression. J Appl Physiol, 92, 1725-1742.

Swamynathan SK, Piatigorsky J, 2007. Regulation of the mouse alpha B-crystallin and MKBP/HspB2 promoter activities by shared and gene specific intergenic elements: the importance of context dependency. Int J Dev Biol, 51, 689.

Taylor RP Benjamin IJ, 2005. Small heat shock proteins: A new classification scheme in mammals. J Mol and Cell Cardiol, 38, 433-444.

Tessier DJ, Komalavilas P, Panitch A, Joshi L, Brophy CM, 2003. The small heat shock protein (HSP) 20 is dynamically associated with the actin cross-linking protein actinin. J Surg Res, 111, 152-157.

Thonel A, Le Mouel A, Mezger V, 2012. Transcriptional regulation of small HSP—HSF1 and beyond. Int J Biochem Cell Biol, 44, 1593-1612.

Vallanat B, Anderson SP, Brown-Borg HM, Ren H, Kersten S, Jonnalagadda S, Corton JC, 2010. Analysis of the heat shock response in mouse liver reveals transcriptional dependence on the nuclear receptor peroxisome proliferator-activated receptor α (PPARα). BMC Genomics, 11, 16.

Wang Y, Xu A, Ye J, Kraegen EW, Cynthia AT, Cooper GJ, 2001. Alteration in phosphorylation of P20 is associated with insulin resistance. Diabetes, 50, 1821-1827.

Zhang HJ, Drake VJ, Morrison JP, Oberley LW, Kregel KC, 2002. Selected contribution: Differential expression of stress-related genes with aging and hyperthermia. J Appl Physiol, 92, 1762-1769.