EXPERIMENTAL STUDY

Adaptive changes of antioxidant status in development of experimental diabetes

Kucharska J, Gvozdjakova A, Stefek M, Sotnikova R, Sumbalova Z

Pharmacobiochemistry Laboratory, IIIrd Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia. **bll@fmed.uniba.sk**

Abstract

Background: **In clinical and experimental reasearch, attention is paid to the role of antioxidant defense systems in the prevention of diabetic complications. Little information is available about regulation of the endogenous level of antioxidants in the state of chronic oxidative stress in relation to the development of diabetes, and particularly about coenzyme Q as one of the most important endogenous antioxidants with an irreplaceable function in mitochondrial bioenergetics.**
Purpose: **To examine changes in concentrations of two important lipophilic antioxidants, coenzyme Q_9 and α-tocopherol, in rat tissues during the development of experimental diabetes.**
Methods: **Experimental diabetes in male Wistar rats, was induced by streptozotocin in the dose of 55 mg/kg intravenously. Coenzyme Q_9 (CoQ_9) and α-tocopherol (α-toc.) were determined in myocardial and skeletal muscles and in kidney tissue after 1, 6 and 8 months of diabetes duration by the method of high-performance liquid chromatography.**
Results: **Myocardial CoQ_9 content increased progressively in the course of diabetes development by 14, 29 and 61 %, while in skeletal muscles and kidney, the increases were not dependent on the duration of diabetes. The content of α-toc. increased in the myocardium after 8 months of diabetes duration, in kidney tissue and skeletal muscles, it did not change in comparison with control rats.**
Conclusions: **An increased content of the lipophilic antioxidants coenzyme Q_9 and α-tocopherol in tissues of diabetic rats is regarded as an adaptation of the antioxidant defense system to chronic oxidative stress. The exact mechanisms of accumulation of these antioxidants in diabetic tissues could be elucidated by studies investigating their relation to changes in lipid content and to the total of bioenergetic and antioxidant capacities.** ***(Fig. 3, Ref. 25.)***
Key words: experimental diabetes, coenzyme Q, α-tocopherol, adaptation.

In clinical and experimental research, great attention is paid to the importance of antioxidant defense systems in the pathogenesis of diabetes and in the prevention of diabetic complications. Oxidative stress based on the disbalance between the production of reactive free radicals and the level of endogenous antioxidants can be either the primary cause of pancreatic ß-cells damage and the development of diabetes or it can contribute to degenerative changes in tissues based on damaged bioenergetic mitochondrial functions leading to the development of cardiomyopathies, myopathies, nephropathies, neuropathies (Luft and Landau, 1995; Wallace et al, 1995). Disturbances in mitochondrial oxidative phosphorylation in pancreatic ß-cells based on oxidative damage of mitochondrial DNA (mtDNA) can affect their response to the secretion of insulin (Kennedy et al, 1998). Despite increasing information about the importance of antioxidants, mechanisms of their regulation in diseases associated with increased oxidative stress, including diabetes mellitus, have not been sufficiently explained so far and results are often contro-

Pharmacobiochemistry Laboratory, IIIrd Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, and Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava

Address for correspondence: J. Kucharska, PharmDr, PhD, Pharmacobiochemistry Laboratory, IIIrd IK LFUK, Hlboka 7, SK-811 05 Bratislava 1, Slovakia.
Phone/Fax: +421.2.52491422

Thanks for technical assistance to V. Jeskova, A. Stetkova, P. Zauskova. This work was financially supported by grants VEGA 2/6026/99 and 1/7547/20.

