Scand J Med Sci Sports 2010: 20: 83–89
DOI: 10.1111/j.1600-0838.2008.00872.x

SCANDINAVIAN JOURNAL OF
MEDICINE & SCIENCE
IN SPORTS

Exercise training and experimental diabetes modulate heat shock protein response in brain

Z. Lappalainen[1], J. Lappalainen[1], N. K. J. Oksala[1,2,3], D. E. Laaksonen[1,4], S. Khanna[5], C. K. Sen[5], M. Atalay[1]

[1]Institute of Biomedicine, Physiology, University of Kuopio, Kuopio, Finland, [2]Institute of Clinical Medicine, Surgery, Kuopio University Hospital, Kuopio, Finland, [3]Department of Surgery, Division of Vascular Surgery, Tampere University Hospital, Tampere, Finland, [4]Institute of Clinical Medicine, Internal Medicine, Kuopio University Hospital, Kuopio, Finland, [5]Laboratory of Molecular Medicine, Department of Surgery, Davis Heart & Lung Research Institute, The Ohio State University Medical Center, Columbus, OH, USA

Corresponding author: Mustafa Atalay, Institute of Biomedicine, Physiology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland. Tel: +358 400 173972, Fax: +358 17 162889, E-mail: mustafa.atalay@uku.fi

Accepted for publication 19 August 2008

In diabetes, defense systems against cellular stress are impaired. Heat shock proteins (HSPs) function primarily as molecular chaperones. Factors that raise tissue HSP levels may slow progression of diabetes and improve diabetic complications that also affect brain tissue. This study tested the effect of an 8-week exercise training on brain HSP response in rats with or without streptozotocin-induced diabetes (SID). In untrained animals, the HSP levels were not different between SID and non-diabetic groups. Endurance training, however, increased HSP72 and HSP90 protein in non-diabetic rats, whereas SID significantly decreased the effect of training on these HSPs. At the mRNA level, HSP60, HSP90 and GRP75 were increased due to training, whereas HSP72 mRNA was only increased in exercise-trained diabetic animals. Training or diabetes had no effect on protein carbonyl content, a marker of oxidative damage. Altogether, our findings suggest that endurance training increases HSP expression in the brain, and that experimental diabetes is associated with an incomplete HSP response at the protein level.

Heat shock proteins (HSPs) are stress-inducible proteins that function primarily as molecular chaperones and have a major role in tissue protection against a number of insults and pathological conditions, including cerebral ischemia, diabetes and its complications (Welsh et al., 1995; Kelly et al., 2002). Endurance exercise training has been shown to upregulate tissue HSP expression (Powers et al., 1998; Noble et al., 1999; Harris & Starnes, 2001) and increase tissue protection (Powers et al., 1998; Smolka et al., 2000). Our group previously reported tissue-specific HSP expression in streptozotocin-induced diabetic (SID) rats in response to exercise training (Atalay et al., 2004). Moreover, in humans, exposure to hyperthermia has been shown to upregulate HSP72 protein levels in skeletal muscle and to improve glucose tolerance and insulin resistance in type 2 diabetic patients (Chung et al., 2008).

Given that certain disease conditions, including diabetes, have been linked to incorrect protein folding (Thomas et al., 1995; Hayden et al., 2005), HSPs may play important roles in minimizing protein damage that may occur from the stressful conditions created by the disease. So far, research on constitutive or inducible expression of HSPs in diabetic state is limited and equivocal as the expression of HSPs in diabetes has been reported to be decreased (Swiecki et al., 2003; Atalay et al., 2004; Chen et al., 2005) or unchanged (Joyeux et al., 1999; Yamagishi et al., 2001), possibly as a result of tissue-specific differences, duration or severity of diabetes, or some other factors.

In brain tissue, prior HSP induction by mild stress has provided protection against further and more severe stress such as ischemia (Kume et al., 1996; Latchman, 2004). Brain cells synthesize the inducible 70 kDa form of HSP (HSP72) in response to a variety of stressors, including hyperthermia (Walters et al., 1998; Leoni et al., 2000), ischemia (Simon et al., 1991), hypoxia (Murphy et al., 1999) and energy depletion (Wang et al., 2005). Moreover, overexpression of HSP70 was shown to protect against both focal and global cerebral ischemia *in vivo* (Kelly et al., 2002; Tsuchiya et al., 2003). Interestingly, HSP70 has recently been suggested to also have anti-inflammatory properties, which may partly explain its neuroprotective function in the post-ischemic brain (Zheng et al., 2008). However, information regarding the effect of physical exercise on HSPs in the brain tissue is limited, and not available

in diabetes. Evidently, the brain seems capable of releasing HSP72 into the bloodstream in response to prolonged exercise due to exercise-induced elevations of brain temperature (Nybo et al., 2003), although this response appears to be subject-dependent (Lancaster et al., 2004).

