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Deficit of Coenzyme Q in Heart and Liver Mitochondria of Rats with Streptozotocin-Induced Diabetes

J. KUCHARSKÁ[1], Z. BRAUNOVÁ[1], O. ULIČNÁ[1], L. ZLATOŠ[2], A. GVOZDJÁKOVÁ[1]

[1]Pharmacobiochemical Laboratory and [2]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

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Summary

Mitochondrial dysfunction and oxidative stress participate in the development of diabetic complications, however, the mechanisms of their origin are not entirely clear. Coenzyme Q has an important function in mitochondrial bioenergetics and is also a powerful antioxidant. Coenzyme Q (CoQ) regenerates alpha-tocopherol to its active form and prevents atherogenesis by protecting low-density lipoproteins against oxidation. The aim of this study was to ascertain whether the experimentally induced diabetes mellitus is associated with changes in the content of endogenous antioxidants (alpha-tocopherol, coenzymes Q_9 and Q_{10}) and in the intensity of lipoperoxidation. These biochemical parameters were investigated in the blood and in the isolated heart and liver mitochondria. Diabetes was induced in male Wistar rats by a single intravenous injection of streptozotocin (45 $mg.kg^{-1}$), insulin was administered once a day for 8 weeks (6 $U.kg^{-1}$). The concentrations of glucose, cholesterol, alpha-tocopherol and CoQ homologues in the blood of the diabetic rats were increased. The CoQ_9/cholesterol ratio was reduced. In heart and liver mitochondria of the diabetic rats we found an increased concentration of alpha-tocopherol, however, the concentrations of CoQ_9 and CoQ_{10} were decreased. The formation of malondialdehyde was enhanced in the plasma and heart mitochondria. The results have demonstrated that experimental diabetes is associated with increased lipoperoxidation, in spite of the increased blood concentrations of antioxidants alpha-tocopherol and CoQ. These changes may be associated with disturbances of lipid metabolism in diabetic rats. An important finding is that heart and liver mitochondria from the diabetic rats contain less CoQ_9 and CoQ_{10} in comparison with the controls. We suppose that the deficit of coenzyme Q can participate in disturbances of mitochondrial energy metabolism of diabetic animals.

Key words
Diabetes mellitus • Mitochondria • Alpha-tocopherol • Coenzyme Q • Oxidative stress

Introduction

Diabetes mellitus (DM) belongs to the chronic diseases associated with increased production of free oxygen radicals. Up to now, it is not clear whether increased oxidative stress is a primary or a secondary indicator of tissue damage in the pathogenesis of diabetic complications (Bayness and Thorpe 1999). Mitochondrial deoxyribonucleic acid (mtDNA) mutations caused by an increased production of free oxygen radicals may lead to

Fax+420 2 293 894
E-mail: physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

the disturbance of mitochondrial bioenergetics (Luft 1995). This may be an important factor in the development of mitochondrial disorders in patients with diabetes mellitus. A significant role in the origin of some complications associated with type 1 and type 2 diabetes (such as cardiomyopathy and hepatopathy) may be played by the consequences of a decreased capacity of oxidative phosphorylation and increased lipoperoxidation. These are the same mechanisms as those occurring at the origin of the degenerative changes associated with the aging process (Linnane 1992, Lawen *et al.* 1994, Luft and Landau 1995). The disturbances of mitochondrial bioenergetics can arise either primarily as a result of mtDNA mutations by free oxygen radicals, or secondarily, due to the lack of substrates and cofactors in the respiratory chain. The intramitochondrial oxidative stress is thus increased as a result of these disturbances (Shoffner and Wallace 1994, Luft and Landau 1995, Wallace *et al.* 1995).

Endogenous antioxidants are important for maintaining the balance between oxidant and antioxidant processes. Coenzyme Q is regarded as one of the most important antioxidants since its biosynthesis decreases with age and its deficit in tissues is associated with degenerative changes appearing in the course of aging (Beyer *et al.* 1985, Kalén *et al.* 1989). Besides its antioxidative properties, coenzyme Q (ubiquinone) has an important function in mitochondrial bioenergetics. It participates as a cofactor of dehydrogenases in the transport of electrons and protons as well as in ATP production (Mitchell 1991, Crane and Navas 1997). The deficit of CoQ_{10} is regarded as the reason for deterioration of the bioenergetics and function of the heart muscle in patients with cardiomyopathies and after heart transplantation (Mortensen *et al.* 1991, Folkers 1993, Kucharská *et al.* 1998, Gvozdjáková *et al.* 1999). The risk of atherogenesis and the development of other degenerative changes is enhanced by a decreased antioxidant capacity of the plasma. The risk is increased in diabetic patients because their low density lipoproteins (LDLs) are more sensitive to oxidation (Reaven 1995, Aguirre *et al.* 1998, Samiec *et al.* 1998). Lipophilic antioxidants alpha-tocopherol and coenzyme Q_{10} can play an important role in the prevention of oxidative modification of LDLs. Supplementation with these antioxidants leads to the several fold increase of their concentrations in the lipoproteins. In comparison with alpha-tocopherol supplementation LDLs enriched with coenzyme Q_{10} are more resistant against peroxidation. Coenzyme Q_{10} prevents from prooxidant effect of alpha-tocopherol (Stocker *et al.* 1991, Thomas *et al.* 1997).

