Liver Mitochondrial Respiratory Function and Coenzyme Q Content in Rats on a Hypercholesterolemic Diet Treated With Atorvastatin

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Summary

Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, are effective drugs in the treatment of hypercholesterolemia, however, their undesirable actions are not fully known. We investigated the effects of atorvastatin on the oxidative phosphorylation and membrane fluidity in liver mitochondria, and also on the coenzyme Q (CoQ) content in the mitochondria, liver tissue, and plasma of rats on a standard (C) and hypercholesterolemic (HCh) diet. Atorvastatin was administered at either low (10 mg·kg^{-1}) or high dose (80 mg·kg^{-1}) for four weeks. The high dose of the drug decreased the concentrations of total cholesterol and triacylglycerols in the plasma and liver of rats on a HCh diet. Administration of atorvastatin was associated with decreased oxygen uptake (state 3), and oxidative phosphorylation rate in the mitochondria of both C and HCh rats. Further, the drug influenced mitochondrial membrane fluidity and dose-dependently reduced concentrations of oxidized and reduced forms of CoQ in the mitochondria. Our findings point to an association between *in vivo* administration of atorvastatin and impaired bioenergetics in the liver mitochondria of rats, regardless of diet, in conjunction with simultaneous depletion of oxidized and reduced CoQ forms from the mitochondria. This fact may play a significant role in the development of statin-induced hepatopathy.

Key words

Hypercholesterolemia • Atorvastatin • Liver mitochondria • Oxidative phosphorylation • Coenzyme Q

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Introduction

Hypercholesterolemia is considered as an important risk factor for the development of atherosclerotic cardiovascular diseases (Rose *et al.* 1977, Martin *et al.* 1986). Statin drugs represent the main therapeutic class of lipid lowering agents. Statins are generally well tolerated, however, they can produce adverse effects including, among others, skeletal muscle associated and dose dependent injuries ranging from muscle pain to clinical manifestations of rhabdomyolysis, as well as liver damages. Statins competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and thus reduce hepatocellular cholesterol production by lowering the synthesis of mevalonate. Mevalonate is a precursor of cholesterol but also of a whole class of other important substances, such as ubiquinone, dolichols and other isoprenoids (Liao 2002). Ubiquinone is also known as coenzyme Q (CoQ). It belongs to a homologous series of compounds that share a common benzoquinone ring structure but differ in the length of the isoprenoid side chain. While CoQ$_9$ is the major CoQ homologue in rats (Lönnrot *et al.* 1998, Bhagavan and Chopra 2006), in humans the side chain

comprises 10 isoprenoid units (CoQ_{10}).

CoQ plays a fundamental role in cellular bioenergetics since it acts as a redox cofactor in the mitochondrial respiratory chain and its cyclic electron-proton (Q-cycle) transport functioning is essential for the optimal production of ATP. In its reduced form (ubiquinol) it is a potent lipophilic antioxidant and, as such, it is capable of recycling and regenerating other antioxidants such as tocopherol and ascorbate (Ernster and Dallner 1995, Crane 2001) thus preventing lipoperoxidation and subsequent changes in mitochondrial membrane fluidity.

It is believed that mitochondrial dysfunction plays a key role in the etiology of myopathy, cardiomyopathy, and hepatopathy. The progressive (cumulative) impairment of mitochondrial function leading to hepatocellular dysfunction cannot be excluded. However, direct evidence for the effect of statins on mitochondrial bioenergetics with respect to the CoQ content is lacking. For ethical reasons such studies cannot be conducted on humans.

The undoubtedly beneficial role of statins in the prevention and inhibition of coronary heart disease has resulted in their frequent use in clinical practice. However, epidemiological data on the increasing incidence of chronic liver diseases (non-alcoholic fatty liver disease − NAFLD) which can be displayed as simple steatosis, non-alcoholic steatohepatitis (NASH), fibrosis and eventually progress to cirrhosis (Angulo 2002), bring about the need for investigating whether statins can cause or contribute to the onset of the abovementioned liver disorders. One of the frequently used statins in clinical practice is atorvastatin, a reversible competitive inhibitor of HMG-CoA-reductase, which decreases tChol in plasma and also very low density lipoproteins (VLDL), low density lipoproteins (LDL), apolipoprotein B and triacylglycerol levels (Bakker-Arkema *et al*. 1996, Gómez-Domínguez *et al*. 2006).

