ELSEVIER

Journal of Diabetes and Its Complications 20 (2006) 257–261

Journal of Diabetes and its Complications

# Heat shock protein 60 response to exercise in diabetes Effects of α-lipoic acid supplementation

Niku K.J. Oksala[a,b,c], David E. Laaksonen[a], Jani Lappalainen[a], Savita Khanna[d], Chitose Nakao[a], Osmo Hänninen[a], Chandan K. Sen[d], Mustafa Atalay[a,*]

[a]*Department of Physiology, University of Kuopio, PO Box 1627, Kuopio FIN-70211, Finland*
[b]*Department of Surgery, Kuopio University Hospital, PO Box 1777, Kuopio FIN-70211, Finland*
[c]*Division of Vascular Surgery, Department of Surgery, Tampere University Hospital, Tampere FIN-33521, Finland*
[d]*Laboratory of Molecular Medicine, Department of Surgery, Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, OH 43210, USA*

Received 21 January 2005; received in revised form 13 May 2005; accepted 12 July 2005

**Abstract**

The pathophysiology of diabetes includes oxidative stress and impaired heat shock protein (HSP) expression. We studied the effects of α-lipoic acid (LA) supplementation for 8 weeks and acute exercise on HSP60 expression and the oxidative stress marker 4-hydroxynonenal adducts (4-HNE) in streptozotocin-induced diabetic (SID) and nondiabetic control rats. Diabetes was associated with decreased HSP60 in the heart and increased levels of HSP60 and 4-HNE in the liver. LA increased HSP60 in the liver of control and diabetic rats and decreased 4-HNE in the liver and heart. Acute exercise increased liver 4-HNE, which was offset by LA. In conclusion, diabetes induced oxidative stress and impaired myocardial HSP60 expression, while LA partially offsets these alterations in a tissue-specific manner.


*Keywords:* Heat shock protein; Exercise; Lipoic acid; Diabetes

## 1. Introduction

Diabetes results in the modification of cellular proteins, oxidative stress, and impairment of cellular defense systems (Atalay & Laaksonen, 2002; Shan, Yang, Mestril, & Wang, 2003). Heat shock proteins (HSPs) are a group of proteins that function in de novo synthesis of proteins and repair damaged proteins, reestablishing their function (Powers, Locke, & Demirel, 2001). HSPs are induced by various stresses, including exhaustive physical exercise (Atalay et al., 2004; Powers et al., 2001). HSPs can be classified into several major families based upon their molecular weight and their function: HSP90 (83–90 kDa), HSP70 (66–78 kDa), HSP60, HSP40, and a family of small HSPs of 15–30 kDa. Among the HSP family, HSP60 is a mitochondrial chaperone that confers protection against cardiac ischemia–reperfusion injury (Powers et al., 2001).

Modification of cellular proteins and oxidative stress in diabetes is associated with disturbances in the HSP-mediated chaperoning system (Bruce, Carey, Hawley, & Febbraio, 2003). The induction of experimental Type 1 diabetes with streptozotocin has decreased levels of HSP60 in the myocardial tissue (Chen et al., 2005), which is associated with the deterioration of cardiac cytoprotection and cardiac myopathy in diabetes (Shan et al., 2003). On the other hand, HSP60 is a beta-cell specific antigen. Injections of its analogues may protect nonobese diabetic

* Corresponding author. Department of Physiology, University of Kuopio, PO Box 1627, Kuopio FIN-70211, Finland. Tel.: +358 17 163108; fax: +358 17 163112.
*E-mail address:* mustafa.atalay@uku.fi (M. Atalay).

