# The effect of simvastatin on coenzyme Q and antioxidant/oxidant balance in diabetic-hypercholesterolaemic rats

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**Abstract.** The effect of simvastatin administered for 10 days on coenzyme Q and antioxidant/oxidant balance in a rat model of diabetes mellitus and hypercholesterolaemia was studied.

In the diabetic-hypercholesterolaemic rats the signs of oxidative stress-decreased α-tocopherol/cholesterol in the plasma ($p < 0.01$) and α-tocopherol in liver ($p < 0.001$) together with increased lipid peroxidation in the liver (TBARS, $p < 0.05$) were found. Increased coenzyme $Q_9$ concentrations in the plasma ($p < 0.05$) and liver ($p < 0.01$), coenzyme $Q_{10}$ in the myocardium ($p < 0.05$) and in the liver ($p < 0.01$) may indicate adaptation to oxidative stress. Administration of simvastatin (10 mg/kg) to the diabetic-hypercholesterolaemic rats counteracted increased myocardial (coenzyme $Q_{10}$, $p < 0.05$) and liver (total coenzyme $Q_9$, $p < 0.05$) coenzyme Q concentrations but did not improve α-tocopherol depletion and increased formation of TBARS in the liver. Even though simvastatin treatment did not induce coenzyme Q deficiency in plasma, heart and liver of the diabetic-hypercholesterolaemic rats as compared to the control levels, it was not able to prevent completely the changes in antioxidant/oxidant balance induced by diabetes and hypercholesterolaemia. The results highlight the importance of studying the effect of statins on the coenzyme Q levels in the animal models of pathological conditions known to change the initial antioxidant defence system.

**Key words:** Experimental diabetic hypercholesterolaemia — Coenzyme Q — α-tocopherol — Lipid peroxidation — Simvastatin

## Introduction

Diabetes mellitus is a metabolic disease associated with an increased generation of free radicals and changes in the cellular antioxidant state. Diabetes-associated hyperlipidaemia related to the increased lipid and protein oxidation contributes to the progression of coronary atherosclerosis and development of cardiovascular diseases. Although experimental diabetes is known as an oxidative stress disease, antioxidant defence system in a double-disease animal model of diabetes and hyperlipidaemia has not been studied yet.

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Over the past decade statins have emerged as one of the most effective classes of drugs for treating hyperlipidaemia. Inhibition of HMG-CoA reductase by statins results in diminished synthesis of cholesterol. However, the effect of statins is not selective for inhibiting cholesterol biosynthesis, and also results in the inhibition of other products downstream of mevalonate pathway, namely coenzyme Q (Crane 2001; Hargreaves et al. 2005), which is an important lipophilic antioxidant and a part of mitochondrial respiratory chain (Kagan et al. 2000).

Many authors have repeatedly shown that different statins reduce plasma coenzyme Q concentration in humans (Folkers et al. 1990; Mortensen et al. 1997; Langsjoen and Langsjoen 2003; Rundek et al. 2004; Mabuchi et al. 2005) and there is a suggestion that the decrease in coenzyme $Q_{10}$ level may be associated with statin-induced myopathy (Nawarskas 2005) and even subclinical cardiomyopathy (Littaru

and Langsjoen 2007). However, reduction of plasma coenzyme Q concentration after statin treatment has not been confirmed in the study of Bleske et al. (2001) and Miyake et al. (1999) where the basal levels of coenzyme $Q_{10}$ were changed by diabetes. Few human studies have investigated tissue coenzyme Q status (Bargossi et al. 1994; Passi et al. 2003) and the results did not clearly show the lowering effect of statins on skeletal muscle coenzyme Q concentration (Laaksonen et al. 1995, 1996). On the other hand there is evidence from animal experiments that statins are able to decrease the tissue content of coenzyme Q in liver, heart and skeletal muscle (Willis et al. 1990; Fukami et al. 1993; Nakahara et al. 1998). Discrepancies between the results obtained from the studies with statins on the antioxidant defence system may be related to the altered basal levels of coenzyme Q in some pathological conditions. These data led us to study the effects of simvastatin on coenzyme Q and antioxidant/oxidant balance in double-disease experimental model of diabetic hypercholesterolaemia.