Here, we hypothesized that exercise training would up-regulate HSP response in brain tissue, and tested whether training can offset the adverse effects of experimental diabetes on HSP response.

Materials and methods

Animals

Twelve-week-old male outbred Wistar rats ($n = 24$ animals) were used in the study. The animals were maintained at 22 ± 2 °C with 12:12 h light–dark cycles and had free access to standard rat chow and water. Animal care and experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985). The experimental protocol was approved by the Ethics Committee for the laboratory animal research of University of Kuopio, Finland.

Preparation of diabetic rats

The animals were first randomly assigned to a non-diabetic control ($n = 12$) and a diabetic ($n = 12$) group. Diabetes was then induced by a single intrapenitoneal injection of streptozotozin (STZ) (60 mg/kg, prepared in 0.1 mol/L citrate buffer, pH 4.5) as described (Atalay et al., 2004), which destroys pancreatic β cells, and is used in experimental models of type 1 diabetes (Wang et al., 1994). Diabetes of the STZ-injected animals was confirmed by glucosuria using glucose test strips (BM-Test-5L, Boehringer Mannheim, Mannheim, Germany) 1 week after the injection, and routinely repeated once a week throughout the study. In addition, blood glucose levels were measured at the end of the study in truncal blood collected immediately after decapitation using a commercial kit (Glucoquant Glucose/HK, Boehringer Mannheim) using the hexokinase reaction. Animals with sustained diabetes (glucosuria of at least 20 mmol/L 2 weeks after the STZ injection) and the non-diabetic control animals were further divided into respective training ($n = 6$) and non-training ($n = 6$) groups.

Training protocol

The rats were trained on a treadmill for 8 weeks, 5 days a week as described previously (Gul et al., 2002). Briefly, after 1 week of familiarizing to the treadmill, training (5 days a week with 1.5 h/day) continued for a total of 8 weeks. All animals tolerated the training well and were able to increase the running distance and intensity according to the training protocol throughout the study. Response to exercise training was confirmed by increased citrate synthase activity in skeletal muscle as reported previously (Atalay et al., 2004).

Tissue harvesting

The animals were sacrificed by decapitation at rest or approximately 72 h after the last training session. Following decapitation, whole brains (cerebrum) of the animals were quickly removed, rinsed in ice-cold saline, blotted, placed in liquid nitrogen, and stored at -70 °C until use.

Analysis of stress proteins and eukaryotic elongation factors by Western blot

To analyze protein expression in brain, standard Western blot techniques were used as previously described (Atalay et al., 2004; Oksala et al., 2006). First, the frozen whole brains were pulverized under liquid nitrogen with a mortar and sonicated in a buffer containing 25% glycerol, 0.42 mol/L NaCl, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L EDTA, 20 mmol/L HEPES, 5 μmol/L DTT and 5 μmol/L PMSF at 4 °C. Protein extracts (30 μg of protein per lane) were electrophoresed together with molecular weight markers on an SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Millipore, Bedford, Massachusetts, USA). Next, after blocking with 5% (w/v) fat-free milk solution at 37 °C for 60 min, the membranes were treated with monoclonal antibodies (Ab) against HSPs (all from StressGen, British Columbia, Victoria, Canada) recognizing the 60 kDa HSP (HSP60), HSP72 and the constitutive cognate form of the 70 kDa HSP (HSC70), the 90 kDa HSP (HSP90) and glucose-regulated protein 75 (GRP75). The polyclonal Ab for eukaryotic elongator factor (eEF)-1α and eEF-2 and EF-2 kinase were purchased from Santa-Cruz Biotechnology (Santa Cruz, California, USA). As secondary Ab, horseradish peroxidase-conjugated anti-mouse (Santa-Cruz Biotechnology) and anti-rat immunoglobulins (Zymed Laboratories, San Francisco, California, USA) were used, respectively. The membranes were developed with the enhanced chemiluminescence method (NEN Life Sciences, Boston, Massachusetts, USA) and quantified using image-analysis software (ScionCorp, Frederick, Maryland, USA). For clarity, all results are expressed relative to values obtained from the respective untrained non-diabetic control group.

Analysis of protein carbonyls as indices of oxidative injury

To evaluate the potential effect of diabetes or exercise training on markers of oxidative stress, protein carbonyls were measured using an ELISA method, as described previously (Oksala et al., 2007b), in brain tissue homogenates. The carbonyl contents are expressed as nmol of protein carbonyl in mg of total protein (nmol/mg protein).