Statins reduce biosynthesis of CoQ which is a mobile electron carrier in the respiratory chain of mitochondria. Based on this we hypothesized that inhibition of CoQ biosynthesis by a statin would decrease CoQ content in the mitochondria and consequently affect the rates of respiration and oxidative phosphorylation. We designed an experiment on rats which were fed a hypercholesterolemic (HCh) diet. Our aim was to assess CoQ content and mitochondrial respiratory function parameters in the livers of rats on a standard or HCh diet given atorvastatin at doses which were suspected to have

some clinical effects. Since both CoQ and atorvastatin are lipophilic, and published evidence suggests that some of the membrane functions may be modulated by changes in membrane fluidity, we also hypothesized that atorvastatin treatment could affect the fluidity of mitochondrial membrane.

Methods

Chemicals

D(-) manitol, saccharose, potassium chloride, ethylenedinitrilotetraacetic acid disodium salt (Titriplex III), potassium dihydrogen phosphate, 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethansulfonate acid (HEPES), hexane, ethanol, methanol, acetonitrile, sodium dodecylsulphate (SDS) were obtained from Merck. Cholesterol, L (+) - glutamic acid monosodium salt monohydrate, adenosine 5'diphosphate sodium salt (ADP), L (-) malic acid sodium salt, dextran, butylhydroxytoluene (BHT) and coenzyme Q_9 standard were obtained from Sigma. The remaining chemicals were of analytical grade purity and obtained from Centralchem.

Animals

Male Wistar rats (210-270 g) were obtained from Dobrá Voda (Slovakia). They were maintained in a 12 h light/dark cycle at a constant temperature of 20-24 °C with free access to tap water and food. All experiments were carried out according to the guidelines for the care and use of experimental animals, and were approved by the State Veterinary Administration of the Slovak Republic.

Experimental procedure

The animals were randomly divided into six groups consisting of 10 rats. Three groups were on the standard diet (KKZ-P/M, Dobrá Voda) for eight weeks. The first group was an untreated control (C). The remaining two groups were treated with atorvastatin at a daily dose, either low 10 mg·kg^{-1} (CA10) or high 80 mg·kg^{-1} (CA80), for four weeks before the end of experiment. The next three groups were on a HCh diet (the standard diet enriched with 4 % cholesterol and 10 % saturated fat prepared as pellets) for eight weeks, untreated (HCh) and treated as mentioned above (HChA10; HChA80). Atorvastatin was given to the animals using the gastric gavage technique. To ensure identical experimental conditions, C and HCh groups

were administered with tap water *via* a gastric tube in the same manner as the experimental groups.

After eight weeks and 24 hours after the last administration of atorvastatin, the rats were anesthetized with thiopental at a dose of 100 mg·kg^{-1}. Blood samples were taken from the abdominal aorta into heparinized tubes. Plasma samples were frozen until analyses. The livers were removed and samples from the right lobe were cut off, weighed and stored at −70 °C for determination of total cholesterol (tChol), triacylglycerols (TAG) and CoQ. The remaining liver tissue was immediately used for mitochondria isolation.

Biochemical analysis

Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as concentrations of tChol and TAG in plasma were evaluated by standard automated techniques using the Hitachi Analyzer Model 911 and adequate kits from the Roche Company (Switzerland). Concentration of tChol in the liver tissue was determined according to Abell *et al.* (1952), and TAG according to Jover (1963). Samples of liver tissues for analysis were homogenized in a chloroform/methanol mixture (1:1) for tChol determination and in a chloroform/methanol mixture (2:1) for TAG determination. Oxidized and reduced forms of CoQ (CoQ$_{9-ox}$ and CoQ$_{9-red}$) were determined by the HPLC method with spectrophotometric detection (Lang *et al.* 1986, Kucharská *et al.* 2007). The liver tissue was homogenized using Ultra-Turrax in water with an addition of BHT and SDS. Homogenate or mitochondrial suspension was extracted by a hexane/ethanol mixture (5:2). The organic phase was evaporated under nitrogen and the residue dissolved in ethanol before measurement.

