Simvastatin Decreased Coenzyme Q in the Left Ventricle and Skeletal Muscle but not in the Brain and Liver in L-NAME-Induced Hypertension

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Running title: Simvastatin decresed myocardial CoQ level in NO-deficient hypertension

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Summary

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (statins) have been proven to effectively reduce cholesterol level and morbidity and mortality in patients with coronary heart disease and also in primary prevention in patients with dyslipoproteinemia. Statins inhibit synthesis of mevalonate, a precursor of both cholesterol and coenzyme Q (CoQ). Inhibited biosynthesis of CoQ may be involved in some undesirable actions of statins. We investigated the effect of simvastatin on tissue CoQ concentrations in the rat model of NO-deficient hypertension induced by chronic L-NAME administration. Male Wistar rats were treated daily for 6 weeks with L-NAME (40 mg/kg) or with simvastatin (10 mg/kg), another group received simultaneously L-NAME and simvastatin in corresponding doses. Coenzyme Q_9 and Q_{10} concentrations were analyzed by high performace liquid chromatography. L-NAME and simvastatin alone had no effect on CoQ concentrations. However, simultaneous application of L-NAME and simvastatin significantly decreased concentrations of both CoQ homologues in the left ventricle and slightly decreased CoQ_9 concentration in the skeletal muscle. No effect was observed on CoQ level in the liver and brain. We conclude that administration of simvastatin in the condition of NO-deficiency reduced the level of CoQ in the heart and skeletal muscle what may participate on adverse effect of statins under certain clinical conditions.

Key words: Simvastatin; NO-deficient hypertension; tissue coenzyme Q; L-NAME

Introduction

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase - statins have been proven to effectively reduce cholesterol level and morbidity and mortality in patients with coronary heart disease and also in primary prevention in patients with dyslipoproteinemia without evident ischemic heart disease. Effects of statin therapy beyond their lipid lowering potential are called pleiotropic effects involving improvement of endothelial function, antioxidant and antiinflamatory properties, imunosupressive activity and effect against pathological remodelling of the heart and vessels (Endres 2006, Harst *et al.* 2006, Simko 2007).

Pleiotropic effect, however, need not be only beneficial, but also neutral and even undesirable. Statins inhibit synthesis of mevalonate, a precursor of both cholesterol and coenzyme Q (CoQ). Indeed, inhibited biosynthesis of CoQ may be involved in some undesirable action of statins (Harst *et al.* 2006), such as liver injury, muscular weakness and even rhabdomyolysis (Bliznakov 2002). Coenzyme Q is an essential part of mitochondrial respiratory chain responsible for ATP production and statins could affect mitochondrial function and worsen the skeletal and cardiac muscle function (Mitchell 1991, De Pinieux *et al.* 1996, Crane 2001). A number of studies confirmed increased risk of coenzyme Q_{10} deficiency in patients with heart failure and improvement the patient´s symptoms with CoQ_{10} supplementation (Folkers 1993, Morisco *et al.* 1993, Gottlieb *et al*. 2000, Tousoulis *et al.* 2007). Moreover, coenzyme Q besides its bioenergetic function in mitochondria is acting as scavenger of free radicals in membranes and its deficit in tissues can contribute to the decreased antioxidant protection

and worsened function of affected organs. Previously we found depletion of CoQ_{10} in plasma and endomyocardial biopsies related to rejection episodes in transplanted heart patients (Kucharská *et al*. 1998), decreased mitochondrial concentrations of CoQ and increased lipoproteinperoxidation in the heart and liver of the rats with streptozotocin-induced diabetes (Kucharská *et al*. 2000), and reduced concentrations of both CoQ homologues in brain mitochondria of spontaneously hypertensive rats (Kucharská *et al*. 2005).

The aim of this study was to show the effect of simvastatin on tissue coenzyme Q concentrations in condition of NO-deficient hypertension induced by L-NAME administration.