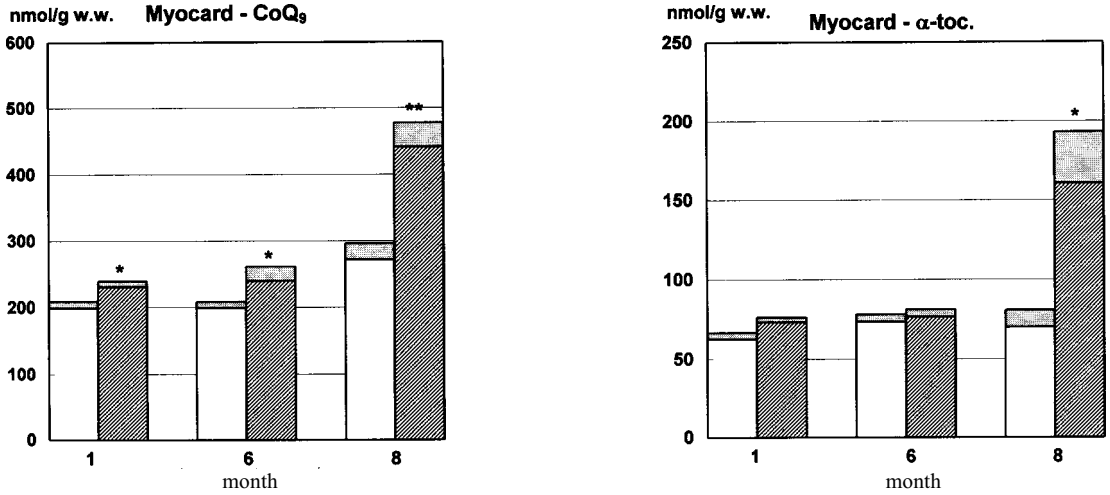

Fig. 1. Concentrations of coenzyme Q_9 and α–tocopherol in myocard of the rats after 1, 6 and 8 months of diabetes duration. Legend: statistical significance $^{*}p<0.05$, $^{**}p<0.005$.

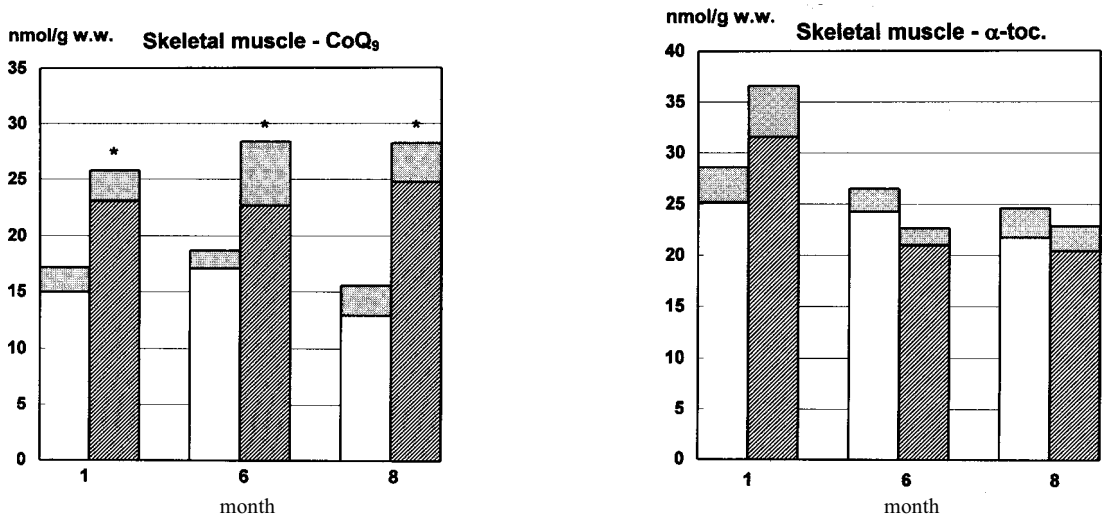

Fig. 2. Concentrations of coenzyme Q_9 and α-tocopherol in skeletal muscle of the rats after 1, 6 and 8 months of diabetes duration. Legend: statistical significance $^{*}p<0.05$.

versial. Due to ethical reasons, the studies of pathobiochemical mechanisms of diabetes at the level of organs and tissues can be conducted mainly on experimental models of diabetes. Based on literary data as well as on our previous clinical and experimental studies (Gvozdjáková et al, 1997; Kucharská et al, 2000; Štefek et al, 2000), we suppose that especially disturbances in the biosynthesis of coenzyme Q, its distribution and mainly in the changes in its function within mitochondria could be important factors affecting the development of diabetic complications. Coenzyme Q (ubiquinone) as part of the respiratory chain has a unique function in mitochondrial bioenergetics, which as a matter of fact is damaged in diabetes. It participates in the transport of electrons in the mitochondrial respiratory chain as well as in the transport of hydrogen protons in the Q-cycle, producing the protonmotive gradient necessary for ATP synthesis. Coenzyme Q occurs in three chemical forms — oxidized, reduced and as a semiquinone radical (Mitchell, 1991). Mainly in its reduced form, it has antioxidant properties, and by reaction with α-tocopherol radical, it contributes to regeneration of vitamin E in membranes and lipoproteins (Kagan et al, 2000; Thomas and Stocker, 2000). Coenzyme Q is the only lipophilic antioxidant which is synthesized in animal cells, its homologues are species dependent — in rats, the dominant homologue is coenzyme Q_9 and in humans it occurs almost entirely in the form of coenzyme Q_{10}.