In our previous study we found decreased levels of coenzyme Q_{10} and β-carotene and increased lipoperoxidation in the plasma of patients with type 1 and type 2 diabetes mellitus (Gvozdjáková *et al.* 1997). The purpose of this study was to investigate the concentrations of alpha-tocopherol, coenzymes Q_9 and Q_{10} in the blood and also in the isolated heart and liver mitochondria of rats with streptozotocin-induced diabetes mellitus. Besides this, we also studied the malondialdehyde formation as an indicator of lipoperoxidation.

Material and Methods

Our experiments were performed on male Wistar rats weighing 250-280 g fed with a standard Larsen diet and water *ad libitum.* The animals were divided into two groups:

Control animals received daily subcutaneous injection of an isotonic saline solution.

Diabetes mellitus was induced in the *experimental animals* by a single intravenous injection of streptozotocin in a dose of 45 mg . kg^{-1}. Insulin was administered once a day subcutaneously for 8 weeks in a dose of 6 $U.kg^{-1}$.

The animals were sacrificed by decapitation, the blood samples were collected and used for determination of glucose (Lachema), cholesterol (Bálint 1962), alpha-tocopherol, coenzyme Q homologues and malon-dialdehyde (MDA). The hearts and livers were placed in an ice-cold isolation solution containing (in $mmol.l^{-1}$) 225 manitol, 75 sucrose and 0.2 EDTA. After homogenization with a teflon pestle, heart and liver mitochondria were isolated by differential centrifugation (Sarma *et al.* 1976, Palmer *et al.* 1977). After extraction, the concentrations of alpha-tocopherol, coenzymes Q_9 and Q_{10} in the blood and mitochondria were determined by a modified method of high-performance liquid chromatography (Takada *et al.* 1982, Lang *et al.* 1986, Kucharská *et al.* 1996). Concentrations of malondialdehyde in the plasma and mitochondria were determined by the reaction with thiobarbituric acid (TBA) spectrophotometrically at 532 nm (Ohkawa *et al.* 1979). Mitochondrial proteins were estimated by the method of Lowry *et al.* (1951). The results were evaluated using Student's t-test for unpaired data, $P<0.05$ was considered as statistically significant.

Results

In comparison with the control rats, the concentrations of glucose and cholesterol in the blood of the diabetic rats were significantly increased (Table 1). The blood concentrations of alpha-tocopherol, coenzymes Q_9 and Q_{10} in the diabetic rats were also significantly higher than those in the controls. In the diabetic animals, however, the standardized coenzyme Q expressed as the CoQ_9/cholesterol ratio was decreased (Table 2). Malondialdehyde formation, considered as an indicator of lipoperoxidation, was significantly increased in the plasma and in heart mitochondria of the diabetic rats (Table 3). In the diabetic rats, the content of alpha-tocopherol in heart and liver mitochondria was elevated, whereas the content of both coenzyme Q homologues (CoQ_9 and CoQ_{10}) in these mitochondria was significantly decreased (Tables 4 and 5).

Table 1. Blood glucose and plasma cholesterol concentrations in control and diabetic rats.

	Glucose ($mmol.l^{-1}$)	**Cholesterol ($mmol.l^{-1}$)**
Controls	6.52±0.15 (11)	1.41±0.05 (8)
Diabetes	16.11±0.99 (12)***	2.79±0.22 (10)*

*Data are means ±S.E.M (n), * $p<0.05$, *** $p<0.001$.*

Table 2. Blood alpha-tocopherol, coenzyme Q_9 and Q_{10} concentrations and CoQ_9/cholesterol ratio in control and diabetic rats.