Analysis of gene expression

To analyze mRNA expression of HSP60, HSC70, HSP72, HSP90, GRP75 and cyclophilin B (CypB) in brain tissue, a quantitative real-time RT-PCR was applied. Briefly, 100 mg of brain tissue was first homogenized with Ultra-Turrax and total cellular RNA was isolated using TRIzol reagent according to the manufacturer's instructions (Life Technologies, Gaithersburg, Maryland, USA). Nucleic acid concentrations were determined by a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA) and their integrity was checked with gel electrophoresis. One microgram of RNA from each sample was then converted to cDNA using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, California, USA) and oligo(dT) primers (Promega, Madison, Wisconsin, USA). For PCR primer design, the annotated nucleotide sequences were retrieved from the GenBank database (National Center for Biotechnology Information, Bethesda, Maryland, USA), and BLAST searches were performed to identify unique stretches of nucleotide sequence, and not to amplify genomic DNA. The primers were synthesized by Oligomer Oy (Helsinki, Finland) as follows (shown in 5′-3′ orientation): HSP60 forward primer (-F) AAAGCTG AACGAGCGACTTG and reverse primer (-R) ATCACTT GTCCCTCCAACCTTC; HSC70-F AGCACCCAGGCCAG

TATTG and HSC70-R CAGCATTCAACTCCTCAAATCG; HSP72-F CAACTGGCTTGACCGAAACC and HSP72-R AGCGCAAGCCTAGTCCACTTC; HSP90-F GTACGAAA CAGCACTCCTGTCTTC and HSP90-R ATCCTCATCAA TACCTAGACCAAGC; GRP75-F ACGAGGATGCCCAA GGTTC and GRP75-R TGAATGGCAGCTCCAATGG; CypB-F GCCTTAGCTACAGGAGAGAAAGGA and CypB-R TCCACCCTGGATCATGAAGTC.

The samples were amplified in duplicate using Brilliant SYBR Green Master Mix (Stratagene, La Jolla, California, USA) with 200 nM of gene-specific primers, and run on an Mx3000P System (Stratagene) with the following program: a 10-min pre-incubation at 95 °C, followed by 40 cycles of 15 s at 95 °C, 20 s at 59 °C, and 25 s at 72 °C. The data were normalized relative to expression of CypB by the previously introduced algorithm (Pfaffl, 2001). Unique amplification products and absence of primer-dimers were evaluated by melt-curve analysis.

Statistics

All calculations were performed using SPSS software (SPSS Inc, Chicago, Illinois, USA). Differences in continuous variables between groups were assessed using Student's *t*-test. To test the effect of diabetes and endurance training, two-way ANOVA with Bonferroni's correction was used. Statistical significance was set at $P<0.05$. Data are represented as means ± SE of the mean (SEM) unless otherwise stated.

Results

Effect of diabetes and training on HSP mRNA and protein in the brain

To determine whether the expression of stress proteins is altered in diabetic brain, we first analyzed the tissue levels of HSPs in non-diabetic and diabetic rats. At rest, the levels of all HSPs investigated did not differ between the groups (Figs 1–4), except GRP75 mRNA, which was slightly depressed ($P = 0.03$) in diabetic animals (Fig. 4).

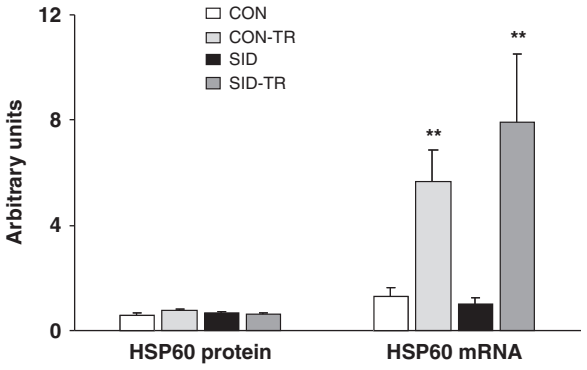

Fig. 1. Effect of 8 weeks of exercise training on HSP60 protein and mRNA levels in non-diabetic and diabetic rat brain. Values are mean ± SEM. The acronyms for the groups ($n = 6$ per group) are as follows: untrained non-diabetic control (CON), non-diabetic control with training (CON-TR), untrained diabetic (SID) and diabetic with training (SID-TR). $^{**}P<0.01$ for the difference due to training. SID, streptozotocin-induced diabetes.

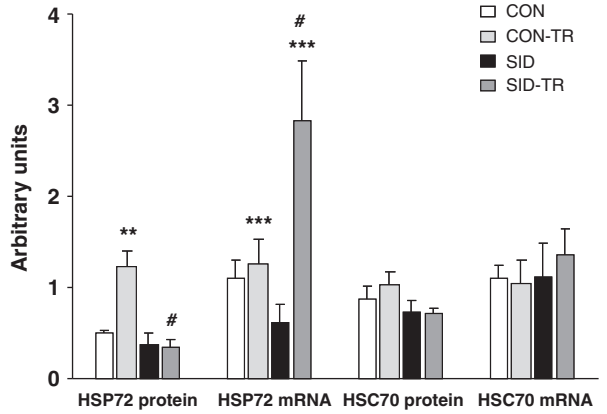

Fig. 2. Effect of 8 weeks of exercise training on HSP72 and HSC70 protein and mRNA levels in non-diabetic and diabetic rat brain. Values are mean ± SEM. The groups are as in Fig. 1. $^{**}P<0.01$, $^{***}P<0.001$ for the difference due to training, two-way ANOVA for training effect and $^{\#}P<0.05$ for the interaction between training and diabetes. ANOVA, analysis of variance.