Isolation of mitochondria

Mitochondria were isolated from freshly excised livers by differential centrifugation as described by Hogeboom (1955), with some modification. The isolation medium was prepared according to Sammut *et al.* (1998), with the following modification: 225 mmol·l^{-1} mannitol, 75 mmol·l^{-1} sucrose, and 0.2 mmol·l^{-1} Titriplex III. Liver was minced in the isolation medium (pH 7.4) at 4 °C, and homogenized using a teflon-to-glass homogenizer. The homogenate was centrifuged at 700 g for 10 min., the supernatant was decanted and centrifuged at 5600 g for 10 min. The mitochondrial pellet was washed twice with isolation medium. The resulting pellet was resuspended in the same medium to a final protein concentration of 20-40 mg·ml^{-1}. All procedures were performed at 4 °C. Proteins were determined according to Lowry *et al.* (1951).

Measurement of mitochondrial function

Respiratory function of the mitochondria was determined using the voltamperometric method on an oxygraph Gilson 5/6 H (USA) with a Clark oxygen electrode at 30 °C. The incubation medium was prepared as described by Rouslin and Millard (1980) with a modification: 12.5 mmol·l^{-1} HEPES, 122 mmol·l^{-1} KCl, 3 mmol·l^{-1} KH$_2$PO$_4$, 0.5 mmol·l^{-1} Titriplex III, and 2 % dextrane. Glutamate/malate at 2.5 mmol·l^{-1} was used as a NAD substrate. For assessing stimulated oxygen consumption, 500 nmol of ADP was added.

The following parameters of oxidative phosphorylation were determined in the liver mitochondria: the rate of oxygen uptake by the mitochondria stimulated with ADP – state 3 [QO$_2$(S$_3$)] expresses the velocity of oxygen consumption by mitochondria in the presence of ADP and substrate. The rate of basal oxygen uptake by mitochondria without ADP – state 4 [QO$_2$(S$_4$)] denotes how fast oxygen is used by mitochondria in the presence of substrate only. Respiratory control ratio (RCR) – ratio of state 3 to state 4 indicates the dependence of respiration rate on availability of ADP. The higher the RCR value, the tighter is the coupling of oxidation to phosphorylation. The oxidative phosphorylation rate (OPR) determines the rate of ATP generation in state 3 (Estabrook 1967).

Biophysical analysis

Membrane fluidity was assessed as a fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH). A high degree of fluorescence anisotropy indicates a high degree of structural order, which indicates lower fluidity. Briefly, isolated mitochondria were diluted to a level at which no significant light scattering had been recorded (30 µg/ml). Each sample was equilibrated with 0.25 µM DPH at 22±0.7 °C in the dark for 20 min. Then the steady-state anisotropy was determined as the mean of ten consecutive recordings taken for every sample during the plateau phase. The mitochondria used in these experiments were intact, in a de-energized (non-respiring) state and were maintained at 4 °C until measured. For details see Waczulíkova *et al.* (2010).

Table 1. Body weight, liver weight and biochemical parameters in plasma and liver after atorvastatin treatment.