1056-8727/06/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jdiacomp.2005.07.008

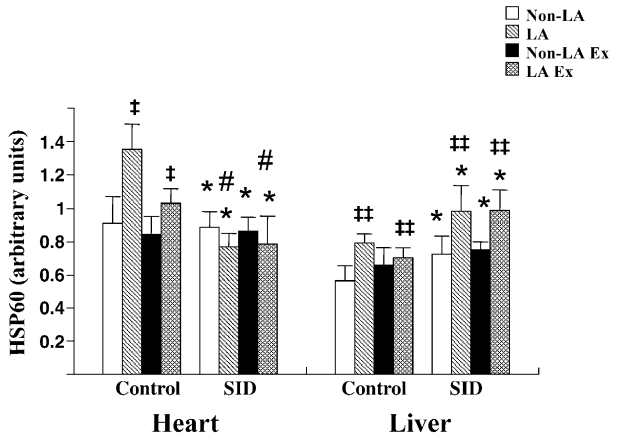


Fig. 1. Effect of streptozotocin-induced diabetes (SID), 8-week α-LA supplementation (LA), and acute exercise (Ex) on the expression of HSP60 in the heart and liver of rat. Densitometric values are means±S.E.M. ($n$=10). Difference due to SID, *$P$<.05; due to LA supplementation, ‡‡$P$<.01. Interaction between diabetes and LA supplementation, #$P$<.05.

(NOD) and low-dose streptozotocin-exposed mice from the development of autoimmune diabetes (Birk et al., 1996). Heat shock preconditioning and up-regulation of HSP expression have also increased the resistance of pancreatic islet cells to the toxic effects of streptozotocin and reactive oxygen species (ROS; Bellmann, Hui, Radons, Burkart, & Kolb, 1997). In contrast, HSP70, the major inducible HSP, seems to be associated with insulin resistance and Type 2 diabetes (Kurucz et al., 2002).

Oxidative stress may have an important role in the pathophysiology of diabetes and its complications (Atalay & Laaksonen, 2002). We have recently shown that experimental diabetes induced oxidative stress and impaired HSP70 responses (Atalay et al., 2004). Regular endurance training, however, restored HSP70 expression (Atalay et al., 2004). Strategies to modulate HSP expression may have important medical implications.

Our group and others have used antioxidant supplementation during physical exercise to overcome oxidative stress (Sen & Packer, 2000). α-Lipoic acid (LA) is a nutritional thiol antioxidant that enhances the endogenous antioxidant defense system and has been used in some countries to treat complications associated with diabetes (Sen & Packer, 2000). Furthermore, LA is an essential cofactor in oxidative metabolism in the mitochondria (Sen & Packer, 2000). LA supplementation has also protected tissues against exercise-induced oxidative stress and stimulated skeletal muscle glucose uptake (Sen & Packer, 2000), which may offset some of the pathophysiological processes of diabetes.

Although antioxidants and HSPs are two major components of cellular defense, their interaction has been studied very little, especially in with regard to diabetes and exercise. We hypothesized that HSP60 synthesis might be altered in streptozotocin-induced diabetes and this alteration could be modulated by antioxidant supplementation and acute exercise.

## 2. Materials and methods

The experimental protocol was approved by the University of Kuopio. The *Principles of laboratory animal care* (NIH publication No. 86-23, revised 1985) was followed for animal care and experimental procedures. Male 11-week-old, outbred Wistar rats ($n$=80) were used. Diabetes was induced by a single intraperitoneal injection of streptozotocin at a dose of 60 mg $kg^{-1}$ (prepared in 0.1 M citrate buffer, pH 4.5) in male 12-week-old Wistar rats ($n$=40; Atalay et al., 2004). Streptozotocin injection has been shown to destroy pancreatic beta cells and is a model of Type 1 diabetes (Wang, Bouwens, & Kloppel, 1994). The state of diabetes was confirmed by glucosuria (BM-Test-5L, Boehringer Mannheim, Germany) and hyperglycemia (Gluco-quant Glucose/HK, Boehringer Mannheim) based on hexokinase/G6P-DH enzymatic method (Gul, Atalay, & Hanninen, 2003; Gul, Laaksonen, Atalay, Vider, & Hänninen, 2002).