Deficit of coenzyme Q_{10} is associated with the development of cardiomyopathies and heart failure (Mortensen et al, 1991; Folkers, 1993; Gvozdjáková et al, 1996; Kucharská et al, 1996). Decreased levels of coenzyme Q_{10} can be associated with the development of myopathies in diabetic patients. Since skeletal muscles have an important role in glucose metabolism, a decre-

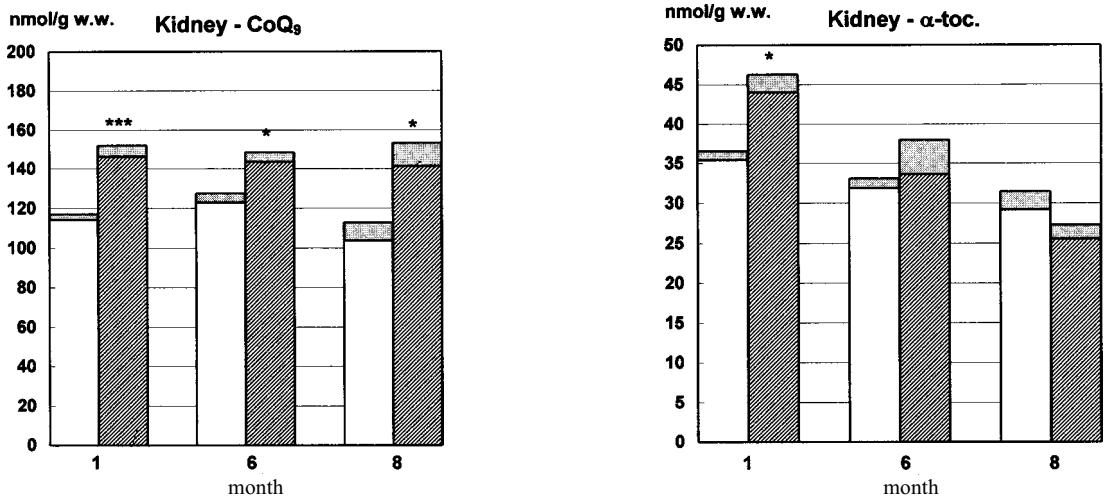

Fig. 3. Concentrations of coenzyme Q_9 and α-tocopherol in kidney of the rats after 1, 6 and 8 months of diabetes duration. Legend: statistical significance $^{*}p<0.01$, $^{***}p<0.0001$.

ase in the amount of CoQ_{10} might lead to impairing of muscle tissue function and consequently to insulin resistance (Eriksson et al, 1999). An increased production of free radicals and a deficit in antioxidants, mainly that of CoQ_{10}, probably participate in the development of nephropathies (Gazdíková et al., 2000). The aim of the presented work was to establish whether changes in the antioxidant status were depend also on the duration of diabetes. We investigated the content of coenzyme Q_9 and α-tocopherol in the myocardium, skeletal muscles and kidney tissue of rats after 1, 6 and 8 months of experimental diabetes induced by streptozotocin.

Methods

Male Wistar rats, 8—9 weeks old, weight 200—230 g, were used in experiments. The animals were fed with standard diet and tap water ad libitum. Experimental diabetes was induced by a single intravenous dose of streptozotocin (Sigma, USA) 55 mg/kg of the body weight. All animals with plasma glucose >20 mmol/l were considered diabetic and were included into the study. Tissue samples from the myocardium, skeletal muscle and kidney were taken after 1, 6 and 8 months of diabetes duration. After homogenization and extraction by a mixture of hexane — ethanol with addition of butylhydroxytoluene and sodium dodecyl sulphate, we determined concentrations of CoQ_9 and α-tocopherol by a modified method of high-performance liquid chromatography (HPLC) with spectrophotometric detection (Takada et al, 1982; Lang et al, 1986; Kucharská et al, 1996) using external standards (Sigma, USA). Concentrations of compounds were calculated in nmol/g of wet weight. The experimental groups consisted of 6—16 animals and were compared with healthy rats in the same age range. The results were evaluated using Student's t-test for unpaired data; $p<0.05$ was considered statistically significant.

