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Statin-induced Myopathy and Ubiquinone Levels in Serum – Results from a Prospective, Observational Study

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Abstract: It has been suggested that an impaired ubiquinone (Q10) synthesis may be responsible for muscular side effects caused by statins. The primary aim of this study was to investigate whether low Q10 levels in serum could be used as a marker to predict the risk of developing statin-induced myopathy. The secondary aim was to compare the change in Q10 levels during statin treatment and differences between men and women. Serum samples from a prospective, observational study in statin-treated patients who were thoroughly followed regarding muscular symptoms were used. In this cohort, 16 developed myopathy and 126 had no muscular symptoms related to statin treatment. Q10 levels were measured with a novel LC-MS method at baseline and after 2 months of statin treatment. Q10 levels showed no correlation with the risk of developing statin-induced myopathy. Individuals with low levels, Q10 < 200 ng/ml, at baseline had no increased risk of developing myopathy. In consistence with earlier reports, we showed that Q10 levels were reduced by 30% during statin treatment. There was no significant difference in the reduction between patients with or without myopathy. Women had approximately 30% lower Q10 levels compared to men both before and after treatment. In this study, there was no association between Q10 levels at baseline and statin-induced muscular side effects during a 2-month follow-up period, and our results indicate that Q10 levels in serum is not a useful marker to predict statin-induced myopathy.

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, are used for treatment of hypercholesterolaemia and have a pronounced, preventive effect on serious cardiovascular events and mortality [1,2]. Today, statins are some of the most prescribed drugs in the Western world, and the consumption is steadily increasing [3]. However, several reports have demonstrated that statin-induced muscular side effects such as myalgia are more common than first demonstrated in clinical trials. In fact, 10–15% of patients prescribed statins may experience muscle symptoms [4–6]. The statin-induced myopathy is considered to be dose dependent [7] but independent of the cholesterol-lowering effect [8].

Statins affect not only the synthesis of cholesterol but also all products in the cholesterol biosynthesis pathway.

Ubiquinone (Q10) is one of these products. The major part of the intracellular Q10 is present in the inner mitochondrial membrane as an essential part of the electron transport chain [9,10]. A smaller fraction is present in the cytosol where it has antioxidative functions [9,11]. Q10 is also present in the blood and is mainly bound to low-density lipoprotein (LDL) and also, to some extent, to high density lipoprotein (HDL) and very low-density lipoprotein (VLDL) [12].

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It has been suggested that the decreased synthesis of Q10 may be involved in statin-induced myopathy [13,14]. In particular, the reduction of mitochondrial Q10 has been suggested to cause myopathy, and statin treatments have been shown to negatively affect the mitochondrial function in skeletal muscle [15]. In contrast, we have recently shown that statin treatment of rats particularly reduces the hepatic cytosolic fraction of Q10 [9].

Several clinical studies have shown that statin treatment reduces Q10 levels in human serum (reviewed in [13]) and the reduction of Q10 varied between 20% and 50% in the different studies. The hypothesis that decreased levels of Q10 during statin treatment might cause adverse events has been investigated, but results have been inconclusive [16–18].

The primary aim of this study was to investigate whether low Q10 levels in serum could be used as a marker to predict the risk of developing statin-induced myopathy. The secondary aim was to compare the change in Q10 levels during statin treatment and differences between men and women. To this end, we used serum samples from a clinical study in which 180 patients starting statin treatment had been followed for 1 year regarding muscular symptoms. Q10 levels were measured with a novel LC-MS method developed by us with higher sensitivity and specificity than currently available methods.

Material and Methods

Study population, cases and controls. We analysed serum from a previously performed clinical study in which 180 patients starting on

statin treatment had been followed for 1 year regarding muscular symptoms with questionnaires. Blood samples had been drawn at baseline and after approximately 2 months of statin treatment. The participants filled in a questionnairy regarding muscular pain at baseline and at every study visit, approximately every third month, up to 1 year after inclusion. Written informed consent was obtained from all participants. The study was approved by the local Ethics Committee (Dnr: 2006/431-31/2) and was performed in accordance with the declaration of Helsinki. Briefly, to define myopathy, we used the criteria of the American College of Cardiology (ACC), American Heart Association (AHA) and National Heart, Lung and Blood institute (NHLBI) [19]. Symptoms meeting these criteria were assessed and classified as 'probable' or 'possible' according to WHO. To be classified as a patient 'with myopathy', only patients with 'probable' were included. To be classified as a patient 'without myopathy', only those with neither 'possible' nor 'probable' were included.