Both control and diabetic rats were randomly divided into LA supplemented and nonsupplemented groups. Briefly, LA was administered orally 150 mg $kg^{-1}$ daily for 8 weeks, while the control animals received a matched volume of phosphate buffered saline. Half of the LA-supplemented and nonsupplemented rats were killed at rest, and the other half immediately after acute exercise by decapitation. Treadmill exercise to exhaustion was performed as described previously (Gul et al., 2002).

Liver and heart tissue samples were analyzed for the expression of HSP60 and 4-hydroxynonenal (4-HNE) protein adducts using standard Western blot techniques as previously described (Atalay et al., 2004; Kinnunen et al., 2005). Multifactorial ANOVA was used to test the effect of

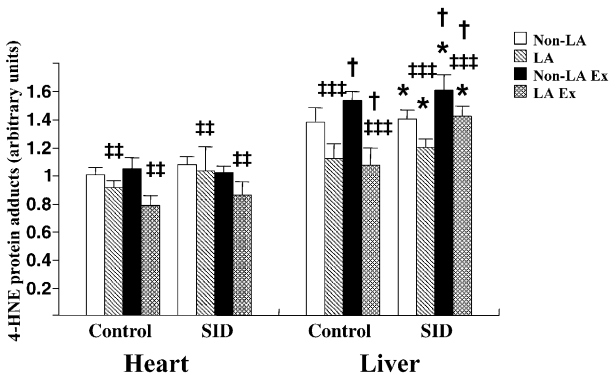


Fig. 2. Effect of streptozotocin-induced diabetes (SID), 8-week α-LA supplementation (LA), and acute exercise (Ex) on the expression of 4-HNE protein adducts in the heart and liver of rat. Densitometric values are means±S.E.M. ($n$=10). Difference due to SID, *$P$<.05; due to LA supplementation, ‡‡$P$<.01, ‡‡‡$P$<.001; due to acute exercise, †$P$<.05.

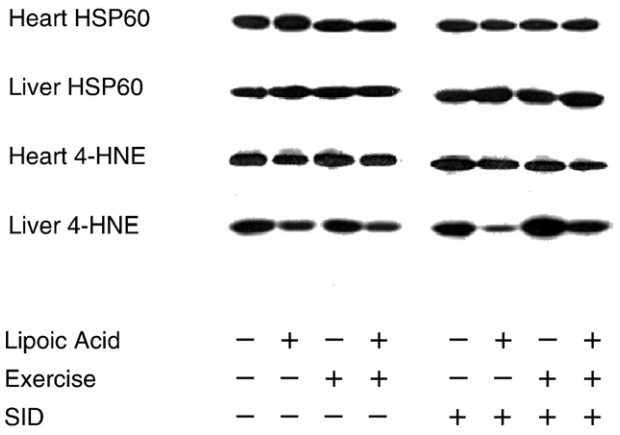


Fig. 3. Western blot images demonstrating tissue HSP60 and 4-HNE protein adducts expression in the heart and liver of rat. Groups are as in Figs. 1 and 2.

diabetes, acute exercise, and lipoate supplementation. $P<.05$ was considered significant.

## 3. Results

The levels of HSP60 in the heart were significantly lower in streptozotocin-induced diabetic (SID) rats than in nondiabetic control animals ($P<.05$, Fig. 1). This decrease was more apparent (43%, $P<.05$) in LA-supplemented diabetic rats than in nondiabetic control animals. However, the levels of HSP60 were higher in the liver of SID animals than in nondiabetic control animals ($P=.014$). In SID animals, increased levels of 4-HNE protein adducts in the liver were detected as a marker of lipid peroxidation ($P<.05$, Fig. 2), but no effect on heart 4-HNE protein adduct levels was observed.

LA increased HSP60 levels significantly in the liver of diabetic and nondiabetic animals ($P<.01$, Fig. 1). The effects of LA supplementation on heart HSP60 levels were less clear (Fig. 1). LA supplementation increased HSP60 levels in the heart of the nondiabetic animals by 49% at rest and by 22% after exhaustive exercise. LA supplementation, however, tended to decrease HSP60 expression in the heart tissue of the diabetic rats. This contrast was significant as an interaction between LA supplementation and diabetes for heart HSP60 levels ($P<.05$).