Material and Methods

Male Wistar rats 15 weeks old were divided into four groups, each consisting of 8 rats: 1/ Control animals (C) 2/ L-NAME (L) was given in the dose 40 mg/kg/day in tap water for 6 weeks 3/ Simvastatin was given in the dose 10 mg/kg/day in tap water for 6 weeks (S) 4/ L-NAME (NG-nitro-L-arginine methyl ester) and simvastatin were administered simultaneously as in the groups 3 and 4 (LS). The animals were fed a standard pellet diet ad libitum. All experiments were carried out according to guidlines for the care and use of laboratory animals.

The systolic blood pressure was measured by non-invasive tail-cuff plethysmography (Hugo Sachs Elektronik KG, Freiburg, Germany) in the sixth week At the end of experiment, animals were sacrified by decapitation, tissues samples 30-100 mg from myocardial left ventricle (LV), skeletal muscle, brain and liver were weighed and frozen until analyses. Concentrations of oxidized

forms of coenzyme Q_9 (CoQ_9) and Q_{10} (CoQ_{10}) were determined by isocratic high-performance liquid chromatography (HPLC) according to Lang *et al.* (1986) with some modifications as follows. Tissues were homogenized in water with addition of t-butylhydroxytoluene (BHT) and sodium dodecylsulphate (SDS) and extracted twice by mixture of hexane/ethanol (5/2, v/v). All steps of sample preparation were carried out in the dark. Collected organic layers were evaporated under nitrogen, the residue was taken up in ethanol and injected on the column SGX C18 7μm (Tessek Ltd, Czech Republic). The mobile phase consisted of methanol/acetonitrile/ethanol (6/2/2, v/v/v). Concentrations of compounds were detected spectrophotometrically at 275 nm using external standards (Sigma, Germany). Data were collected and processed using CSW32 chromatographic station (DataApex Ltd, Czech Republic). The results were evaluated using Student´s t-test for unpaired data, values of $p<0.05$ were considered statistically significant.

Results

After six weeks of L-NAME administration systolic blood pressure (SBP) increased significantly in comparison with controls (182.4 ± 0.31 vs 123.8 ± 2.06 mm Hg, $p<0.001$). In the L-NAME + simvastatin (LS) group SBP decreased to 162.2 ± 1.46 mm Hg but was higher than in controls ($p<0.001$) (Fig. 1).

L-NAME and simvastatin had no significant effects on coenzyme Q_9 and Q_{10} concentration in the left ventricle, skeletal muscle, liver and brain tissues in comparison with control rats (Fig. 2, 3, 4, 5). However, the simultaneous application of L-NAME and simvastatin significantly decreased concentrations of

CoQ_9 and CoQ_{10} in the left ventricle of myocardium when compared to controls (126.0 ± 11.7 vs 204.0 ± 18.6 nmol/g ww and 11.2 ± 1.73 vs 17.5 ± 1.88 nmol/g ww, respectively) (Fig. 2). In the skeletal muscle, CoQ_9 concentration decreased marginally significant in LS group 19.1 ± 1.47 nmol/g ww compared to controls (26.7 ± 3.12 nmol/g ww, $p<0.069$) (Fig. 3).

The concentrations of CoQ were not changed in the liver and brain tissues in LS group (Fig. 4 and 5).

Discussion

We investigated the effect of simvastatin on coenzyme Q concentrations in different tissues in condition of NO-deficient hypertension induced by L-NAME administration. L-NAME and simvastatin alone had no effect on CoQ concentrations. However, simultaneous application of L-NAME and simvastatin significantly decreased concentrations of both CoQ homologues in the left ventricle and slightly decreased CoQ_9 concentration in the skeletal muscle. No effect was observed on CoQ levels in the kidney and brain.

A number of studies documented the effect of statins on coenzyme Q concentrations in animals. Lovastatin treatment during 4 weeks (400 mg/kg) decreased CoQ_9 and CoQ_{10} concentrations in the blood, heart and liver of rats, and supplementation with CoQ_{10} completely reversed these effects (Willis *et al.* 1990). Nakahara *et al.* (1998) reported decreased skeletal muscle ubiquinone content in rabbits treated with simvastatin (50 mg/kg) and pravastatin (100 mg/kg) for 4 weeks. However, mitochondrial activities were not affected.