	α-tocopherol ($\mu mol.l^{-1}$)	**CoQ_9 ($\mu mol.l^{-1}$)**	**CoQ_{10} ($\mu mol.l^{-1}$)**	**CoQ_9 / cholesterol ($\mu mol.mmol^{-1}$)**
Controls (n=11)	4.55±0.32	0.297±0.020	0.074±0.005	0.211±0.006
Diabetes (n=12)	9.87±0.91****	0.395±0.028*	0.381±0.039****	0.142±0.008****

*Data are means ±S.E.M, * $p<0.05$, **** $p<0.0001$.*

Discussion

The data concerning a deficit of mitochondrial function in the diabetic rats under various experimental conditions were published by a number of authors (Hall *et al.* 1960, Mackerer *et al.* 1971, Pierce and Dhalla 1985, Tomita *et al.* 1996, Tanaka *et al.* 1992). In the same experimental model of diabetes mellitus (Uličná *et al.* 1996), decreased efficacy of oxidative phosphorylation was found in liver mitochondria. This decrease was more marked in the group with 8-week duration of diabetes than in the group with diabetes lasting 8 days. Damaged bioenergetics in heart and liver mitochondria was also demonstrated in 3-month-old rats with neonatally induced diabetes (Zlatoš *et al.* 1997, Uličná *et al.* 1999). The mechanisms of mitochondrial function damage are mostly related to the oxidative stress. Assuming that free oxygen radicals production is increased in diabetes, we investigated the concentrations of alpha-tocopherol and coenzyme Q homologues – naturally occurring mitochondrial antioxidants in our experimental study. Glucose concentration in the blood of diabetic rats was 2.5 times higher than in the blood of control animals. In the diabetic rats, the disturbances of lipid metabolism were manifested by significantly increased plasma cholesterol concentrations (Table 1). In spite of the increase of mitochondrial alpha-tocopherol concentration the heart mitochondria of the diabetic rats were exposed to oxidative stress as was indicated by the increased malondialdehyde production (Tables 3 and 4). In comparison with the controls, the mitochondrial alpha-tocopherol concentration in the liver of diabetic rats was also significantly increased (Table 5).

Table 3. Malondialdehyde concentrations in the plasma and heart mitochondria of control and diabetic rats.

	MDA - plasma ($\mu mol.l^{-1}$)	MDA - mitochondria ($nmol.mg\ prot.^{-1}$)
Controls	7.13±0.39 (11)	24.9±2.19 (8)
Diabetes	10.3±0.51 (12)****	57.3±2.97 (10)****

*Data are means ± S.E.M (n), * p<0.05, ****p<0.0001.*

The increased concentration of alpha-tocopherol in the liver mitochondria of rats with 4 weeks' persisting diabetes was also found by Sukalski *et al.* (1993). However, these mitochondria were less susceptible to *in vitro* oxidative damage. Increased liver alpha-tocopherol could not be normalized in the diabetic rats by restricted intake of this vitamin. The authors supposed that a decreased susceptibility of liver mitochondria to oxidative damage may be lost with the progression of the disease. An accumulation of vitamin E and increased lipoperoxidation in the heart ventricles were found in the rats with 2 months' persisting diabetes. Insulin treatment of the diabetic rats did not cause any change in the vitamin E levels but it did prevent an increase of lipid peroxidation (Jain and Levine 1995).

Table 4. Alpha-tocopherol, coenzyme Q_9 and Q_{10} concentrations in heart mitochondria of control and diabetic rats.

	α-tocopherol ($nmol.mg\ prot.^{-1}$)	CoQ_9 ($nmol.mg\ prot.^{-1}$)	CoQ_{10} ($nmol.mg\ prot.^{-1}$)
Controls (n=8)	0.57±0.18	7.71±0.54	0.83±0.048
Diabetes (=10)	1.14±0.05**	5.75±0.41**	0.48±0.024***

*Data are means ± S.E.M., * p<0.05, **p<0.01, ***p<0.001.*

Table 5. Alpha-tocopherol, coenzyme Q_9 and Q_{10} concentrations in liver mitochondria of control and diabetic rats.

	α-tocopherol ($nmol.mg\ prot.^{-1}$)	CoQ_9 ($nmol.mg\ prot.^{-1}$)	CoQ_{10} ($nmol.mg\ prot.^{-1}$)
Controls (n=8)	0.244±0.23	2.45±0.54	0.225±0.016
Diabetes (=10)	1.07±0.18**	0.62±0.13**	0.132±0.018**

*Data are means ± S.E.M., * p<0.05, **p<0.01.*

As has already been mentioned, coenzyme Q is a part of the mitochondrial respiratory chain, as well as an important endogenous antioxidant. However, data about the mitochondrial concentrations of coenzyme Q in diabetes mellitus are lacking. In the present study, we found that the concentrations of coenzyme Q_9 and coenzyme Q_{10} were significantly decreased in heart and liver mitochondria of the diabetic rats. These results indicate that the deficit of coenzyme Q in diabetes could be a reason for deteriorated mitochondrial function and could contribute to the increase of mitochondrial oxidative stress. Lenaz *et al.* (1994) demonstrated that lipoperoxidation is accompanied by reduced mitochondrial coenzyme Q concentrations concomitantly with the decreased activities of respiratory chain enzymes, such as NADH- and succinate oxidases. These authors also found negative correlation between coenzyme Q and cholesterol in healthy rats. Forsmark-Andrée *et al.* (1997) suppose that ubiquinone (coenzyme Q) is oxidatively modified and destroyed during

lipoperoxidation to a form which can no longer function as a component of the respiratory chain. After diabetes of 3 months' duration in rats, Kristal *et al.* (1997) found disturbances in the Q-cycle of complex III of the respiratory chain. These disturbances were characterized by electron leakage and increased production of free oxygen radicals. Mitochondrial dysfunctions may contribute to diabetic complications by producing potentially toxic free radicals and diffusible prooxidants.