## Materials and Methods

### *Experimental animals*

The study conformed to the European Community guidelines for the use of experimental animals, Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) and was approved by the Animal Care and Use Committee of the Slovak Republic.

Male Wistar rats (250–300 g body weight), fed a standard diet and tap water *ad libitum*, were randomly assigned to the control group, diabetic, diabetic-hypercholesterolaemic group and diabetic-hypercholesterolaemic group treated with simvastatin.

### *Induction of acute phase of experimental diabetes mellitus, hypercholesterolaemia and treatment with simvastatin*

A single intraperitoneal injection of streptozotocin (80 mg/kg; Sigma Chemical Co., USA) diluted in buffer solution (0.05 mol/l citrate buffer, 0.1 mol/l NaCl; pH 4.5) was administered to induce the acute phase of diabetes mellitus. Rats fasted overnight before the administration of streptozotocin. Immediately after the application, the rats were allowed to drink 5% glucose solution for the first 24 h and afterwards they were fed a normal diet. To induce hypercholesterolaemia in diabetic rats, a fat-cholesterol diet (20 g/day) containing cholesterol and coconut oil 1% equally was used according to the modified models described by Jiao et al. (1988) and Kusunoki et al. (2000). The diet was administered for 10 days. Development of the acute phase of diabetes mellitus and hypercholesterolaemia was confirmed by the enhanced plasma glucose and total cholesterol levels (see Results). Simvastatin obtained from Zentiva, a.s. (Hlohovec, Slovak Republic) was administered in the dose of 10 mg/kg/day as an admixture to the fat-cholesterol diet for 10 days. After ten days, the animals were decapitated and blood and tissue samples used for determination of biochemical parameters.

### *Measurement of biochemical parameters*

The samples of plasma for estimation of glucose, total cholesterol, coenzyme $Q_9$, coenzyme $Q_{10}$ and α-tocopherol concentrations were prepared from blood collected into tubes containing heparin after centrifugation at $1.96 \times g$ for 15 min. Tissue samples of myocardium and liver were used for determination of coenzyme $Q_9$, reduced coenzyme $Q_9$, coenzyme $Q_{10}$ and α-tocopherol concentrations.

Glucose and total cholesterol in plasma were measured enzymatically using a commercial assay kits (PLIVA-Lachema Diagnostika s.r.o. (Czech Republic) and Cormay (Poland), respectively) and the bioanalyzer ELISA 200 (USA).

Concentrations of oxidized and reduced forms of coenzyme $Q_9$, oxidized form of coenzyme $Q_{10}$ and α-tocopherol were determined simultaneously by isocratic high-performance liquid chromatography (LKB, Sweden) according to Lang et al. (1986) with some modifications as follows. Plasma samples (500 µl) were extracted by mixture of hexane/ethanol (5/2, v/v; Merck). Tissue samples from myocardial left ventricle (30–50 mg) and liver (80–100 mg) were homogenized in water with addition of t-butylhydroxytoluene and sodium dodecylsulphate and extracted in the same way. Organic layer was evaporated under nitrogen, the residue was taken up in 99.9% ethanol and injected on the 7 µm column SGX C18 (Tessek Ltd., Czech Republic). The mobile phase consisted of methanol/acetonitrile/ethanol (6/2/2, v/v/v; Merck). Concentrations of compounds were detected spectrophotometrically at 275 nm using external standards (Sigma, Germany). Reduced coenzyme $Q_9$ standard was prepared by the reduction of coenzyme $Q_9$ with sodium dithionite. Data were collected and processed using CSW32 chromatographic station (DataApex Ltd., Czech Republic).

Formation of lipid peroxides in myocardial and liver tissue was determined spectrophotometrically as the concentration of thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa et al. (1979).

### *Statistical analysis*

Data were statistically analyzed using first a one-way ANOVA. In cases of significance, a two-tailed unpaired Student's *t*-test was applied. Values of $p < 0.05$ were considered statistically significant.