Similar results were achieved with cerivastatin in rats. It was observed the elevation of plasma level of creatine kinase (a clinical marker of myopathy), histologically proved damaging of myofibrills and slightly decreased CoQ_9 in the skeletal muscle, yet without any changes in mitochondria (Schaefer *et al.* 2004). The dose 50 mg/kg of simvastatin used by Fukami *et al.* (1993) in rabbits did not affect skeletal muscle ubiquinone content, but severe lesions in skeletal muscles associated with abnormal elevation of creatine kinase and lactate dehydrogenase activities occured. These authors found significantly reduced ubiquinone content in the liver and cardiac muscle. Others observed decreased content of ATP and creatine phosphate in the heart muscle of the rats treated with simvastatin for 30 days (Pisarenko *et al.* 2001) or myopathy and worsening of myocardial function in rabbits, when simvastatin was administered for 14 days (50 mg/kg) (Jasinska *et al.* 2006).

Although a number of studies demonstrated a significant decline in plasma coenzyme Q_{10} level in patients treated with statins (Folkers *et al*. 1990, Watts *et al*. 1993, De Pinieux *et al.* 1996, Rundek *et al*. 2004), only few studies investigated effect of statins on tissue coenzyme Q in humans and with contradictive findings. Laaksonen *et al.* (1996) found no significant changes in muscle high-energy phosphates and CoQ_{10} concentrations in muscle biopsies of hypercholesterolemic patients treated with simvastatin (20 mg/day) for 6 months and Lamperti *et al.* (2005) found only a mild-decrease in muscle CoQ_{10} concentration in patients with statin-related myopathy. On the other hand hig-dose of simvastatin (80 mg/day) administered for 8 weeks decreased CoQ_{10} concentration and respiratory chain enzyme activities in human skeletal muscle biopsies (Päivä *et al.* 2005). Moreover authors of these studies suggested that

serum and muscle ubiquinone are differently regulated and that serum ubiquinone levels can not be used as a marker for tissue ubiquinone level. In addition, lactic acidosis and higher blood lactate/pyruvate ration observed in patients treated with statins indicate an interference of statins with the mitochondrial respiratory chain (De Pinieux *et al.* 1996, Goli *et al*. 2002), leading potentially to energy depletion and oxidative stress, damaging muscles and other tissues (Parker *et al.* 2003, Al-Ruzzeh *et al.* 2004).

In our experiment, the sole use of simvastatin did not induce a significant change in CoQ concentration in the left ventricle, skeletal muscle, brain or kidney. It is generally known that statin induced myopathy is dose dependent. The dose of simvastatin was lower in our experiment (10 mg/kg/day), compared to 50 mg/kg/day used by Nakahara *et al.* (1998) or Fukami *et al.* (1993) in rabbits, what migh have determined the different effects of simvastatin on skeletal muscle observed in these experiments.

On the other hand, the simultaneous treatment with simvastatin and L-NAME decreased CoQ_9 and CoQ_{10} in the LV in this experiment. Previously we have shown that in L-NAME-induced hypertension simvastatin partly prevented the decrease of nitric oxide synthase (NOS) activity in the brain and kidney induced by L-NAME, however, not in the LV (Simko *et al.* 2004). It may be supposed that decreased NOS activity in the LV with presumed reduction of NO synthesis and attenuated vasodilatative ability of coronary arteries may increase the oxidative stress in the LV. The free radicals production may even be supported by decreased CoQ concentration, having antioxidant and free radicals scavenging properties. Enhanced oxidative stress may have participated on the

development of the hypertrophy and fibrosis of the LV observed in our previous work despite chronic simvastatin treatment (Simko *et al.* 2004).

We conclude that administration of simvastatin in the condition of NO-deficiency reduced the level of coenzyme Q in the heart and skeletal muscle what may participate on adverse effect of statins under certain clinical condition.

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References

AL-RUZZEH S, SCHMIDT I, NAKAMURA K, CHESTER A, ILSLEY C, AMRANI M: Cerivastatin does not prevent oxidative injury of human aortic endothelial cells. *Tex Heart Inst J* **31**: 127-131, 2004.