From the original study of 180 patients, 142 patients were included in this study. There were 17 patients with obvious muscular symptoms assessed to be 'probably' related to statin therapy. Thus, 17 patients were defined as 'patients with myopathy'. However, serum samples from one of these patients were missing, and 16 patients with myopathy could be included in this study. Patients with muscular symptoms that were not 'probably' related to statin therapy were excluded (n = 15). One hundred and twenty-six patients from the original cohort had no statin-related muscular symptoms (assessed as possible or probable) but could have other adverse reactions. These were defined as 'patients without myopathy'. Patients lost to follow-up or whose serum samples were missing were excluded (22 controls, 1 case mentioned above). All patients with and without myopathy had CK values within the normal range throughout the study. Compliance to treatment was assured as all patients had decreased cholesterol levels.

Ubiquinone measurements. All chemicals and reagents were of the highest purity available. Methanol, 2-propanol (J.T. Baker, Denventer, the Netherlands) and 1-propanol (Merck KGaA, Darmstadt, Germany) were of HPLC grade. Co-enzyme standards Q10 and Q9 as internal standard were obtained from Sigma-Aldrich (St. Louis, MO, USA). For preparation of standards, Q10 and Q9 powder was dissolved in toluene (Merck KGaA, Darmstadt, Germany) and further diluted with 2-propanol.

Q10 extraction. One hundred microlitre 100 µl of 1-propanol, containing 100 ng/ml of Q9 internal standard, was added to 50 µl of serum, then vortexed for 1 min. and the solution centrifuged at 5000 × *g* for 5 min. to separate proteins from solution. The liquid phase was extracted three times with n-hexane (250 µl), and the organic phases, which contain 1-propanol and hexane, were pooled and evaporated to dryness under argon atmosphere; then, the extract was reconstituted with 150 µl of a 33:67 water:1-propanol mixture and analysed.

Liquid chromatography/mass spectrometry (LC-MS). The samples were run on Waters 2695 separation module coupled with Micromass Quattro micro mass spectrometer (QAA452). 100 µl of each sample (temperature was 6°C) was injected into the liquid chromatograph inlet via autosampler. Separation was performed on a Waters SunFire column (C18, 3.5 µm, 4.6 × 100 mm) at 40°C, using solvents A (95% methanol, 5% 2-propanol) and B (100% 2-propanol). The gradient at a flow of 1.2 ml/min. was as follows: isocratic A solvent for 3 min., gradient from 100%A to 70%A and 30%B for 1 min., then isocratic 50%A and 50%B for 4 min., gradient from 50%A and 50%B to 100%A for 1 min. and isocratic 100%A for 1 min. The total time was 10 min.

The mass spectrometer was optimized for different Qs using positive atmospheric pressure chemical ionization mode (APCI+). Probe temperature was 525°C, and single ion monitoring (SIR) of 795.60 m/z with cone voltage 24 V was used for Q9 and for Q10 SIR of 863.60 m/z with cone voltage 40 V was used. Retention times were 7.01 and 7.80 min. for Q9 and Q10, respectively. The concentrations of Q10 were estimated on the basis of adequate standard curve (R^2 = 0.994) using standards with MassLynx V4.1 software (Waters Sverige AB, Sollentuna, Sweden).

Statistical analysis. All statistical tests were performed using GraphPad Prism v. 6.00, and values of $p < 0.05$ were considered statistically significant. The data had a Gaussian distribution. A paired, two-tailed t-test was used in all patients to investigate whether there was any statistically significant difference between values prior to, and during treatment. For analysis of patients with and without myopathy and women and men, an unpaired two-tailed t-test was used. The bars in the figures and the figures in the text show mean ± S.E.M. To evaluate whether Q10 levels less than 200 or 100 ng/ml could be used as a predictor for statin-induced myopathy, Fisher's exact test was used.

Results

Baseline demography.