## Results

### *Development of acute diabetes and hypercholesterolaemia and effect of simvastatin*

In comparison with the control rats, the concentration of glucose in the plasma of the acute diabetic rats was significantly increased ($p < 0.05$). Simultaneous induction of acute diabetes and administration of fat cholesterol diet resulted in significantly increased plasma levels of glucose ($p < 0.05$) and total cholesterol ($p < 0.001$) as compared with the control group (Table 1).

Administration of simvastatin to the diabetic-hypercholesterolaemic rats tended to decrease total plasma cholesterol and glucose concentrations compared with the diabetic-hypercholesterolaemic group (Table 1).

**Table 1.** Plasma glucose and cholesterol levels in the control (C), diabetic (DM), diabetic-hypercholesterolaemic (DM-HCH) and simvastatin-treated diabetic-hypercholesterolaemic (DM-HCH+Sim) rats

| Group | Glucose (mmol/l) | Cholesterol (mmol/l) |
|---|---|---|
| C | 11.2 ± 0.65 | 1.5 ± 0.09 |
| DM | 17.0 ± 2.03[a] | 1.6 ± 0.06 |
| DM-HCH | 18.8 ± 2.35[b] | 3.1 ± 0.24[bbb] |
| DM-HCH+Sim | 15.0 ± 1.48 | 2.5 ± 0.13 |

The data are expressed as means ± S.E.M. for 5–6 measurements. [a] $p < 0.05$, DM vs. C rats; [b] $p < 0.05$ and [bbb] $p < 0.001$, DM-HCH vs. C rats.

### *Coenzyme Q, α-tocopherol and TBARS in acute diabetic, diabetic-hypercholesterolaemic rats and effect of simvastatin*

*Plasma*

Not only in the diabetic but also in the diabetic-hypercholesterolaemic rats, the plasma content of coenzyme $Q_9$ was significantly increased (0.152 ± 0.026 and 0.134 ± 0.017, respectively vs. 0.091 ± 0.007 μmol/l in the control group; $p < 0.05$). The cholesterol-corrected plasma coenzyme $Q_9$ concentration was increased in the diabetic rats (0.095 ± 0.015 vs. 0.060 ± 0.004 μmol/mmol in the control group; $p < 0.05$). On the other hand, both absolute and cholesterol-corrected α-tocopherol concentrations were significantly decreased in the acute diabetic animals (4.91 ± 1.17 and 3.20 ± 0.85 vs. 10.23 ± 1.31 μmol/l and 6.75 ± 0.91 μmol/mmol, respectively; $p < 0.05$) when compared to the control rats. In the diabetic-hypercholesterolaemic animals, the decrease in plasma cholesterol-corrected α-tocopherol was even more pronounced (2.50 ± 0.42 vs. 6.75 ± 0.91 μmol/mmol; $p < 0.01$) (Fig. 1). Plasma coenzyme $Q_{10}$ was not detectable in any experimental group.