LA supplementation markedly decreased 4-HNE protein adduct levels in both the heart and liver tissues ($P<.01$ and $P<.001$, respectively, Fig. 2). The inhibition of LA on 4-HNE protein adducts was significant at rest and after exercise in diabetic and control rats.

A single bout of acute exhaustive exercise did not affect HSP60 expression in the heart or liver tissue of the control and diabetic rats (Fig. 1). Acute exhaustive exercise significantly increased 4-HNE protein adducts in the liver ($P<.05$, Fig. 2). Western blot images of HSP60 and 4-HNE protein adducts expression are demonstrated in Fig. 3.

## 4. Discussion

In this study, the effects of the natural thiol antioxidant LA and acute exercise on HSP60 expression in experimental diabetes were tested for the first time. In SID rats, the levels of HSP60 were attenuated in the heart, but induced in the liver. This implicates a deterioration of cardiac cytoprotective capacity in SID rats. In line with our hypothesis, lipoate supplementation increased the expression of HSP60 in the liver of diabetic rats. Supplementation of the antioxidant LA also up-regulated HSP60 levels in myocardial tissue of nondiabetic animals, but this effect was abolished in diabetic animals. This suggests a different mechanism of LA action on the cytoprotective capacity in the liver and heart. These results confirm our previous findings that the expression of HSPs is altered in diabetes, resulting in increased susceptibility of the tissues to injury (Atalay et al., 2004). In line with decreased myocardial HSP60 expression, we recently showed a tendency of another mitochondrial HSP, glucose regulated protein (GRP75), to be decreased in the heart tissue of SID rats (Atalay et al., 2004). The role of HSP60 in the protection of myocardium has been demonstrated (Powers et al., 2001). The capacity of HSP60 to stabilize mitochondrial proteins, promote mitochondrial protein biosynthesis, and prevent the induction of mitochondrial apoptosis seems to be crucial for this function (Gupta & Knowlton, 2005).

Long-lasting hyperglycemia results in the modification of cellular proteins that may alter the expression of HSPs. The levels of the mitochondrial chaperones HSP60 and GRP75 and mitochondrial electron transport proteins have recently been shown to be decreased in the heart tissue mitochondria of SID rats (Turko & Murad, 2003). The increased hepatic HSP60 expression in SID rats might reflect compensation for the impaired protein turnover, accumulation of glycated protein adducts, and impaired cellular defense (Atalay et al., 2004).

An acute bout of exercise had no effect on HSP60 expression, which, to our knowledge, has not been tested before in diabetic rats. Both HSP60 and HSP72 have been demonstrated to be induced by a single submaximal bout of aerobic exercise in human skeletal muscle (Khassaf et al., 2001).

The exercise protocol used induced oxidative stress, as shown by increased levels of 4-HNE protein adducts in liver tissue. This is in agreement with our previous study in which lipid peroxidation, as measured by thiobarbituric acid reactive substances (TBARS), was more pronounced in liver tissues than in other tissues (Gul et al., 2002). Therefore, as the major site of detoxification, liver tissue was more prone to exercise-induced oxidative stress.

Dietary antioxidant supplement of a mixture of beta carotene, vitamins C and E, selenium, and zinc can modify heat shock-induced gene expression in vivo, conferring protection of rat tissues against oxidative stress via