Sixteen patients with myopathy and 126 patients without myopathy were included in this study. The average blood pressure in the cohort was systolic 139 ± 19 and diastolic 81 ± 10 mm Hg. The average BMI was 27.21 ± 4.44. The average cholesterol levels were before treatment 6.8 ± 1.1 and 6.6 ± 1.1 mmol/l among the patients without and with myopathy. The average decrease in cholesterol levels was 28% ± 13% and 24% ± 8% among patients without and with myopathy, respectively.

Twelve myopathy patients (75%) and 61 of the patients without myopathy (48%) were women. The median age among patients with myopathy was 65 years (range 39–86) and among patients without myopathy 65 years (range 32–89). The patients with myopathy were all treated with a daily dose of simvastatin, 10–40 mg, average dose 25.3 mg. Of the 126 patients without myopathy, 72 were treated with a daily dose of simvastatin (10–40 mg), 40 with rosuvastatin (5–20 mg), 11 atorvastatin (10–40 mg), two with 20 mg pravastatin and one with 10 mg fluvastatin.

Q10 in patients with and without myopathy.

At baseline, the mean Q10 level in all participants was 222 ng/ml (±12) ranging from 48 to 1076 ng/ml. After 2 months of treatment, the mean Q10 levels had decreased to 155 ng/ml (±7) corresponding to an average decrease of 30% (fig. 1). There was no statistically significant difference between the Q10 levels in patients with and without myopathy at baseline (195 ± 32 and 226 ± 13) or after statin treatment; 159 ± 26 ng/ml compared to 161 ± 7 ng/ml (fig. 2). The average reduction of Q10 levels was 20% in patients with myopathy and 30% in patients without myopathy, a difference that was not statistically significant.

Q10 in women and men.

At baseline, the mean level of Q10 was significantly lower in women (n = 73) than in men (n = 69), 190 ± 13 ng/ml

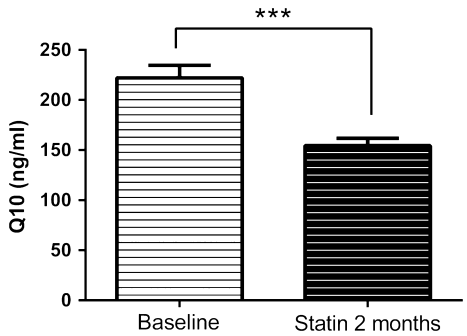

Fig. 1. Mean levels + S.E.M. of Q10 in serum from all (n = 142) individuals at baseline and after 2 months of statin treatment. The decrease of more than 30% during statin treatment was statistically significant (***$p < 0.001$).

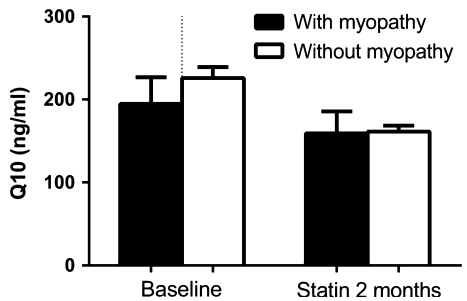

Fig. 2. Mean levels + S.E.M. of Q10 in serum from patients with myopathy (n = 16) and without myopathy (n = 126). There was no difference in mean Q10 level between cases and controls at baseline or during statin treatment.

compared to 256 ± 21 ng/ml ($p < 0.01$). This difference was also present after statin treatment; mean level of Q10 in women was 129 ± 8 ng/ml compared to 182 ± 11 ng/ml ($p < 0.001$) in men (fig. 3). Women had approximately 30% lower Q10 levels than men.

Low levels of Q10 levels as a predictor of statin-induced myopathy.

To evaluate whether baseline Q10 levels less than 200 or 100 ng/ml could be used as a predictor for statin-induced myopathy, Fisher's exact test was used. Eleven cases and 73 controls had Q10 levels < 200 ng/ml at baseline. The statistical analysis showed no increased risk of myopathy among the individuals with Q10 levels < 200 ng/ml; relative risk 1.56; 95% CI 0.57–4.23; $p = 0.43$. Four cases and fourteen controls had Q10 levels < 100 ng/ml, and no increased risk of myopathy among the individuals with Q10 levels < 100 ng/ml could be observed; relative risk 2.30; 95% CI 0.83–6.36; $p = 0.12$.