BLIZNAKOV EG: Lipid-lowering drugs (statins), cholesterol, and coenzyme Q_{10}. The Baycol case - a modern Pandora´s box. *Biomed Pharmacother* **5**6: 56-59, 2002.

CRANE FL: Biochemical functions of coenzyme Q_{10}. *J Am Coll Nutr* **20**: 591-598, 2001.

DE PINIEUX G, CHARIOT P, AMMI-SAID M, LOUARN F, LEJONC JL, ASTIER A, JACOTOT B, GHERARDI R: Lipid-lowering drugs and mitochondrial function: effects of HMG-CoA reductase inhibitors on serum ubiquinone and blood lactate/pyruvate ratio. *Br J Clin Pharmacol* **42**: 333-337, 1996.

ENDRES M, LAUFS U: Effects of statins on endothelium and signalling mechanisms. *Stroke* **35** (Suppl 1): 2708-2711, 2006.

FOLKERS K: Heart failure is a dominant deficiency of coenzyme Q_{10} and challenges for future clinical research on coenzyme Q_{10}. *Clin Investig* **71**: 551-554, 1993.

FOLKERS K, LANGSJOEN P, WILLIS R, RICHARDSON P, XIA LJ, YE CQ, TAMAGAWA H: Lovastatin decreases coenzyme Q levels in humans. *Proc Natl Acad Sci USA* **87**: 8931-8934, 1990.

FUKAMI M, MAEDA N, FUKUSHIGE J, KOGURE Y, SHIMADA Y, OGAWA T, TSUJITA Y: Effects of HMG-CoA reductase inhibitors on skeletal muscles of rabbits. *Res Exp Med (Berl)* **193**: 263-273, 1993.

GOLI AK, GOLI SA, BYRD RP Jr, ROY TM: Simvastatin-induced lactic acidosis: a rare adverse reaction? *Clin Pharmacol Ther* **72**: 461-464, 2002.

GOTTLIEB SS, KHATTA M, FISHER ML: Coenzyme Q_{10} and congestive heary failure. *Ann Intern Med* **133**: 745-746.

HARST P, VOORS AA, GILST WH, BÖHM M, VELDHUISEN DJ: Statins in the treatment of chronic heart failure: Biological and clinical considerations. *Cardiovasc Res* **71**: 443-454, 2006.

JASIŃSKA M, OWCZAREK J, ORSZULAK-MICHALAK D: Influence of simvastatin at high dose and nifedipine on hemodynamic parameters in rabbits. *Pharmacol Reports* **58**: 48-59, 2006.

KUCHARSKÁ J, GVOZDJÁKOVÁ A, MIZERA S, BRAUNOVÁ Z, SCHREINEROVÁ Z, SCHRAMEKOVÁ E, PECHÁŇ I, FABIÁN J: Participation of coenzyme Q_{10} in the rejection development of the transplanted heart: a clinical study. *Physiol Res* **47**: 399-404, 1998.

KUCHARSKÁ J, BRAUNOVÁ Z, ULIČNÁ O, ZLATOŠ L, GVOZDJÁKOVÁ A: Deficit of coenzyme Q in heart and liver mitochondria of rats with streptozotocin-induced diabetes. *Physiol Res* **49**: 411-418, 2000.

KUCHARSKÁ J, SUMBALOVÁ Z, GVOZDJÁKOVÁ A, BADA V, ANDRIANTSITOHAINA R, BERNÁTOVÁ I, PECHÁŇOVÁ O: Red wine polyphenolic compounds prevented depletion of brain mitochondrial coenzyme Q in spontaneously hypertensive rats. Possible mechanism of brain protection in hypertension? *Physiol Res* **54**: 55P, 2005.

LAAKSONEN R, JOKELAINEN K, LAAKSO J, SAHI T HARKONEN M, TIKKANEN J, HIMGERG JJ: The effect of simvastatin treatment on natural antioxidants in low-density lipoproteins and high-energy

phosphates and ubiquinone in skeletal muscle. *Am J Cardiol* **15**: 851-854, 1996.

LAMPERTI C, NAINI AB, LUCCHINI V, PRELLE A, BRESOLIN N, MOGGIO M, SCIACCO M, KAUFMANN P, DI MAURO S: Muscle coenzyme Q_{10} levels in statin-related myopathy. *Arch Neurol* **62**: 1709-1712, 2005.