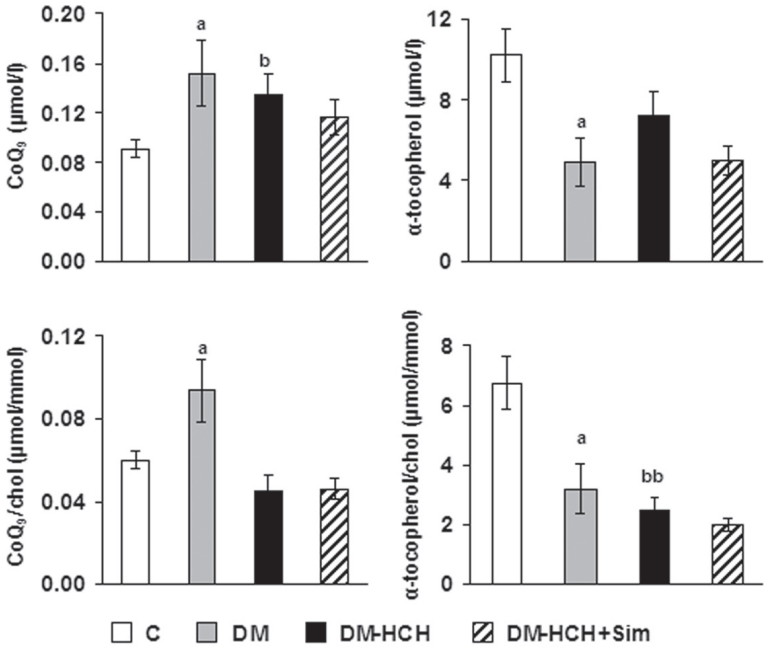


**Figure 1.** Plasma coenzyme $Q_9$ ($CoQ_9$), coenzyme $Q_9$/cholesterol ratio ($CoQ_9$/chol), α-tocopherol and α-tocopherol/cholesterol ratio (α-tocopherol/chol) in the control (C), diabetic (DM), diabetic-hypercholesterolaemic (DM-HCH) and simvastatin-treated diabetic-hypercholesterolemic (DM-HCH+Sim) rats. Values are means ± S.E.M. for 5–6 measurements. [a] $p < 0.01$, DM vs. C rats; [b] $p < 0.05$ and [bb] $p < 0.01$, DM-HCH vs. C rats.

Administration of simvastatin to the rats developing diabetes and hypercholesterolaemia for 10 days had a tendency to decrease the plasma coenzyme $Q_9$ and α-tocopherol concentrations, whereas the cholesterol-corrected coenzyme $Q_9$ and α-tocopherol values were unchanged (Fig. 1).

*Myocardium*

Myocardial coenzyme $Q_{10}$ content was significantly increased in both diabetic and diabetic-hypercholesterolaemic group (15.0 ± 0.44 and 14.23 ± 1.05, respectively vs. 11.17 ± 0.89 nmol/g ww in controls; $p < 0.01$ and $p < 0.05$, respectively) and myocardial coenzyme $Q_9$ content had a tendency to increase as compared with the control group. The content of α-tocopherol in myocardium of the diabetic and diabetic-hypercholesterolaemic rats was not changed (Fig. 2).

Simvastatin treatment significantly reduced the myocardial coenzyme $Q_{10}$ content (11.39 ± 0.67 vs. 14.23 ± 1.05 nmol/g ww in diabetic-hypercholesterolaemic rats; $p < 0.01$), near to the control value, with the tendency to decrease coenzyme $Q_9$ values (Fig. 2). No changes were found in myocardial TBARS formation (Fig. 3).

*Liver*

The concentration of reduced coenzyme $Q_9$, total coenzyme $Q_9$ and coenzyme $Q_{10}$ was significantly increased in the liver of the diabetic and the diabetic-hypercholesterolaemic animals ($p < 0.01$) in comparison with the control group. α-tocopherol levels were significantly decreased in the diabetic and the diabetic-hypercholesterolaemic animals ($p < 0.01$ and $p < 0.001$, respectively; Table 2). The TBARS concentration in liver tissue increased both in diabetic and the diabetic-hypercholesterolaemic rats (131.7 ±12.98 and 115.7 ± 6.82, respectively vs. 87.5 ± 9.19 nmol/g ww in controls; $p < 0.05$) (Fig. 3).

In the simvastatin-treated diabetic-hypercholesterolaemic rats, total coenzyme $Q_9$ decreased significantly ($p < 0.05$), and reduced coenzyme $Q_9$ together with coenzyme $Q_{10}$ had a tendency to decrease in comparison with the