Discussion

Here, we demonstrate that baseline Q10 levels showed no correlation with risk of developing statin-induced myopathy in 142 Swedish patients in primary health care. In consistence with earlier reports, we showed reduced Q10 levels during

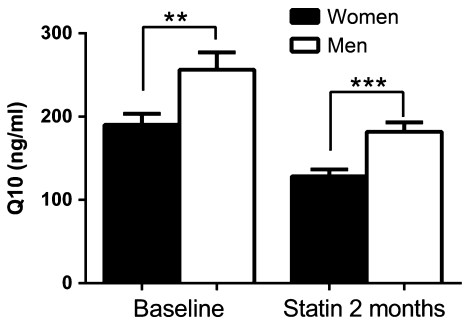

Fig. 3. Mean levels + S.E.M. of Q10 levels in serum in women (n = 73) and men (n = 69) before and after 2 months of statin treatment. Women had statistically significantly lower levels of Q10 than men both before and during statin treatment (**$p < 0.01$; ***$p < 0.001$).

statin treatment by about 30%. We also demonstrated that women generally had lower Q10 levels compared to men, approximately 30% lower levels, and women and men had the same percentage of decrease in their Q10 levels during statin treatment.

The hypothesis that decreased levels of Q10 during statin treatment might cause adverse events in general and muscular symptoms in particular has been debated for a long time, and the question was raised already in 1989 [20]. Attempts to treat or avoid statin-induced adverse events by Q10 supplementation have also been performed. In two small intervention studies, n = 44 [18] and n = 49 [17], patients on statin therapy were randomized to Q10 or placebo for 12 or 16 weeks, respectively. No correlation between Q10 supplementation and change in myalgia score [18] or creatine kinase (CK) values [17] were found in these two studies. In a third study, 76 patients on statin therapy who had developed new onset myalgia were randomized to 120 mg Q10 daily or placebo for 3 months in combination with continued statin therapy [16]. No protective effect of Q10 supplementation could be demonstrated in this study either.

However, all these three studies were small and probably underpowered to be conclusive regarding the effect of Q10 supplementation and statin-induced myopathy.

In a large clinical study where 10 mg rosuvastatin or placebo was given to 1191 patients (the Corona study), Q10 levels of all participants were measured [21]. In this study, the primary aim was to investigate whether Q10 was an independent predictor of prognosis in heart failure, and adverse events were not in main focus. As expected, Q10 levels were reduced by rosuvastatin treatment, but low levels of Q10 were not associated with a worse outcome regarding heart failure or all-cause mortality [21]. In this study, low Q10 levels were not associated with less adverse reactions. However, muscular symptoms seem not to have been thoroughly studied.

The strength of the present prospective study, compared to previously performed studies, is that the patients were thoroughly followed regarding muscular symptoms with interviews and questionnaires and not only with CK measurements and the careful evaluation if the myalgia was associated with statin

treatment or not. For example, all of the 'patients with myopathy' had myalgia despite CK values within the normal reference interval throughout the study.

A limitation of this study, as well as of previously performed studies, is that Q10 levels are measured in serum. Q10 is transported by LDL and a statin-induced reduction of LDL levels will also lead to reduction in the ability to transport Q10 in the blood [12]. Q10 is mainly present in the mitochondria, but the mitochondrial Q10 is not available for analysis. The role of Q10 in serum is not fully known, but it has most probably an antioxidative function. To which extent serum levels correlate with intracellular levels, and especially mitochondrial levels, of Q10 is not clear.

Animal studies have shown that statin treatment leads to reduced Q10 levels in liver [9] and cardiac tissue [22], while the results in skeletal muscle are more inconclusive [22–24]. How the intracellular Q10 levels are affected during statin treatment in human beings is not thoroughly investigated, although a small study in 19 simvastatin-treated individuals indicated that there was no change in Q10 levels in skeletal muscle tissue during statin treatment [25].

In conclusion, we did not find any association between low Q10 levels and statin-induced myopathy in this study. Our results indicate that the Q10 level in serum cannot serve as a marker to predict statin-induced myopathy.

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