	C	CA10	CA80	HCh	HChA10	HChA80
Initial body weight [g]	229 ±14.49	230 ±10.54	222.5 ±11.65	236 ±15.06	237.8 ±19.11	236.5 ±17.65
Final body weight [g]	370.5±35.94	363±27.41	349.4±29.57	401±59.53	394.4±48.31	377.5±35.99
Liver weight [g]	12.21±1.46	11.94±1.65	11.76±1.87	16.2±2.93**	15.6±2.22	13.9±1.31[#]
Plasma						
tChol [mmol.l⁻¹]	1.46±0.26	1.19±0.15	1.39±0.30	3.15±0.47**	2.95±0.57	2.70 ±0.30[#]
TAG [mmol.l⁻¹]	1.27±0.47	0.95±0.22	0.75±0.24*	1.59±0.56	1.57±0.68	1.43±0.61
ALT [μkat.l⁻¹]	0.95±0.19	1.25±0.48	1.06±0.15	1.22±0.25*	1.13±0.28	1.53±0.45[+]
AST [μkat.l⁻¹]	1.68±0.41	1.84±0.54	1.80±0.10	1.92±0.53	1.66±0.36	1.74±0.25
$CoQ_{9\text{-}ox}$	0.155	0.165	0.068** [++]	0.166	0.151	0.062[##+]
[μmol.l⁻¹]	(0.134-0.188)	(0.124-0.175)	(0.049-0.086)	(0.147-0.194)	(0.120-0.174)	(0.056-0.083)
Liver						
tChol	5.45	5.52	6.22	119.33**	100.82	36.29[##++]
[mmol.kg⁻¹]	(5.14-5.74)	(5.43-5.68)	(5.56-6.49)	(83.12-127.84)	(76.19-113.52)	(26.57-54.60)
TAG	8.93	8.06	7.35	39.34**	44.04	20.34[##++]
[mmol.kg⁻¹]	(8.03-9.35)	(7.07-8.66)	(5.84-9.22)	(33.46-47.73)	(37.65-49.19)	(17.24-23.97)
$CoQ_{9\text{-}ox}$	67.7	54.4	61.4	38.5**	27.7[#]	30.9[#]
[nmol.g⁻¹]	(59.6-73.7)	(46.3-66.5)	(55.5-62.8)	(32.2-41.9)	(24.0-31.9)	(24.8-33.1)
$CoQ_{9\text{-}red}$	57.3	54.5	49.6	21.5**	21.4	25.4
[nmol.g⁻¹]	(54.1-75.6)	(47.6-59.1)	(46.1-66.3)	(17.0-22.9)	(16.5-21.7)	(23.9-30.4)

Experimental groups: C – control, CA10 – control + atorvastatin 10 mg.kg⁻¹, CA80 – control + atorvastatin 80 mg.kg⁻¹, HCh – hypercholesterolemic, HChA10 – hypercholesterolemic + atorvastatin 10 mg.kg⁻¹, HChA80 – hypercholesterolemic + atorvastatin 80 mg.kg⁻¹. Data are expressed as mean ± S.D. (standard deviation) for the normally distributed variables; otherwise median (M) and lower quartile Q_1-upper quartile Q_3, $M(Q_1$-$Q_3)$, are used. Sample means of normally distributed variables were compared with one-way analysis of variance. If the overall test statistic was significant, multiple comparisons were performed with the Tukey-Kramer test. For non-normal variables, significance of differences was tested by means of the Kruskal-Wallis test. *Post hoc* pairwise comparisons were performed with the Connover-Inman test. Indices: * $p<0.05$, ** $p<0.005$ vs. C, # $p<0.05$, ## $p<0.005$ vs. HCh, + $p<0.05$, ++ $p<0.005$ vs. either CA10 or HChA10.

Statistical analysis

Data were checked for normality with the Shapiro-Wilk W-test. Normally distributed variables are described as mean ± S.D. (standard deviation); otherwise median (M) and quartile ranges from the lower quartile Q_1 to the upper quartile Q_3 $(Q_1$-$Q_3)$, are used (Table 1). One-way analysis of variance, followed either with the Dunnett test for multiple comparisons with the control or Tukey-Kramer test for all pairwise comparisons, was used. In the case of non-normality and/or unequal variances in between groups being compared, a non-parametric alternative (the Kruskal-Wallis test and the *post hoc* pairwise comparisons with the Connover-Inman test) was performed.

Results

At the end of the experiment, the mean body weight of C and HCh groups did not significantly differ.

Atorvastatin administration did not influence the weights of rats on a standard, or on a HCh diet. However, the average weight of the liver increased significantly in the group on the HCh diet in comparison with the control group on the standard diet. Liver weights of rats on the standard diet were not affected, but those of rats on the HCh diet and administered the higher dose of atorvastatin were decreased (HChA80; $p<0.05$; Table 1).