Results

In diabetic rats, the myocardial CoQ_9 content increased progressively in dependence on diabetes duration: after 1 month by 14 %, after 6 months by 29 % and after 8 months by 61 %; all changes were statistically significant in comparison with controls (Fig. 1). In skeletal muscle and kidney tissue the content of CoQ_9 increased significantly from the first month of diabetes and the levels maintained elevated up to the 8th month (Figs 2 and 3). Myocardial α-tocopherol in diabetic rats did not change after 1 and 6 months of diabetes duration and was significantly increased after 8 months (Fig. 1). On the other hand α-tocopherol concentration in kidney tissue increased significantly after 1 month and the concentrations kept unchanged after 6 and 8 months (Fig. 3). In skeletal muscle no significant changes were found in the content of α-tocopherol (Fig. 2).

Discussion

It is generally accepted that reactive oxygen species participate in the development of chronic diabetic complications. There are still unresolved questions of the functional capacity of antioxidant systems in diabetes and mainly that of the mechanisms of their regulation in the state of chronic oxidative stress. Conclusions of clinical studies are virtually in agreement, particularly that increased oxidative stress and decreased antioxidant capacity contribute to the progression of complications in diabetes. Results of experimental studies are often controversial, probably in consequence of different experimental models, duration of diabetes as well as in result of antioxidants or antioxidant enzymes examined. In our study, we found that during the development of diabetes, the changes in the amount of antioxidants were time dependent and there were also differences between the levels of coenzyme Q_9 and α-tocopherol. Myocardial concentration of α-tocopherol did not change after 1 and 6 months,

and it increased significantly only after 8 months of diabetes duration in comparison with control rats. On the other hand, α-tocopherol increased in kidney tissue after 1 month, and other changes were not significant. In skeletal muscle, the content of α-tocopherol was unchanged. In spite of increased concentrations of α-tocopherol after 8 months of diabetes duration, myocardial tissue exhibited an increased sensitivity to oxidative damage, as found in our previous study (Štefek et al, 2000). According to some authors (Thomas and Stocker, 2000), there can be a manifested prooxidant effect of high concentrations of α-tocopherol and a deficit in reduced CoQ. Increased concentrations of α-tocopherol were found also in isolated myocardial and liver mitochondria of diabetic rats, simultaneously with increased lipoperoxidation as a manifestation of oxidative stress (Kucharská et al, 2000). More important changes in diabetes development occur probably in the metabolism of coenzyme Q, which is the only lipophilic antioxidant synthesized in animal cells. Therefore the content of CoQ is dependent not only on exogenous intake and distribution within organism but also on its endogenous biosynthesis and degradation. In the myocardium of diabetic rats, the content of coenzyme Q_9 increased after 1 month significantly and this increase remained practically on the same level after 6 and 8 months of diabetes duration. We suppose that the increased content of CoQ_9 may manifest the adaptation of antioxidant defense systems in diabetes, while different changes in different organs can be caused by different antioxidant capacities and abilities of adaptation. Increased activities of antioxidant enzymes and the level of antioxidants in models of experimental diabetes were reported also by other authors (Jain and Levine, 1995; Volkovová et al, 1997; Štefek et al, 2000). Adaptive changes are operative also in other regulative mechanisms, e.g. in the development of diabetic cardiomyopathy, an increased tolerance to calcium overload was found (Ziegelhoffer et al, 1997). To date, little attention has been paid to the role of coenzyme Q in diabetes. Clinical studies examined only coenzyme Q_{10} levels in blood of diabetic patients or the effects of coenzyme Q_{10} supplementation (Andersen et al, 1997; Gvozdjáková et al, 1998; Eriksson et al, 1999; Tomasetti et al, 1999). In our previous study (Kucharská et al, 2000), we found decreased concentrations of CoQ_9 in isolated myocardial and liver mitochondria of rats after 8 weeks of diabetes duration, and we assumed that it may be the one of the reasons of disturbances in oxidative phosphorylation occurring in diabetes. The increased content of CoQ_9 and α-tocopherol in tissues of diabetic rats found in this work may represent an adaptation to chronic oxidative stress rather than a manifestation of increased antioxidant activity. This assumption is supported by previous findings (Štefek et al, 2000) when in an 8-month model of diabetes, simultaneously with the increased content of myocardial antioxidants, there was an increase in lipoperoxidation in myocardial tissue. Supplementation of diabetic rats with synthetic antioxidant stobadine normalized the level of antioxidants, which can be regarded as a manifestation of normalized oxidative status of the diabetic heart. Similarly as α-tocopherol, coenzyme Q has a prooxidant effect, as can be shown in pathological conditions (Nohl et al, 2000). Since α-tocopherol and coenzyme Q belong to lipophilic antioxidants, we can suppose that their accumulation in diabetic tissues is associated with lipolysis and increased storage of lipophilic substances in tissues, mainly in the liver. These assumptions have not been sufficiently confirmed yet. In the rat liver, after 8 weeks of diabetes duration, no increased levels of cholesterol and triglycerides were found (Uličná et al, 1996). Other authors (Jain and Levine, 1995) do not suppose that accumulation of vitamin E in heart ventricles of diabetic rats is associated with lipolysis, since insulin treatment did not lower the vitamin E level. Sukalski et al (1993) found no changes of α-tocopherol level in kidney mitochondria of diabetic rats. There are no references about coenzyme Q in tissues in experimental models of diabetes. Studies investigating the level of antioxidants in relation to changes in lipid content and to the total antioxidant capacity of tissues may contribute to elucidation of mechanisms involved in the regulation of antioxidant levels in diabetes. Prospective reasearch in the area of diabetes will include studies on disturbances in the function of mitochondria, where coenzyme Q directly affects their bioenergetic and antioxidant capacities. Clinical evidence of mitochondrial damage based on oxidative stress allows to classify diabetes mellitus also as a mitochondrial disease in the new developing area of mitochondrial medicine.