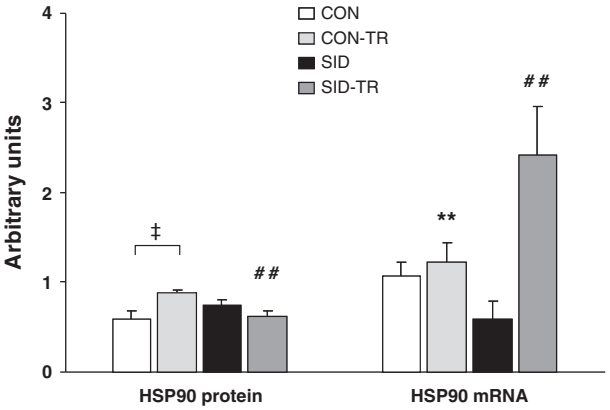

Fig. 3. Effect of 8 weeks of exercise training on HSP90 protein and mRNA levels in non-diabetic and diabetic rat brain. Values are mean ± SEM. The groups are as in Fig. 1. $^{\ddagger}P<0.05$, $^{**}P<0.01$ for the difference due to training, $^{\#\#}P<0.01$ for the interaction between training and diabetes.

We also observed that in response to 8-week endurance training, all HSP mRNAs, excluding HSC70, were significantly up-regulated in diabetic animals (Figs 1–4), whereas HSP60, HSP90 and GRP75 mRNAs were increased in non-diabetic animals (Figs 1, 3 and 4, respectively). Interestingly, diabetes significantly inhibited the effect of training on HSP72 and HSP90 proteins (Figs 2 and 3, respectively), whereas the levels of constitutive HSC70 protein were slightly, but non-significantly ($P = 0.08$) lower in diabetic animals (Fig. 2).

Training and diabetes showed an interaction only on HSP72 and HSP90 proteins (Figs 2 and 4, respectively), whereas at the mRNA level, this interaction was observed on HSP72, HSP90 and GRP75 (Figs 1, 3 and 4, respectively).

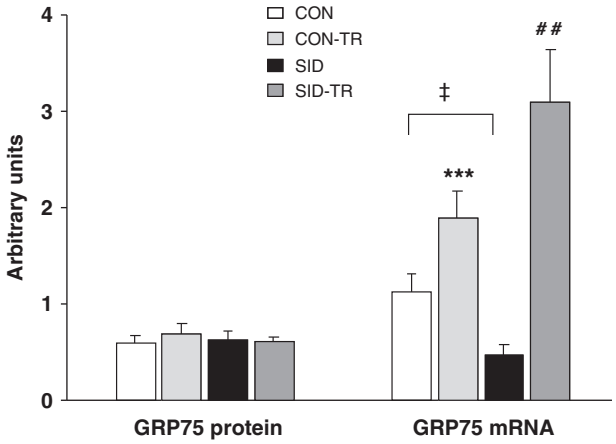

Fig. 4. Effect of 8 weeks of exercise training on GRP75 protein and mRNA levels in non-diabetic and diabetic rat brain. Values are mean ± SEM. The groups are as in Fig. 1. $^{‡}P<0.05$, $^{***}P<0.001$ for the difference due to training, $^{\#\#}P<0.01$ for the interaction between training and diabetes.

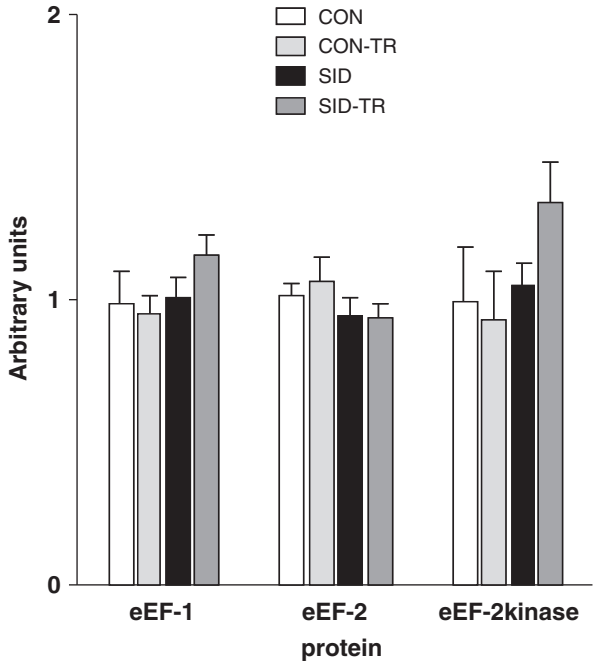

Fig. 5. Effect of 8 weeks of exercise training on eEF-1, eEF-2 and eEF-2kinase protein levels in non-diabetic and diabetic rat brain. Values are mean ± SEM. The groups are as in Fig. 1.