LANG JK, GOHIL K, PACKER L: Simultaneous determination of tocopherols, ubiquinols, and ubiquinones in blood, plasma, tissue, and subcellular fractions. *Anal Biochem* **157**: 106-116, 1986.

MITCHELL P: The vital protonmotive role of coenzyme Q. In: *Biomedical and Clinical Aspects of Coenzyme Q*. K FOLKERS, GP LITTARRU, T YAMAGAMI (eds), Vol. 6, Elsevier Science Publisher, Amsterdam, 1991, pp 3-10.

MORISCO C, TRIMARCO B, CONDORELLI M: Effect of coenzyme Q_{10} therapy in patients with congestive heart failure: a long-term multicenter randomized study. *Clin Investig* **71** (Suppl 8): S134-136, 1993.

NAKAHARA K, KURIYAMA M, SONODA Y, YOSHIDOME H, NAKAGAWA H, FUJIYAMA J, HIGUCHI I, OSAME M: Myopathy induced by HMG-CoA inhibitors in rabbits: a pathological, electrophysiological, and biochemical study. *Toxicol Appl Pharmacol* **152**: 99-106, 1998.

PÄIVÄ H, THELEN KM, VAN COSTER R, SMET J, DE PAEPE B, MATTILA KM, LAAKSO J, LEHTIMÄKI T, VON BERGMANN K, LÜTJOHANN D, LAAKSONEN R: High/dose statins and skeletal muscle metabolism in humans: A randomized, controlled trial. *Clin Pharmacol Ther* **78**: 60-68, 2005.

PARKER RA, HUANG Q, TESFAMARIAN B: Influence of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors on endothelial nitric oxide synthase and the formation of oxidants in the vasculature. *Atherosclerosis* **169**: 19-29, 2003.

PISARENKO OI, STUDNEVA IM, LANKIN VZ, KONOVALOVA GG, TIKHAZE AK, KAMINNAYA VI, BELENKOV YN: Inhibitor of beta-hydroxy-beta-methylglutaryl coenzyme A reductase decreases energy supply to the myocardium in the rats. *Bull Exp Biol Med* **132**: 956-958, 2001.

RUNDEK T, NAINI A, SACCO R, COATES K, DI MAURO S: Atorvastatin decreases the coenzyme Q_{10} level in the blood of patients at risk for cardiovascular disease and stroke. *Arch Neurol* **61**: 889-892, 2004.

SCHAEFER WH, LAWRENCE JW, LOUGHLIN AF, STOFFREGEN DA, MIXSON LA, DEAN DC, RAAB CE, YU NX, LANKAS GR, FREDERICK CB: Evaluation of ubiquinone concentration and mitochondrial function relative to cerivastatin-induced skeletal myopathy in rats. *Toxicol Appl Pharmacol* **194**: 10-23, 2004.

ŠIMKO F: Statins - a perspective for left ventricular hypertrophy treatment (review). *Europ J Clin Invest* **37**:681-691, *2007.*

ŠIMKO F, MATÚŠKOVÁ J, LUPTÁK I, KRAJČÍROVIČOVÁ K, KUCHARSKÁ J, GVOZDJÁKOVÁ A, BABÁL P, PECHÁŇOVÁ O: Effect of simvastatin on remodeling of the left ventricle and aorta in L-NAME-induced hypertension. *Life Sci* **74**: 1211-1224, 2004.

TOUSOULIS D, CHARAKIDA M, STEFANADI E, SIASOS G, LATSIOS G, STEFANADIS C: Statins in heart failure. Beyond the lipid lowering effect. *Int J Cardiol* **115**: 144-150, 2007.

WATTS GF, CASTELLUCIO C, RICE-EVANS C, TAUB NA, BAUM H, QUINN PJ: Plasma coenzyme Q (ubiquinone) concentrations in patients treated with simvastatin. *J Clin Pathol* **46**: 1055-1057, 1993.

WILLIS RA, FOLKERS K, TUCKER L, YE CH-Q, XIA L-J, TAMAGAWA H: Lovastatin decreases coenzyme Q levels in rats. *Proc Natl Acad Sci* **87**: 8928-8930, 1990.