Animals on the HCh diet had an increased tChol concentration in their plasma ($p<0.005$), which was reduced by the higher dose of atorvastatin (HChA80; $p<0.05$). The plasma concentrations of TAG were not affected by the HCh diet. In the group on the standard diet, TAG concentration in plasma decreased by the high dose of atorvastatin (CA80; $p<0.05$). In the group on the HCh diet atorvastatin had no effect. ALT activity was increased in the HCh group ($p<0.05$), whereas AST activity was unchanged in comparison with the control
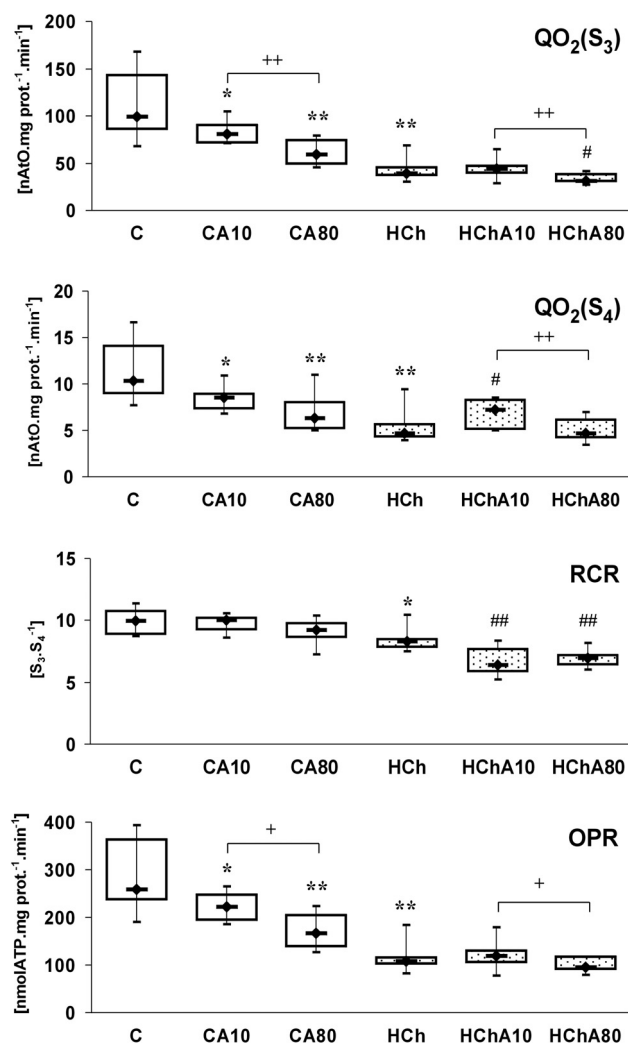
Fig. 1. Parameters of liver mitochondrial function following atorvastatin treatment. $QO_2(S_3)$ – ADP stimulated respiration rate, $QO_2(S_4)$ – basal respiration rate, RCR – respiratory control ratio, OPR – oxidative phosphorylation rate. Experimental groups: C – control, CA10 – control + atorvastatin 10 mg.kg^{-1}, CA80 – control + atorvastatin 80 mg.kg^{-1}, HCh – hypercholesterolemic, HChA10 – hypercholesterolemic + atorvastatin 10 mg.kg^{-1}, HChA80 – hypercholesterolemic + atorvastatin 80 mg.kg^{-1}. Data are presented as median (horizontal bar with a diamond sign) and lower-upper quartile range and minimal and maximal value for rats on standard diet (open boxes) and on HCh diet (dotted boxes). Significance of differences was tested by means of the Kruskal-Wallis test. *Post hoc* pairwise comparisons were performed with the Connover-Inman test. Indices: * $p<0.05$, ** $p<0.005$ vs. C, # $p<0.05$ vs. HCh, + $p<0.05$, ++ $p<0.005$ vs. either CA10 or HChA10.

group. Atorvastatin did not affect the activities of aminotransferases in the plasma. The plasma concentration of $CoQ_{9\text{-}ox}$ decreased following the higher dose of atorvastatin in both the group on standard diet (CA80; $p<0.005$), and the group on the HCh diet (HChA80; $p<0.005$) (Table 1).

There were significantly increased concentrations of tChol and TAG in the livers of the

group of rats on the HCh diet ($p<0.005$) in comparison with the control group. Unlike the lower statin dose, the higher dose effectively reduced these elevated concentrations (HChA80; $p<0.005$; Table 1).

We found markedly decreased parameters of oxidative phosphorylation ($p<0.005$ for oxygen consumption rate in state 3, state 4 and OPR; $p<0.05$ for RCR) in the liver mitochondria of HCh animals in comparison with control values (C). Oxygen consumption rate in state 3 was reduced in the HCh group on higher dose of atorvastatin (HChA80; $p<0.05$) and RCR was lowered by both doses of atorvastatin (HChA10, HChA80; $p<0.005$) when compared with the HCh group. In the control animals oxygen consumption rates in state 3, state 4 and OPR decreased upon administration of both, the lower (CA10; $p<0.05$) and the higher (CA80; $p<0.005$) doses of atorvastatin, whereas RCRs were not affected. Our findings at low and high atorvastatin doses indicate a dose-dependent impairment of mitochondrial function. The lowest rate of oxygen consumption in state 3 and ATP production was found in the HCh group given the higher dose of atorvastatin (Fig. 1).