Effect of diabetes and training on key elongation factors

The levels of elongation factor eEF-1 and eEF-2 were similar in diabetic and control animals ($P = 0.39$) (Fig. 5). Exercise training increased eEF-1 and eEF-2 kinase levels slightly, but non-significantly, in diabetic animals only ($P = 0.07$ and 0.097, respectively) (Fig. 5).

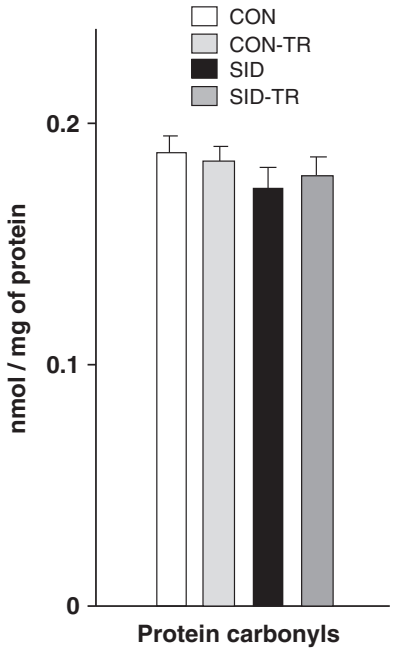

Fig. 6. Effect of 8 weeks of exercise training on protein carbonyl content in non-diabetic and diabetic rat brain. Values are mean ± SEM. The groups are as in Fig. 1.

Effect of diabetes and training on protein carbonyls

The protein carbonyl contents were similar in diabetic and non-diabetic animals, and exercise training had no effect on these values (Fig. 6).

Discussion

The brain is a unique tissue regarding the HSP response, because the transcriptional regulation in neuronal cells is different from other tissues (Kaarniranta et al., 2002). In the present study, HSP72 and HSP90 levels were found to be similar between diabetic and non-diabetic animals at rest. On the other hand, endurance training induced an HSP response in the brain in non-diabetic animals that was blunted in diabetic animals at the protein level. These observations are novel, and, to our knowledge, no data exist on brain HSP expression using exercise training in a diabetes model. Earlier studies indicate that some HSPs are released in the brain in response to acute exercise (Walters et al., 1998; Leoni et al., 2000; Febbraio et al., 2002; Nybo et al., 2003; Lancaster et al., 2004), and that habitual exercise induces HSP72 expression in specific brain areas (Campisi et al., 2003), suggesting a potentiating effect of physical activity on HSP72 expression.

We found similar levels of HSP60 in the brain of diabetic and non-diabetic animals at rest, whereas the mRNA for GRP75 was slightly decreased in diabetic animals. Recently, HSP60 levels have been

found to be reduced in the heart (Shan et al., 2003; Oksala et al., 2006) and increased in the kidney and liver (Oksala et al., 2006; Oksala et al., 2007a) of diabetic animals. To our knowledge, however, only one study has investigated the levels of HSP60 in the diabetic brain (Yuan et al., 2006). The authors reported elevated levels of mitochondrial HSP60 in the hippocampal region. No information was available previously on HSP60 response of whole brain to diabetes and exercise. Elevated levels of HSP60, HSP90 and GRP78 have also been reported in the skeletal muscle in diabetic patients (Höjlund et al., 2003). On the contrary, (Yamagishi et al., 2001) found significantly decreased levels of the constitutive HSC70 in the liver, but not in the brain of diabetic rats, while HSP90 expression remained unchanged. Similarly, our group has previously reported that experimental diabetes decreased HSP72 levels in the heart, liver and skeletal muscle, whereas HSP90 was increased in the heart and decreased in the liver (Atalay et al., 2004). Hence, the available studies indicate that the diabetic state may exert variable and tissue-specific effects on HSP expression.

We found increased levels of HSP72 and HSP90 protein in non-diabetic animals by exercise training. This is consistent with a previous report where the brain tissue was shown to release HSP72 protein actively during prolonged exercise (Nybo et al., 2003). On the other hand, exhaustive exercise has been shown to induce an HSP72 response only in the active brain regions (Campisi et al., 2003), also suggesting a role for increased metabolic rate in HSP72 induction. Therefore, the existing literature and our findings support a role for exercise-induced HSP72 response in brain tissue.