References

1. Andersen CB, Henriksen JE, Hother-Nielsen O, Vaag A, Mortensen SA, Bech-Nielsen H: The effect of coenzyme Q_{10} on blood glucose and insulin requirement in patients with insulin dependent diabetes mellitus. Mol Aspects Med 1997; 18 (Suppl): 307—309.

2. Eriksson JG, Forsén TJ, Mortensen SA, Rohde M: The effect of coenzyme Q_{10} administration on metabolic control in patients with type 2 diabetes mellitus. BioFactors 1999; 9: 315—318.

3. Folkers K: Heart failure is a dominant deficiency of coenzyme Q_{10} and challenges for future clinical research on CoQ_{10}. Clin Invest 1993; 71 (Suppl): 51—54.

4. Gazdikova K, Gvozdjakova A, Kucharska J, Spustova V, Braunova Z, Dzurik R: Hladiny malondialdehydu (MDA) a vabraných antioxidantov v plazme u konzervatívne liečených pacientov s chorobami obličiek. Bratisl Lek Listy 2000; 101 (9): 490—494.

5. Gvozdjakova A, Kucharska J, Mizera S, Snircova M, Schreinerova Z, Schramekova E, Pechan I, Fabian J: Metabolické štúdie mitochondrií v biopsiách myokardu u pacientov s kardiopatiami nejasného pôvodu. Bratisl Lek Listy 1996; 97 (6): 348—350.

6. Gvozjakova A, Kucharska J, Braunova Z, Kolesar P: Beneficial effect of coenzyme Q_{10} on the antioxodative status and metabolism of fats and sugars in diabetic patients. First Conference of the International Coenzyme Q_{10} Association, Boston 1998, 153 p.

7. Jain SK, Levine SN: Elevated lipid peroxidation and vitamin E-quinone levels in heart ventricles of streptozotocin-treated diabetic rats. Free Radic Biol Med 1995; 18: 337—341.

8. Kagan VE, Fabisiak YP, Tyurina YY: Independent and concerted antioxidant functions of coenzyme Q. P. 119—129. In: Kagan VE, Quinn DJ (Eds): Coenzyme Q: Molecular mechanisms in health and disease. CRC Press LLC 2000.

9. Kennedy ED, Maechler P, Wollheim CB: Effects of depletion of mitochondrial DNA in metabolism secretion coupling in INS-1 cells. Diabetes 1998; 47: 374—380.