Figure captions

Figure 1 Effect of L-NAME, simvastatin and L-NAME + simvastatin on systolic blood pressure after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M., **p<0.001.

Figure 2 Effect of L-NAME, simvastatin and L-NAME + simvastatin on coenzyme Q_9 and Q_{10} concentrations in the myocardial left ventricle after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M., *p<0.05.

Figure 3 Effect of L-NAME, simvastatin and L-NAME + simvastatin on coenzyme Q_9 and Q_{10} concentrations in the skeletal muscle after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M., m.s. - marginally significant.

Figure 4 Effect of L-NAME, simvastatin and L-NAME + simvastatin on coenzyme Q_9 and Q_{10} concentrations in the liver after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M.

Figure 5 Effect of L-NAME, simvastatin and L-NAME + simvastatin on coenzyme Q_9 and Q_{10} concentrations in the brain after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M.

Figure 1

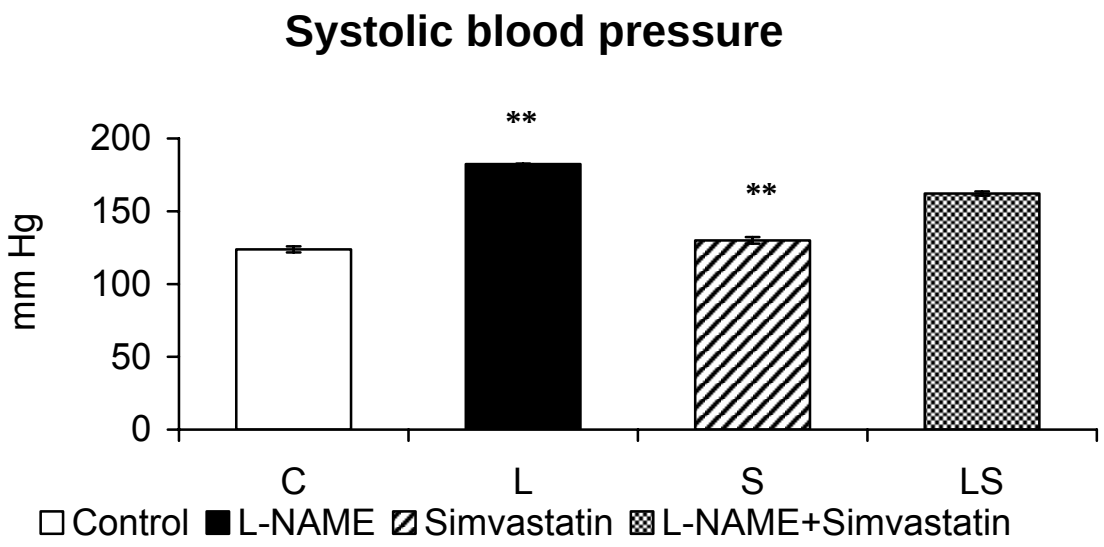

Systolic blood pressure
mm Hg
200
150
100
50
0
**
**
C
L
S
LS
Control
L-NAME
Simvastatin
L-NAME+Simvastatin