restoration of baseline tissue glutathione (GSH) levels and HSP70 expression (Ushakova et al., 1996). GSH is the most important intracellular thiol antioxidant and a major determinant of the intracellular redox status. Intracellular GSH levels and GSH redox status play a central role in regulating a wide variety of cell responses, including signal transduction, immune regulation, maintenance of protein structure cell proliferation, and apoptosis (Sen & Packer, 2000). No previous information regarding the efficacy of oral LA supplementation on HSP60 expression has been available. In the present study, LA supplementation increased hepatic HSP60 levels in both diabetic and nondiabetic animals. LA increased cardiac HSP60 levels in nondiabetic rats, but not in diabetic rats, suggesting that the diabetic state may decrease the efficacy of LA in up-regulating HSP60 in the heart. We have previously shown in nondiabetic rats that LA supplementation was more effective in increasing total GSH levels in the liver than in heart tissue (Khanna et al., 1999). LA is a naturally occurring antioxidant present in alpha-keto acid dehydrogenase and pyruvate dehydrogenase complexes bound to a protein–lysyl residue, and it functions as a cofactor in oxidative metabolism in mitochondria (Sen & Packer, 2000).

Furthermore, a recent study suggests that insulin deficiency causes HSP60 down-regulation in the cardiac muscle of diabetic mice (Chen et al., 2005). LA is also known as an insulin-mimetic nutrient that stimulates skeletal muscle glucose uptake by the favorable redistribution of glucose transporters (Estrada et al., 1996; Khanna et al., 1999; Sen & Packer, 2000). Moreover, insulin and LA share the same signaling pathway (Estrada et al., 1996). Although the induction of HSP expression could be interpreted as an indicator of sublethal levels of tissue damage (Fehrenbach & Northoff, 2001), the restoration of HSP60 levels after LA supplementation is possibly due to the insulin-mimetic effects of LA on HSP synthesis (Chen et al., 2005), rather than being a consequence of tissue damage.

LA supplementation decreased 4-HNE protein adduct levels in both the liver and heart, suggesting a potent antioxidative action. LA is rapidly reduced to dihydrolipoate (6,8 dithiooctanoic acid, DHLA), which acts as a strong reductant that can regenerate major physiological antioxidants (Sen & Packer, 2000). We have previously shown that Type 1 diabetic men were more susceptible to exercise-induced oxidative stress than were nondiabetic men (Laaksonen et al., 1996). Decreasing the exercise-induced oxidative stress by LA supplementation may enhance the health benefits of physical exercise in diabetics.

In conclusion, experimental diabetes decreased myocardial HSP60 expression and induced lipid peroxidation as measured by 4-HNE protein adducts in the liver. Eight weeks of LA supplementation may offset some of the adverse effects of diabetes by up-regulating tissue HSP60 expression and decreasing oxidative stress. Our results provide further evidence that LA supplementation may enhance antioxidant protection against exercise-induced oxidative stress both in the presence and absence of experimental diabetes.

## Acknowledgments

This work has been supported by the grants from the Finnish Ministry of Education, Yrjö Jahnsson Foundation, Helsinki, Finland, and the High Technology Foundation of Eastern Finland. J.L. is supported by TULES Graduate School, Finland.

## References

Atalay, M., & Laaksonen, D. E. (2002). Diabetes, oxidative stress and physical exercise. *Journal of Sports Science and Medicine*, *1*, 1–14.

Atalay, M., Oksala, N. K., Laaksonen, D. E., Khanna, S., Nakao, C., Lappalainen, J., Roy, S., Hänninen, O., & Sen, C. K. (2004). Exercise training modulates heat shock protein response in diabetic rats. *Journal of Applied Physiology*, *97*, 605–611.

Bellmann, K., Hui, L., Radons, J., Burkart, V., & Kolb, H. (1997). Low stress response enhances vulnerability of islet cells in diabetes-prone BB rats. *Diabetes*, *46*, 232–236.

Birk, O. S., Douek, D. C., Elias, D., Takacs, K., Dewchand, H., Gur, S. L., Walker, M. D., van der Zee, R., Cohen, I. R., & Altmann, D. M. (1996). A role of Hsp60 in autoimmune diabetes: Analysis in a transgenic model. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 1032–1037.

Bruce, C. R., Carey, A. L., Hawley, J. A., & Febbraio, M. A. (2003). Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: Evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism. *Diabetes*, *52*, 2338–2345.