10. Kucharska J, Gvozdjakova A, Snircova M, Mizera S, Schramekova E, Schreinerova Z, Pechan I, Fabian J: Stanovenie hladín koenzýmu Q_{10} a alfatokoferolu u pacientov s kardiopatiami nejasného pôvodu: perspektívne diagnostické možnosti. Bratisl Lek Listy 1996; 97: 351—354.

11. Kucharska J, Braunova Z, Ulicna O, Zlatos L, Gvozdjakova A: Deficit of coenzyme Q in heart and liver mitochondria of rats with streptozotocin-induced diabetes. Physiol Res 2000; 49: 411—418.

12. Lang JK, Gohil K, Packer L: Simultaneous determination of tocopherol, ubiquinols, and ubiquinones in blood, plasma, tissue homogenates, and subcellular fractions. Anal Biochem 1986; 157: 106—116.

13. Luft R, Landau BR: Mitochondrial medicine. J Int Med 1995; 238: 405—421.

14. Mitchel P: The vital protonmotive role of coenzyme Q. P. 3—10. In: Folkers K, Littarru GP, Yamagami T (Eds): Biomedical and clinical aspects of coenzyme Q. Amsterdam, Elsevier 1991.

15. Mortensen SA, Kondrup P, Folkers K: Myocardial deficiency of coenzyme Q_{10} and carnitine in cardiomypathy. P. 269—281. In: Folkers K, Littarru GP, Yamagami T (Eds): Biomedical and clinical aspects of coenzyme Q. Amsterdam, Elsevier 1991.

16. Nohl H, Gille L, Kozlov AV, Staniek K: Coenzyme Q as a generator of reactive oxygen metabolites. P. 163—180. In: Kagan VE, Quinne DJ (Eds): Molecular mechanisms in health and disease. CRC Press LLC 2000.

17. Stefek M, Sotnikova R, Okruhlicova L, Volkovova K, Kucharska J, Gajdosik A, Gajdosikova K, Mihalova D, Hozova R, Tribulova N, Gvozdjakova A: Effect of dietary supplementation with pyridoindole antioxidant stobadine on antioxidant state and ultrastructure of diabetic rat myocardium. Acta Diabetol 2000; 37: 111—117.

18. Takada M, Ikenoya S, Yuzuriha T, Katayama K: Studies on reduced and oxidized coenzyme Q. The determination of oxidation-reduction levels of coenzyme Q in mitochondria, microsomes and plasma by high-performance liquid chromatography. Biochim Biophys Acta 1982; 679: 308—314.

19. Thomas RS, Stocker R: Mechanism of antioxidant action of ubiquinol-10 for low density lipoprotein. P. 131—145. In: Kagan VE, Quinne DJ (Eds): Molecular mechanisms in health and disease. CRC Press LLC 2000.

20. Tomasetti M, Aleeva R, Solenghi MD, Littarru GP: Distribution of antioxidants among blood components and lipoprotein: significance of lipids/CoQ_{10} ratio as a possible marker of increase risk for atherosclerosis. BioFactors 1999; 9: 231—240.

21. Torres MD, Canal JR, Pérez C: Oxidative stress in normal and diabetic rats. Physiol Res 1999; 48: 203—208.

22. Ulicna O, Volkovova K, Istvanova B: Bioenergetika mitochondrií pečene potkana pri experimentálnom inzulíndependentnom diabete. Bratisl Lek Listy 1996; 97 (10): 619—624.

23. Volkovova K, Ziegelhoffer A, Staruchova M, Ondreicka R: Vzťah medzi niektorými biochemickými ukazovateľmi v krvi a v tkanive myokardu a toleranciou srdca k vápniku v rôznych štádiách experimentálneho diabetu. Cardiol 1997; 6 (4): 152—157.

24. Wallace DC, Shaffner JM, Trounce I, Broen MD, Ballinger MP, Coral-Debrinski M, Horton T, Sun AS, Lott MT: Mitochondrial DNA mutations in human degenerative disease and aging. Biochim Biophys Acta 1995; 127: 141—151.

25. Ziegelhoffer A, Ravingerova T, Styk T, Sebokova J, Waczulikova I, Breier A, Dzurba A, Volkovova K, Carsky J, Turecky L: Mechanisms that may be involved in calcium tolerance of the diabetic heart. Mol Cell Biochem 1997; 176: 191—198.

Received June 21, 2001.
Accepted October 12, 2001.