Figure 2

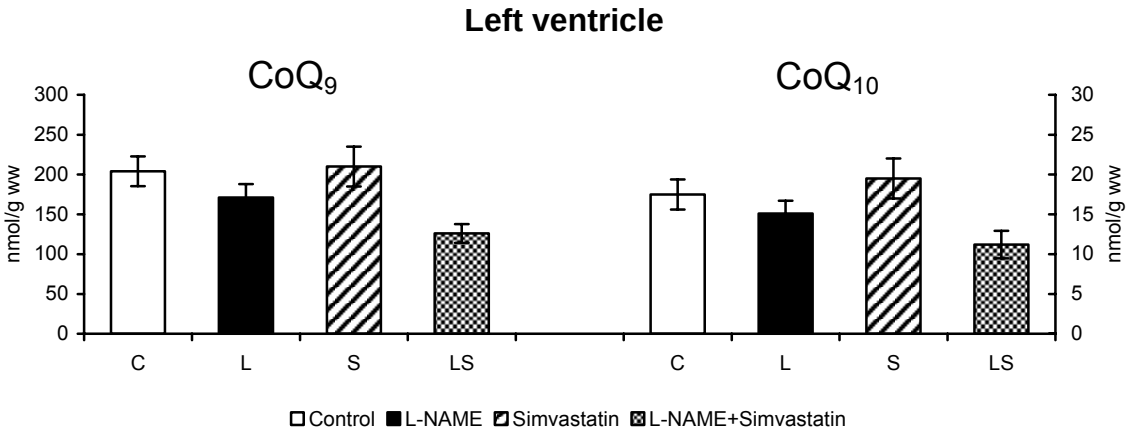

Left ventricle
CoQ9
CoQ10
300
250
200
150
100
50
0
30
25
20
15
10
5
0
nmol/g ww
nmol/g ww
C
L
S
LS
C
L
S
LS
Control
L-NAME
Simvastatin
L-NAME+Simvastatin

Figure 3

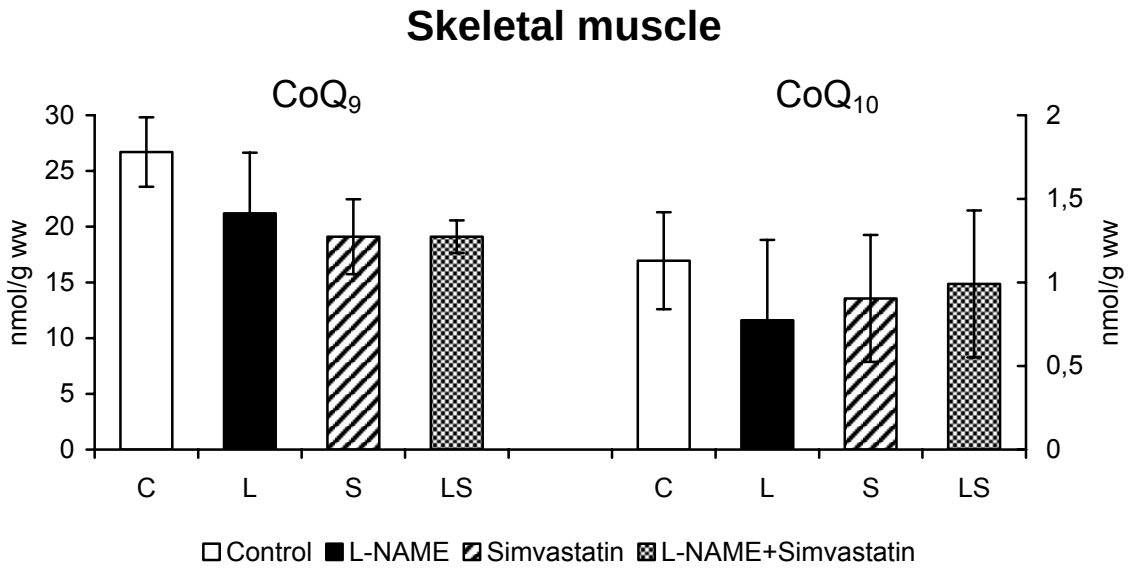

Skeletal muscle
CoQ9
CoQ10
nmol/g ww
nmol/g ww
30
25
20
15
10
5
0
2
1,5
1
0,5
0
C
L
S
LS
C
L
S
LS
Control
L-NAME
Simvastatin
L-NAME+Simvastatin