The results concerning CoQ levels showed decreased concentrations of both oxidized and reduced CoQ_9 in the liver mitochondria of rats on the HCh diet (HCh; $p<0.05$) in comparison with the control group. Administration of atorvastatin at both doses further deepened this trend (HChA10, HChA80; $p<0.005$). In the groups on the standard diet atorvastatin decreased concentrations of both forms of CoQ (all $p<0.05$). The most pronounced decrease in reduced CoQ_9 was in HChA80 ($p<0.005$) (Fig. 2). In the liver tissue, the levels of $CoQ_{9\text{-}ox}$ and CoQ_{9red} were lowered by the HCh diet ($p<0.005$) in comparison with the control group. The $CoQ_{9\text{-}ox}$ level was further reduced by atorvastatin treatment (HChA10, HChA80; $p<0.05$; Table 1).

The steady-state fluorescence anisotropy of DPH was used to study the effect of atorvastatin on the physical state of mitochondrial membrane lipids. As expected, membrane fluidity (inversely related to anisotropy) decreased in the animals on HCh diet ($p<0.05$). The diet-induced increase in the lipid order was completely prevented in the rats on higher dose of atorvastatin ($p<0.005$; Fig. 3) thus approaching values found in the controls. The increase in lipid order in the control groups cannot be considered conclusive ($p=0.11$ and 0.18 for lower and higher doses, respectively).
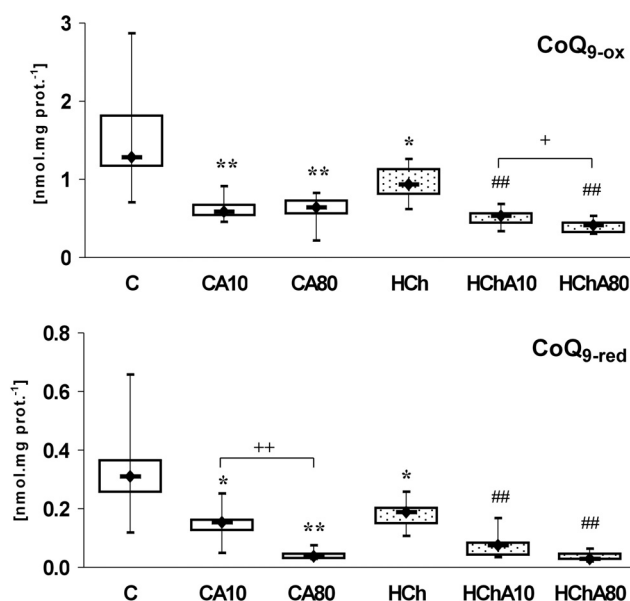
Fig. 2. Concentrations of oxidized and reduced coenzyme Q_9 (CoQ_9) in liver mitochondria after atorvastatin treatment. Experimental groups: C – control, CA10 – control + atorvastatin 10 mg.kg^{-1}, CA80 – control + atorvastatin 80 mg.kg^{-1}, HCh – hypercholesterolemic, HChA10 – hypercholesterolemic + atorvastatin 10 mg.kg^{-1}, HChA80 – hypercholesterolemic + atorvastatin 80 mg.kg^{-1}. Data are presented as median (horizontal bar with a diamond sign) and lower-upper quartile range and minimal and maximal value for rats on standard diet (open boxes) and on HCh diet (dotted boxes). Significance of differences was tested by means of the Kruskal-Wallis test. *Post hoc* pairwise comparisons were performed with the Connover-Inman test. Indices: * $p<0.05$, ** $p<0.005$ vs. C, ## $p<0.005$ vs. HCh, + $p<0.05$, ++ $p<0.005$ vs. either CA10 or HChA10.