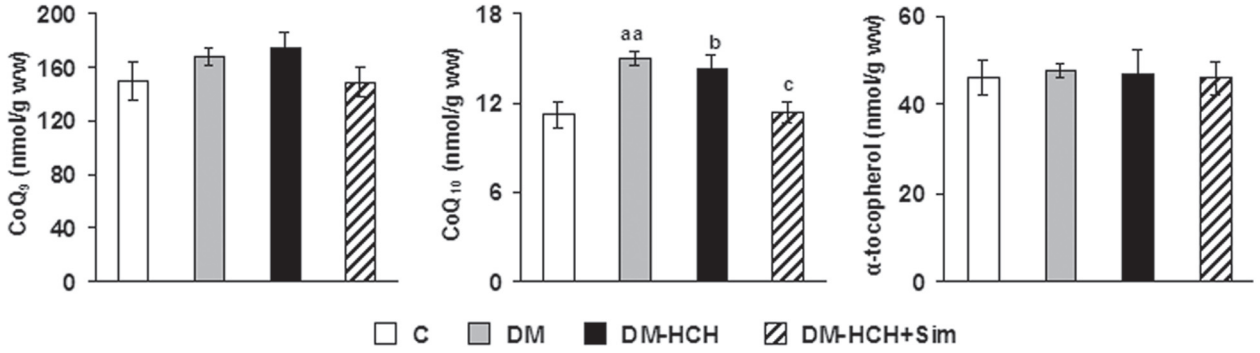


**Figure 2.** Myocardial coenzyme $Q_9$ ($CoQ_9$), coenzyme $Q_{10}$ ($CoQ_{10}$) and α-tocopherol in the control (C), diabetic (DM), diabetic-hypercholesterolaemic (DM-HCH) and simvastatin-treated diabetic-hypercholesterolaemic (DM-HCH+Sim) rats. Values are means ± S.E.M. for 5–6 measurements. [aa] $p < 0.01$, DM vs. C rats; [b] $p < 0.05$, DM-HCH vs. C rats; [c] $p < 0.05$, DM-HCH+Sim vs. untreated DM-HCH rats.

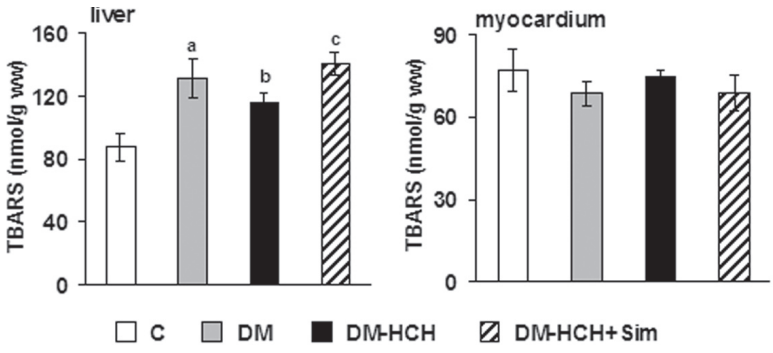


**Figure 3.** Thiobarbituric acid reactive substances (TBARS) concentration in the liver and myocardium in the control (C), diabetic (DM), diabetic-hypercholesterolaemic (DM-HCH) and simvastatin-treated diabetic-hypercholesterolemic (DM-HCH+Sim) rats. Values are means ± S.E.M. for 5–6 measurements. [a] $p < 0.05$, DM vs. C rats; [b] $p < 0.05$, DM-HCH vs. C rats; [c] $p < 0.05$, DM-HCH+Sim vs. untreated DM-HCH rats.

**Table 2.** Reduced coenzyme $Q_9$ ( $CoQ_9H_2$), total coenzyme $Q_9$ (total $CoQ_9$), coenzyme $Q_{10}$ ($CoQ_{10}$) concentrations and α-tocopherol in the liver of control (C), diabetic (DM), diabetic-hypercholesterolaemic (DM-HCH) and simvastatin-treated diabetic-hypercholesterolaemic (DM-HCH+Sim) rats

| Group | $CoQ_9H_2$ (nmol/g ww) | total $CoQ_9$ (nmol/g ww) | $CoQ_{10}$ (nmol/g ww) | α-tocopherol (nmol/g ww) |
|---|---|---|---|---|
| C | 24.9 ± 1.67 | 60.0 ± 4.19 | 3.12 ± 0.23 | 79.2 ± 4.49 |
| DM | 50.3 ± 6.79$^{aa}$ | 110.8 ± 11.20$^{aa}$ | 4.97 ± 0.45$^{aa}$ | 53.1 ± 5.15$^{aa}$ |
| DM-HCH | 52.7 ± 8.18$^{bb}$ | 105.4 ± 9.05$^{bb}$ | 4.72 ± 0.38$^{bb}$ | 29.0 ± 3.01$^{bbb}$ |
| DM-HCH+Sim | 39.2 ± 4.94 | 81.4 ± 5.90$^{c}$ | 4.11 ± 0.44 | 29.7± 2.94 |