Figure 4

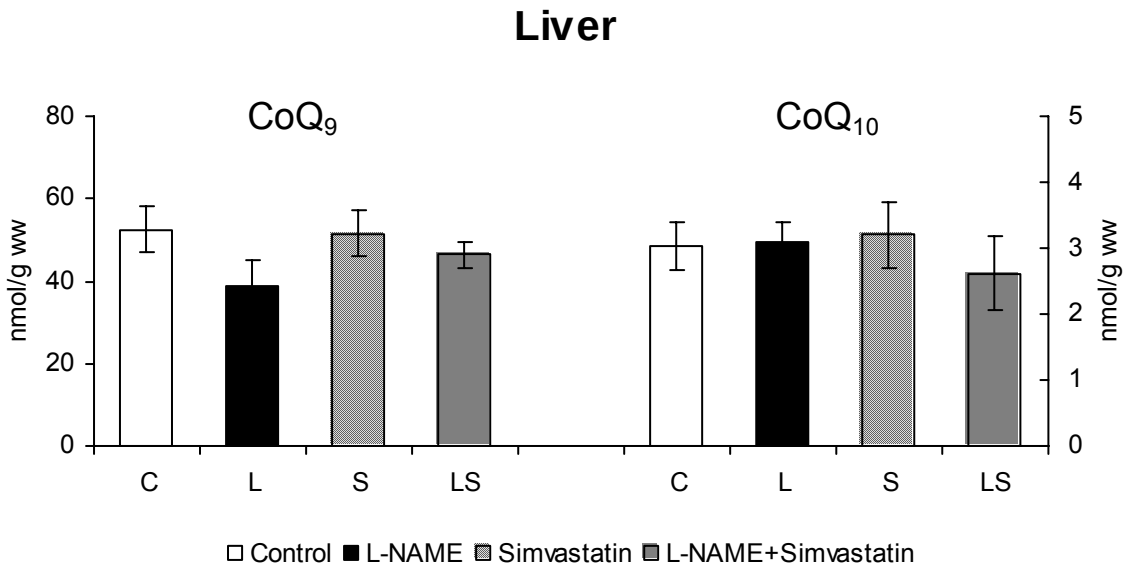
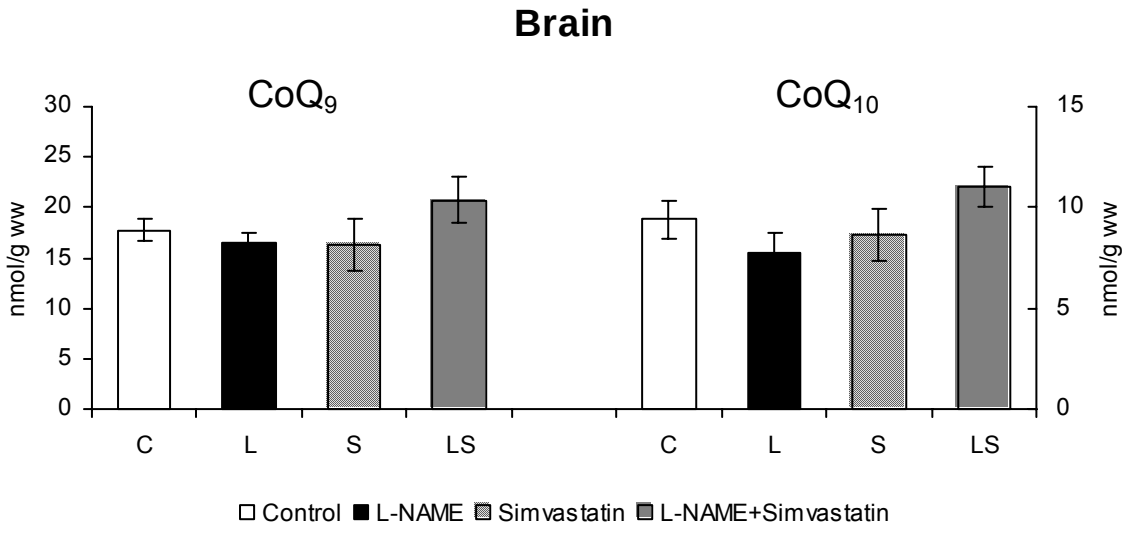

Liver
CoQ9
CoQ10
nmol/g ww
nmol/g ww
80
60
40
20
0
5
4
3
2
1
0
C
L
S
LS
C
L
S
LS
Control
L-NAME
Simvastatin
L-NAME+Simvastatin

Figure 5


Brain
CoQ9
CoQ10
nmol/g ww
nmol/g ww
30
25
20
15
10
5
0
15
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5
0
C
L
S
LS
C
L
S
LS
Control
L-NAME
Simvastatin
L-NAME+Simvastatin