Fig. 3. Mitochondrial membrane fluidity expressed as a reciprocal of fluorescence anisotropy of DPH following atorvastatin treatment. Experimental groups: C – control, CA10 – control + atorvastatin 10 mg.kg^{-1}, CA80 – control + atorvastatin 80 mg.kg^{-1}, HCh – hypercholesterolemic, HChA10 – hypercholesterolemic + atorvastatin 10 mg.kg^{-1}, HChA80 – hypercholesterolemic + atorvastatin 80 mg.kg^{-1}. Data are presented as median (horizontal bar with diamond sign) and lower-upper quartile range and minimal and maximal value for rats on standard diet (open boxes) and on HCh diet (dotted boxes). Significance of differences was tested by means of the Kruskal-Wallis test. *Post hoc* pairwise comparisons were performed with the Connover-Inman test. Indices: * $p<0.05$ vs. C, ## $p<0.005$ vs. HCh, ++ $p<0.005$ vs. either CA10 or HChA10.

Discussion

Overconsumption of highly calorific foods, in particular a high-fat diet, is important in the development of hyperlipidemia, non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). An increase in tChol and TAG in the plasma, together with the onset and development of steatosis after the administration of a HCh diet, has been evidenced in several animal studies (Matos *et al.* 2005, Kovář *et al.* 2009, Kučera *et al.* 2010). We also showed that the HCh diet significantly increased tChol concentration in plasma and liver tissue. In addition, it reduced membrane lipid fluidity in the liver mitochondria. Plasma TAG concentration increased only slightly, but their accumulation in the liver induced a fatty liver. We found increased ALT activity in rats on the HCh diet, whereas AST activity was not affected. In the groups on the standard diet or for those on the HCh diet, atorvastatin did not affect the activities of aminotransferases. This suggests that the administration of atorvastatin did not
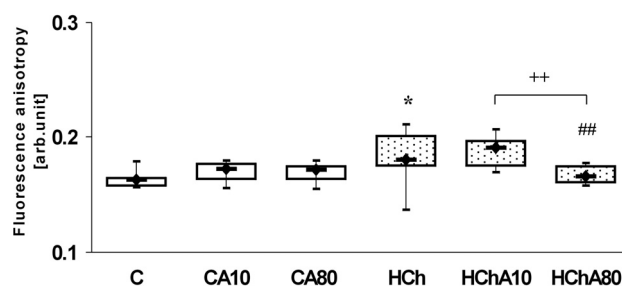
impair the membranes of hepatocytes. However, the drug significantly influenced membrane fluidity in the liver mitochondria of rats on the HCh diet. It has long been known that exogenously induced changes in the molar ratio of cholesterol/phospholipid of cell membranes induced by a change in cholesterol content may affect a number of membrane properties. Further, lipophilic compounds, such as atorvastatin, are able to interact with membranes in a transient manner, which itself may change membrane dynamics. Although the extent to which atorvastatin alters mitochondrial function *in vivo* is not yet understood, it is anticipated to alter both CoQ levels (and thus antioxidant defence) and membrane lipid composition, which might depress oxygen consumption and energy production in the mitochondria. Our study was not aimed at investigating whether, and to which extent the diet- or drug-induced alterations in mitochondrial cholesterol/phospholipid ratio (and concomitantly in mitochondrial membrane fluidity) could be directly responsible for the impairment of mitochondrial function. However, we attribute the observed alterations in mitochondrial bioenergetics to the drug-induced decrease in the concentration of CoQ, since fast lateral diffusion of small lipophilic quinones is assumed to be fluidity-independent (Lenaz 1987). To summarize the above: administration of the high dose of atorvastatin resulted in a reduction of tChol in the plasma and liver of rats on the HCh diet, and significantly reduced the degree of steatosis and diet-induced membrane rigidity in liver mitochondria.

These findings are consistent with clinical studies showing that atorvastatin is beneficial in patients with NASH and NAFLD (Gómez-Domínguez *et al.* 2006, Kimura *et al.* 2010). Atorvastatin effectively improves NAFLD hyperlipidemia and inhibits liver steatosis in rats on a high-fat diet (Ji *et al.* 2011).