Interestingly, diabetes inhibited the effect of exercise training on HSP72 induction at the protein level, whereas the mRNA level was significantly up-regulated. Although no comparable information on training is available in brain tissue, other studies have shown an accumulation of myocardial HSP72 following heat stress in both diabetic and non-diabetic animals (Joyeux et al., 1999; Swiecki et al., 2003). This discrepancy may be explained by tissue-specific differences, the duration or severity of diabetes, type of stressor or other still unknown factors. Moreover, because endurance training up-regulated HSP72 and HSP90 protein levels in non-diabetic animals, it is likely that these proteins play an important role in protecting brain tissue during stress as suggested earlier (Stahnke et al., 2007), but in diabetes, this response was depressed. We also found significantly increased HSP60 and GRP75 mRNA expression in response to training in both diabetic and non-diabetic animals, although the protein levels remained unchanged, providing additional evidence that not all mRNAs are translated into protein.

Protein carbonyl content in the brain was not affected by training or diabetes, suggesting that oxidative stress may not explain the differential effect of exercise training on HSP expression in diabetic and non-diabetic animals. We have reported previously that exercise training increased the protein carbonyl content in the heart, liver and skeletal muscle in SID rats (Atalay et al., 2004). Our findings are consistent with a previous study (Radak et al., 1995) suggesting that brain tissue may be less susceptible to exercise-induced to oxidative damage.

The initial level of mRNA translation into protein has been acknowledged for its crucial role in controlling net protein synthesis (Proud, 2006). The elongation step of protein synthesis may also be affected, especially with oxidant compounds (Parrado et al., 2003). Many pathologic states, including diabetes, have been associated with changes in the elongation factors (Kimball et al., 1994). Indeed, experimental diabetes was shown to decrease the rate of peptide chain elongation, which was further associated with reduced levels of the elongation factor eEF-2 (Bergstedt et al., 1993). However, in our study, the decrease (on average 7.5%) in total eEF-2 protein in diabetic animals was non-significant, and exercise had no significant effect on this protein. The eEF-2 kinase is highly specific for phosphorylation eEF-2 and also inactivates eEF-2, and thus can modulate the rate of polypeptide chain elongation during translation. We found slightly, but non-significantly increased levels of eEF-2 kinase in trained diabetic rats. Furthermore, we could not analyze the phosphorylated form of the eEF-2 protein, which limits the available data.

Taken together, our findings suggest that endurance training increases HSP expression in brain tissue, and that experimental diabetes impairs the HSP response at the protein level. Thus, endurance training may act as a potential tool for enhancing chaperone-mediated cellular regulation in the brain.

Perspectives

Physical exercise has a protective effect on the brain and its mental processes. This is important as diabetes is a risk factor for the decline in cognitive function and ischemic stroke. In addition to the well-known role of HSPs in protein homeostasis and cell survival, recent reports provide evidence that an increased HSP72 expression in the brain provides protection from ischemia and also has anti-inflammatory effects (Kelly et al., 2002; Zheng et al., 2008). However, there is little information available about how the beneficial effects of physical exercise are mediated in the brain compared with the information

about other tissues such as skeletal muscle. We noted that endurance exercise training selectively increases HSP expression in brain tissue, and that experimental diabetes impairs this response at the protein level. This in turn indicates that the beneficial effects of physical exercise are affected by the diabetic state, which is an important area for further investigation. Nevertheless, the potential importance of strategies to elevate HSP expression in a safe and physiological manner using physical exercise provides cost-effective means for improving brain health by chaperone-mediated cellular regulation.

Key words: diabetes, brain, exercise, heat shock protein.

Acknowledgements

The authors thank Taija Hukkanen, Satu Mattila and Taina Vihavainen for technical assistance. This work was supported by the grants from the Finnish Ministry of Education, Centre for International Mobility (CIMO), Foundation of Pajulahti College of Sports, Juho Vainio and Yrjö Jahnsson Foundations, Helsinki, Finland, High Technology Foundation of Eastern Finland, and by COST actions B35 and BM0602.

References

Atalay M, Oksala NK, Laaksonen DE, Khanna S, Nakao C, Lappalainen J, Roy S, Hänninen O, Sen CK. Exercise training modulates heat shock protein response in diabetic rats. J Appl Physiol 2004: 97: 605–611.

Bergstedt K, Hu BR, Wieloch T. Initiation of protein synthesis and heat-shock protein-72 expression in the rat brain following severe insulin-induced hypoglycemia. Acta Neuropathol 1993: 86: 145–153.

Campisi J, Leem TH, Greenwood BN, Hansen MK, Moraska A, Higgins K, Smith TP, Fleshner M. Habitual physical activity facilitates stress-induced HSP72 induction in brain, peripheral, and immune tissues. Am J Physiol Regul Integr Comp Physiol 2003: 284: 520–530.