The data are expressed as means ± S.E.M. for 5–6 measurements. $^{aa}$ $p < 0.01$, DM vs. C rats; $^{bb}$ $p < 0.01$ and $^{bbb}$ $p < 0.001$, DM-HCH vs. C rats; $^{c}$ $p < 0.05$, DM-HCH+Sim vs. untreated DM-HCH rats.

untreated diabetic-hypercholesterolaemic rats. No effect of simvastatin was observed on α-tocopherol content in the liver (Table 2).

Simvastatin treatment increased liver TBARS concentration in comparison with diabetic-hypercholesterolaemic group (141.0 ±6.71 vs. 115.7 ± 6.82 nmol/g ww; $p < 0.05$) (Fig. 3).

## Discussion

The lowering effect of statins on plasma concentration of coenzyme Q, a compound essential in mitochondrial ATP production and one of the most important endogenous antioxidant, has been reported since the end of the last century (Hargreaves et al. 2005). There is still insufficient evidence from both human and animal studies clearly to elucidate this phenomenon associated with the adverse effect of statins. In the present study, we investigated the effect of simvastatin on both plasma and tissue concentrations of coenzyme Q, α-tocopherol and lipid peroxidation in the animal model of acute diabetes and hypercholesterolaemia.

Diabetes mellitus belongs to the diseases associated with the increased production of reactive oxygen species, changes in antioxidant status and deterioration of mitochondria energy metabolism, potentially leading to cumulative action of radicals (Uličná et al. 1996; Ferko et al. 2006; Ziegelhöffer 2007). Lipophilic antioxidants, such as coenzyme Q and α-tocopherol, play an important role in the prevention of oxidative stress resulting in diminished oxidation of low-density lipoproteins. The studies investigating antioxidant status have found coenzyme $Q_{10}$ to be significantly decreased (Miyake et al. 1999) or even increased (Salardi et al. 2004) in the serum of diabetic patients. In the experimental studies, deficit of both coenzyme Q homologues in the heart and liver mitochondria of 8-week streptozotocin-induced diabetic rats treated with insulin was demonstrated together with elevated mitochondrial α-tocopherol and lipoperoxidation (Kucharská et al. 2000). On the other hand, coenzyme $Q_9$ levels in plasma were increased in diabetic rats together with significantly augmented plasma/serum malondialdehyde (Hermans et al. 2007). Jain and Levine (1995) found an accumulation of vitamin E and increased lipoperoxidation in the heart ventricles of 2-months diabetic rats. When studying regulation of the endogenous level of antioxidants in the state of chronic oxidative stress caused by diabetes, myocardial coenzyme $Q_9$ content increased progressively in the course of diabetes development, whereas the content of α-tocopherol increased at the end of the 8-months study. Simultaneously, increased lipoperoxidation in the myocardial tissue was documented (Stefek et al. 2000; Kucharska et al. 2001). In accordance with these data, increased content of coenzyme Q in plasma, liver and myocardium of diabetic and diabetic-hypercholesterolaemic rats found in our study could be regarded as an adaptive response to oxidative stress confirmed by increased lipid peroxidation in liver without changes in the heart. In contrast, contents of α-tocopherol, which is not synthesized endogenously, was decreased in plasma and liver of diabetic rats which could reflect its attenuated regeneration from α-tocopheroxyl radical formed by action of free radicals.

Administration of the fat-cholesterol diet to the animals developing diabetes after injection of streptozotocin resulted in hypercholesterolaemia. The effects on coenzyme Q status in plasma, liver and myocardium were similar to the diabetic animals. On the other hand, the concentration of α-tocopherol in the liver was lower in the diabetic-hypercholesterolaemic rats in comparison with the diabetic animals, indicating a decreased antioxidant capacity in double-diseases experimental conditions.