Chen, H. S., Shan, Y. X., Yang, T. L., Lin, H. D., Chen, J. W., Lin, S. J., & Wang, P. H. (2005). Insulin deficiency downregulated heat shock protein 60 and IGF-1 receptor signaling in diabetic myocardium. *Diabetes*, *54*, 175–181.

Estrada, D. E., Ewart, H. S., Tsakiridis, T., Volchuk, A., Ramlal, T., Tritschler, H., & Klip, A. (1996). Stimulation of glucose uptake by the natural coenzyme alpha-lipoic acid/thioctic acid: Participation of elements of the insulin signaling pathway. *Diabetes*, *45*, 1798–1804.

Fehrenbach, E., & Northoff, H. (2001). Free radicals, exercise, apoptosis, and heat shock proteins. *Exercise Immunology Review*, *7*, 66–89.

Gul, M., Atalay, M., & Hanninen, O. (2003). Endurance training and glutathione-dependent antioxidant defense mechanism in heart of the diabetic rats. *Journal of Sports Science and Medicine*, *2*, 52–61.

Gul, M., Laaksonen, D. E., Atalay, M., Vider, L., & Hänninen, O. (2002). Effects of endurance training on tissue glutathione homeostasis and lipid peroxidation in streptozotocin-induced diabetic rats. *Scandinavian Journal of Medicine & Science in Sports*, *12*, 163–170.

Gupta, S., & Knowlton, A. A. (2005). HSP60, Bax, apoptosis and the heart. *Journal of Cellular and Molecular Medicine*, *9*, 51–58.

Khanna, S., Atalay, M., Laaksonen, D. E., Gul, M., Roy, S., & Sen, C. K. (1999). Alpha-lipoic acid supplementation: Tissue glutathione homeostasis at rest and after exercise. *Journal of Applied Physiology*, *86*, 1191–1196.

Khassaf, M., Child, R. B., McArdle, A., Brodie, D. A., Esanu, C., & Jackson, M. J. (2001). Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise. *Journal of Applied Physiology*, *90*, 1031–1035.

Kinnunen, S., Hyyppä, S., Lappalainen, J., Oksala, N., Venojärvi, M., Nakao, C., Hänninen, O., Sen, C. K., & Atalay, M. (2005). Exercise-induced oxidative stress and muscle stress protein responses in trotters. *European Journal of Applied Physiology*, *93*, 496–501.

Kurucz, I., Morva, A., Vaag, A., Eriksson, K. F., Huang, X., Groop, L., & Koranyi, L. (2002). Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance. *Diabetes*, *51*, 1102–1109.

Laaksonen, D. E., Atalay, M., Niskanen, L., Uusitupa, M., Hanninen, O., & Sen, C. K. (1996). Increased resting and exercise-induced oxidative stress in young IDDM men. *Diabetes Care*, *19*, 569–574.

Powers, S. K., Locke, M., & Demirel, H. A. (2001). Exercise, heat shock proteins, and myocardial protection from I-R injury. *Medicine and Science in Sports and Exercise*, *33*, 386–392.

Sen, C. K., & Packer, L. (2000). Thiol homeostasis and supplements in physical exercise. *American Journal of Clinical Nutrition*, *72*, 653S–669S.

Shan, Y. X., Yang, T. L., Mestril, R., & Wang, P. H. (2003). 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. *Journal of Biological Chemistry*, *278*, 45492–45498.

Turko, I. V., & Murad, F. (2003). Quantitative protein profiling in heart mitochondria from diabetic rats. *Journal of Biological Chemistry*, *278*, 35844–35849.

Ushakova, T., Melkonyan, H., Nikonova, L., Mudrik, N., Gogvadze, V., Zhukova, A., Gaziev, A. I., & Bradbury, R. (1996). The effect of dietary supplements on gene expression in mice tissues. *Free Radical Biology & Medicine*, *20*, 279–284.

Wang, R. N., Bouwens, L., & Kloppel, G. (1994). Beta-cell proliferation in normal and streptozotocin-treated newborn rats: Site, dynamics and capacity. *Diabetologia*, *37*, 1088–1096.