Chen HS, Shan YX, Yang TL, Lin HD, Chen JW, Lin SJ, Wang PH. Insulin deficiency downregulated heat shock protein 60 and IGF-1 receptor signaling in diabetic myocardium. Diabetes 2005: 54: 175–181.

Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, Lancaster GI, Southgate RJ, Bruce CR, Duffy SJ, Horvath I, Mestril R, Watt MJ, Hooper PL, Kingwell BA, Vigh L, Hevener A, Febbraio MA. HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci USA 2008: 105: 1739–1744.

Febbraio MA, Steensberg A, Walsh R, Koukoulas I, van Hall G, Saltin B, Pedersen BK. Reduced glycogen availability is associated with an elevation in HSP72 in contracting human skeletal muscle. J Physiol 2002: 538: 911–917.

Gul M, Laaksonen DE, Atalay M, Vider L, Hänninen O. Effects of endurance training on tissue glutathione homeostasis and lipid peroxidation in streptozotocin-induced diabetic rats. Scand J Med Sci Sports 2002: 12: 163–170.

Harris MB, Starnes JW. Effects of body temperature during exercise training on myocardial adaptations. Am J Physiol Heart Circ Physiol 2001: 280: 2271–2280.

Hayden MR, Tyagi SC, Kerklo MM, Nicolls MR. Type 2 diabetes mellitus as a conformational disease. JOP 2005: 6: 287–302.

Höjlund K, Wrzesinski K, Larsen PM, Fey SJ, Röpstorff P, Handberg A, Dela F, Vinten J, McCormack JG, Reynet C, Beck-Nielsen H. Proteome analysis reveals phosphorylation of ATP synthase beta-subunit in human skeletal muscle and proteins with potential roles in type 2 diabetes. J Biol Chem 2003: 278: 10436–10442.

Joyeux M, Faure P, Godin-Ribuot D, Halimi S, Patel A, Yellon DM, Demenge P, Ribuot C. Heat stress fails to protect myocardium of streptozotocin-induced diabetic rats against infarction. Cardiovasc Res 1999: 43: 939–946.

Kaarniranta K, Oksala N, Karjalainen HM, Suuronen T, Sistonen L, Helminen HJ, Salminen A, Lammi MJ. Neuronal cells show regulatory differences in the hsp70 gene response. Brain Res Mol Brain Res 2002: 101: 136–140.

Kelly S, Zhang ZJ, Zhao H, Xu L, Giffard RG, Sapolsky RM, Yenari MA, Steinberg GK. Gene transfer of HSP72 protects cornu ammonis 1 region of the hippocampus neurons from global ischemia: influence of Bcl-2. Ann Neurol 2002: 52: 160–167.

Kimball SR, Vary TC, Jefferson LS. Regulation of protein synthesis by insulin. Annu Rev Physiol 1994: 56: 321–348.

Kume M, Yamamoto Y, Saad S, Gomi T, Kimoto S, Shimabukuro T, Yagi T, Nakagami M, Takada Y, Morimoto T, Yamaoka Y. Ischemic preconditioning of the liver in rats: implications of heat shock protein induction to increase tolerance of ischemia-reperfusion injury. J Lab Clin Med 1996: 128: 251–258.

Lancaster GI, Moller K, Nielsen B, Secher NH, Febbraio MA, Nybo L. Exercise induces the release of heat shock protein 72 from the human brain *in vivo*. Cell Stress Chaperones 2004: 9: 276–280.

Latchman DS. Protective effect of heat shock proteins in the nervous system. Curr Neurovasc Res 2004: 1: 21–27.

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

Murphy SJ, Song D, Welsh FA, Wilson DF, Pastuszko A. Regional expression of heat shock protein 72 mRNA following mild and severe hypoxia in neonatal piglet brain. Adv Exp Med Biol 1999: 471: 155–163.

Noble EG, Moraska A, Mazzeo RS, Roth DA, Olsson MC, Moore RL, Fleshner M. Differential expression of stress proteins in rat myocardium after free wheel or treadmill run training. J Appl Physiol 1999: 86: 1696–1701.

Nybo L, Moller K, Pedersen BK, Nielsen B, Secher NH. Association between fatigue and failure to preserve cerebral energy turnover during prolonged exercise. Acta Physiol Scand 2003: 179: 67–74.

Oksala NK, Laaksonen DE, Lappalainen J, Khanna S, Nakao C, Hänninen O, Sen CK, Atalay M. Heat shock protein 60 response to exercise in diabetes: effects of alpha-lipoic acid supplementation. J Diabetes Complications 2006: 20: 257–261.

Oksala NK, Lappalainen J, Laaksonen DE, Khanna S, Kaarniranta K, Sen CK, Atalay M. Alpha-lipoic acid modulates heat shock factor-1 expression in streptozotocin-induced diabetic rat kidney. Antioxid Redox Signal 2007a: 9: 497–506.