It was demonstrated several times that statins reduce plasma coenzyme Q concentration (Folkers et al. 1990; Mortensen et al. 1997; Langsjoen and Langsjoen 2003; Rundek et al. 2004; Mabuchi et al. 2005). However, plasma concentration of coenzyme Q is influenced by a number of physiological factors and may not represent cellular concentrations. Determination of tissue concentration of coenzyme Q may provide suitable alternatives for these measurements

(Hargreaves 2003) and a number of studies documented the effect of statins on coenzyme Q concentrations in animal tissue. First demonstration was provided by Willis et al. (1990) who have found that the treatment of rats with lovastatin for 4 weeks (400 mg/kg) decreased concentration of coenzyme Q in heart and liver. Administration of simvastatin (50 mg/kg) for 4 weeks decreased concentration of coenzyme Q in skeletal muscle in rabbits (Nakahara et al. 1998). In another work of Fukami et al. (1993), rabbits´ heart and liver, but not skeletal muscle coenzyme Q was diminished after simvastatin treatment. Decreased coenzyme Q in the left ventricle and skeletal muscle was found in the rats treated with simvastatin (10 mg/kg, 6 weeks) in condition of NO-deficient hypertension, the use of simvastatin in control rats did not induce significant changes (Kucharska et al. 2007).

Either in human or in animal studies, the changed initial coenzyme Q levels in some pathological conditions were often not taken into consideration when assessing statins effect on coenzyme $Q_{10}$ level. The basal level of coenzyme $Q_{10}$ was found to be changed in some cardiovascular diseases as congestive heart disease (Sarter 2002; Hargreaves et al. 2005), coronary heart disease (Hanaki et al. 1993; Yalcin et al. 2004) and diabetes (Miyake et al. 1999; Wittenstein et al. 2002; Salardi et al. 2004) or in diabetic patients with hypercholesterolaemia (Miyake et al. 1999).

Simvastatin in the model of acute diabetes and hypercholesterolaemia with initially changed antioxidative defence system had a tendency to decrease cholesterol in plasma. In our previous experiment, the same dose of simvastatin administered to animals for 5 days had also a tendency to decrease plasma cholesterol levels. Significantly decreased total liver cholesterol levels confirmed the lipid lowering effect of simvastatin in the model where cholesterol was continuously administered to the animals (Adameová et al. 2006).

Simvastatin treatment counteracted increased concentrations of coenzyme $Q_9$ in plasma and liver and coenzyme $Q_{10}$ in myocardium of the diabetic-hypercholesterolaemic animals without a tendency to induce coenzyme Q depletion, when compared to the healthy animals. When compared to diabetic-hypercholesterolaemic rats with elevated coenzyme Q levels, simvastatin decreased their concentrations in myocardial and liver tissues. Despite the findings that HMG-CoA reductase inhibitors possess antioxidant properties (Davignon et al. 2004) proved in patients (Shin et al. 2007 ) as well as in experimental diabetic rats (Riad et al. 2007) and in hypercholesterolaemic rabbits (Bolayirli et al. 2007), it has been supposed that under some conditions such as decreased activity of nitric oxide synthase they may promote formation of reactive oxygen and nitrogen species and increase the potential for tissue damage (Parker et al. 2003). Recent study showed that simvastatin administration to mice decreased tissue coenzyme Q levels and the body resistance to oxidative stress and these effects were alleviated by coadministering of coenzyme $Q_{10}$ with statin (Kettawan et al. 2007).

Our results suppose that administration of simvastatin to the diabetic-hypercholesterolaemic rats in the tested dose did not induce coenzyme Q deficiency in the plasma, heart and liver when compared to the levels in control rats. Simvastatin prevented the increases in myocardial and partially in liver coenzyme Q concentrations found in the animal model of acute diabetes and hypercholesterolaemia but it was not able to prevent depletion of α-tocopherol in plasma and liver as well as increased formation of lipid peroxides in the liver.

The results highlight the importance of studying the effect of statins on the coenzyme Q levels and antioxidant/oxidant balance in the animal models of pathological conditions known to change the initial antioxidative defence system.

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