Statins have been known to reduce circulating CoQ in animal models (Willis *et al.* 1990) and humans (Folkers *et al.* 1990, Rundek *et al.* 2004). In our study, we found a decreased concentration of CoQ in the plasma following the higher dose of atorvastatin in the animals given a standard or HCh diet. Reduction of CoQ may be considered as a significant adverse effect of statin therapy. Moreover, unsatisfactory synthesis of CoQ may reduce plasma and liver antioxidant capacity and negatively influence the membranes as they are the primary targets of reactive oxygen species. Oxidation can affect lipid fluidity, permeability and consequently the function of the membrane. Thus, since the majority of CoQ in hepatocytes is present in membrane systems including mitochondrial ones (Bentinger *et al.* 2003), and since CoQ is an integral part of the so-called Q-cycle in the electron transport chain, any decrease in the endogenous synthesis of CoQ will lead to a decreased capacity of the respiratory chain. Moreover, modifications of membrane structure and dynamics change the working environment for mitochondrial enzymes and redox carrier molecules, which might also contribute to impaired mitochondrial bioenergetics.

Fat accumulation is associated with ultrastructural abnormalities of liver mitochondria. Electron microscope images have revealed that mitochondria in NAFLD are large and swollen, scarce in number, and the matrix is hypodense with paracrystalline inclusions. These mitochondrial changes were found in a mouse model with a fatty acid oxidation defect and hepatic steatosis (Wei *et al.* 2008). Hepatic mitochondrial abnormalities in patients (Caldwell *et al.* 1999) and rats (Rector *et al.* 2010), with hepatic steatosis and reduced mitochondrial oxidative capacity in the livers of diabetic rats on HCh diet (Uličná and Volkovová 1996) were found.

This was also confirmed by our results on the respiratory function of mitochondria isolated from livers of rats on a HCh diet. These mitochondria showed a reduced rate of oxygen consumption, phosphorylation rate and RCR in comparison with the group on a standard diet. Atorvastatin significantly reduced oxidative phosphorylation parameters in the rats on a standard diet.

In the HCh groups, treatment with atorvastatin at both doses was associated with a decrease in RCR, which indicates a lowered coupling of the oxidation to phosphorylation. Oxygen consumption rate in state 3 was decreased only at the higher dose of atorvastatin. However, it should be stressed that the lowest values of the monitored parameters were found in the mitochondria isolated from livers of animals on the HCh diet following the higher dose of atorvastatin where, in fact, the extent of steatosis was vastly reduced. Concentrations of oxidized and reduced forms of CoQ were lowered in the liver mitochondria of rats on the standard as well as the HCh diet following atorvastatin treatment. We assume that the decreased capacity of the respiratory chain may be a consequence of a reduced synthesis of CoQ. Tavintharan *et al.* (2007) found that simvastatin at higher concentrations decreased mitochondrial CoQ_{10} levels, reduced ATP synthesis and increased DNA oxidative damage and the rate of HepG2 cell death. Treatment of the HepG2 cell line with simvastatin at a dose up to 10 μmol/l reduces HMG-CoA reductase activity in a dose-dependent manner. The authors concluded that simvastatin does not significantly affect the permeability of the mitochondrial inner membrane to ions, however, it does reduce ATP content. Since CoQ_{10} supplementation protected the hepatic cells line they suggest that its deficiency plays an important role in statin-induced hepatopathy. At the present time we cannot unambiguously assign our findings as direct contributors to the observed loss of efficiency in the electron transport chain. Conceivably, observed changes in CoQ levels and mitochondrial membrane lipid fluidity and function are interrelated in a complex manner.

In conclusion, we found a decreased CoQ_9 content and reduced capacity of the respiratory chain and rate of ATP synthesis in the liver mitochondria after administration of atorvastatin *in vivo*. We were unable to find research dealing with the *in vivo* effects of atorvastatin on mitochondrial function in rat liver. We revealed an association between decreased CoQ in the plasma, depletion of mitochondrial CoQ and, at the same time, decreased respiratory function of the liver mitochondria of rats given standard and HCh diets with atorvastatin. We attribute the observed alterations in mitochondrial bioenergetics to the reduced CoQ concentration, since it appears unlikely that ubiquinone diffusion can be the rate-limiting step in electron transfer. A dose-dependent effect of atorvastatin on mitochondrial CoQ depletion and compromised bioenergetic function

might play an important role in statin-induced hepatopathy. Our results contribute to existing views concerning adverse effects of statins on the liver, mainly those associated with the use of higher doses.

Conflict of Interest
There is no conflict of interest.

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