Oksala NK, Paimela H, Alhava E, Atalay M. Heat shock preconditioning induces protein carbonylation and alters antioxidant protection in superficially injured guinea pig gastric mucosa *in vitro*. Dig Dis Sci 2007b: 52: 1897–1905.

Parrado J, Absi EH, Machado A, Ayala A. "In vitro" effect of cumene hydroperoxide on hepatic elongation factor-2 and its protection by melatonin. Biochim Biophys Acta 2003: 1624: 139–144.

Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001: 29: 2002–2007.

Powers SK, Demirel HA, Vincent HK, Coombes JS, Naito H, Hamilton KL, Shanely RA, Jessup J. Exercise training improves myocardial tolerance to *in vivo* ischemia–reperfusion in the rat. Am J Physiol 1998: 275: 1468–1477.

Proud CG. Regulation of protein synthesis by insulin. Biochem Soc Trans 2006: 34: 213–216.

Radak Z, Asano K, Inoue M, Kizaki T, Ohishi S, Suzuki K, Taniguchi N, Ohno H. Acute bout of exercise does not alter the antioxidant enzyme status and lipid peroxidation in rat hippocampus and cerebellum. Pathophysiology 1995: 2: 243–245.

Shan YX, Yang TL, Mestril R, Wang PH. Hsp10 and Hsp60 suppress ubiquitination of insulin-like growth factor-1 receptor and augment insulin-like growth factor-1 receptor signaling in cardiac muscle: implications on decreased myocardial protection in diabetic cardiomyopathy. J Biol Chem 2003: 278: 45492–45498.

Simon RP, Cho H, Gwinn R, Lowenstein DH. The temporal profile of 72-kDa heat-shock protein expression following global ischemia. J Neurosci 1991: 11: 881–889.

Smolka MB, Zoppi CC, Alves AA, Silveira LR, Marangoni S, Pereira-Da-Silva L, Novello JC, Macedo DV. HSP72 as a complementary protection against oxidative stress induced by exercise in the soleus muscle of rats. Am J Physiol Regul Integr Comp Physiol 2000: 279: R1539–R1545.

Stahnke T, Stadelmann C, Netzler A, Bruck W, Richter-Landsberg C. Differential upregulation of heme oxygenase-1 (HSP32) in glial cells after oxidative stress and in demyelinating disorders. J Mol Neurosci 2007: 32: 25–37.

Swiecki C, Stojadinovic A, Anderson J, Zhao A, Dawson H, Shea-Donohue T. Effect of hyperglycemia and nitric oxide synthase inhibition on heat tolerance and induction of heat shock protein 72 kDa *in vivo*. Am Surg 2003: 69: 587–592.

Thomas PJ, Qu BH, Pedersen PL. Defective protein folding as a basis of human disease. Trends Biochem Sci 1995: 20: 456–459.

Tsuchiya D, Hong S, Matsumori Y, Kayama T, Swanson RA, Dillman WH, Liu J, Panter SS, Weinstein PR. Overexpression of rat heat shock protein 70 reduces neuronal injury after transient focal ischemia, transient global ischemia, or kainic acid-induced seizures. Neurosurgery 2003: 53: 1179–1187.

Walters TJ, Ryan KL, Tehrany MR, Jones MB, Paulus LA, Mason PA. HSP70 expression in the CNS in response to exercise and heat stress in rats. J Appl Physiol 1998: 84: 1269–1277.

Wang JL, Ke DS, Lin MT. Heat shock pretreatment may protect against heatstroke-induced circulatory shock and cerebral ischemia by reducing oxidative stress and energy depletion. Shock 2005: 23: 161–167.

Wang RN, Bouwens L, Kloppel G. Beta-cell proliferation in normal and streptozotocin-treated newborn rats: site, dynamics and capacity. Diabetologia 1994: 37: 1088–1096.

Welsh N, Margulis B, Borg LA, Wiklund HJ, Saldeen J, Flodstrom M, Mello MA, Andersson A, Pipeleers DG, Hellerstrom C. Differences in the expression of heat-shock proteins and antioxidant enzymes between human and rodent pancreatic islets: implications for the pathogenesis of insulin-dependent diabetes mellitus. Mol Med 1995: 1: 806–820.

Yamagishi N, Nakayama K, Wakatsuki T, Hatayama T. Characteristic changes of stress protein expression in streptozotocin-induced diabetic rats. Life Sci 2001: 69: 2603–2609.

Yuan J, Young BJ, Martinus RD. Expression of chaperonin 60 in the hippocampus of the streptozotocin diabetic rat. Neuroreport 2006: 17: 239–242.

Zheng Z, Kim JY, Ma H, Lee JE, Yenari MA. Anti-inflammatory effects of the 70 kDa heat shock protein in experimental stroke. J Cereb Blood Flow Metab 2008: 28: 53–63.