In our experimental conditions, important changes also occurred in the concentrations of investigated antioxidants in the blood of diabetic rats. We found increased concentrations of alpha-tocopherol and both coenzyme Q homologues. The blood concentrations of lipophilic antioxidants can also be expressed as the ratio of their concentration to the concentration of cholesterol. In the diabetic rats, the CoQ_9/cholesterol ratio was significantly lower. The decrease of this ratio can be an indicator of insufficient antioxidant capacity in spite of the increased blood concentration of coenzyme Q. This assumption supports the fact that the malondialdehyde formation working as an indicator of lipoperoxidation was increased in the diabetic rats. Coenzyme Q_9 is a dominant form of coenzyme Q in rats. Its concentration in mitochondria is about 10 times higher than the concentration of coenzyme Q_{10}. In the blood, the concentrations of CoQ_{10} in control rats are close to the near detection limit and we therefore regard changes in the CoQ_9 and CoQ_9/cholesterol ratio as more significant. We suppose that the changes of lipophilic antioxidant concentrations in diabetic rats are associated with disturbances of lipid metabolism.

For ethical reasons, the study of pathological mechanisms of the development of mitochondrial disorders is possible only in experimental models. However, changes in the relation between the plasma antioxidant capacity and lipoperoxidation, considered as the cause of a number of diabetic complications, is also a topical theme for research in clinical diabetology. In previous study, we found that the blood levels of coenzyme Q_{10} and β-carotene were diminished in patients with type 1 and type 2 diabetes (Gvozdjáková *et al.* 1997). The blood concentration of alpha-tocopherol in patients with type 1 diabetes was in the range of reference values, however, it was slightly increased. Plasma malondialdehyde concentrations were increased in both groups of diabetic patients. After 6 weeks' treatment of these patients with 30 mg of coenzyme Q_{10} in the form of a dietary nutritional supplement, the blood level of coenzyme Q_{10} significantly increased, HDL-cholesterol increased only slightly and the total and LDL-cholesterol slightly decreased. Glycemia, glycated hemoglobin, liver enzymatic activities and kidney functions were not affected. The patients reported an improved vitality and a better quality of life.

Positive correlation between coenzyme Q_{10} and cholesterol in the plasma was documented in healthy men (Johansen *et al.* 1991). Caye-Vaugien *et al.* (1990) found increased levels of plasma alpha-tocopherol and values of the alpha-tocopherol/cholesterol ratio in diabetic patients. Jameson (1991) did not find any changes in alpha-tocopherol levels in the sera of patients with type 1 and type 2 diabetes mellitus. He also investigated coenzyme Q_{10} in the plasma. The diabetic patients had increased CoQ_{10} levels which, however, declined with the severity of damage of their organs. The patients with the lowest levels of coenzyme Q_{10} died of heart failure. Tomasetti *et al.* (1999) reported higher serum levels of CoQ_{10} in diabetic patients and the levels decreased with advanced organ damage. The authors supposed that modifications of CoQ_{10} levels may predispose the patients to pathological conditions. Though the results of various studies concerning the extent of changes of endogenous antioxidants in diabetic patients are not always in agreement, their conclusions are usually similar: increased oxidative stress and a decreased antioxidant capacity contribute to the progression of atherogenesis and other chronic diabetic complications. The supplementation with antioxidants, mainly alpha-tocopherol and coenzyme Q_{10}, may be an important factor in preventing these diabetic complications (Stocker *et al.* 1991, Reaven 1995, Thompson an Godin 1995, Thomas *et al.* 1997). In the patients with the coenzyme Q_{10} deficit, supplementation with coenzyme Q_{10} improves their bioenergetics and function of the heart and other organs (Folkers 1993, Langsjoen *et al.* 1994).

Diabetes mellitus belongs to the chronic diseases associated with oxidative stress and disturbances of mitochondrial function. We suppose that one of the causes leading to mitochondrial dysfunction can be the decreased level of coenzyme Q found in heart and liver mitochondria of rats with experimental diabetes mellitus.

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Reprint requests

J. Kucharská, Pharm.D., Ph.D., Pharmacobiochemical Laboratory, Faculty of Medicine, Comenius University, Hlboká 7, 811 05 Bratislava, Slovak Republic. Fax: 00421 7 5249 1422.