Childhood initiated statin therapy in familial hypercholesterolemia

Avis, H.J.

Publication date
2013

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date: 27 Jul 2023
ROSUVASTATIN LOWERS COENZYME Q10 LEVELS, BUT NOT MITOCHONDRIAL ADENOSINE TRIPHOSPHATE SYNTHESIS, IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA


ABSTRACT

Objective: To investigate whether statin therapy affects coenzyme Q10 (CoQ10) status in children with heterozygous familial hypercholesterolemia (FH).

Study design: Samples were obtained at baseline (treatment naïve) and after dose titration with rosuvastatin, aiming for a low-density lipoprotein cholesterol level of 110 mg/dL. Twenty-nine patients were treated with 5, 10, or 20 mg of rosuvastatin for a mean period of 29 weeks.

Results: We found a significant (32%) decrease in peripheral blood mononuclear cell (PBMC) CoQ10 level (P = .02), but no change in PBMC adenosine triphosphate synthesis (P = .60). Uncorrected plasma CoQ10 values were decreased significantly, by 45% (P < .01). In contrast, ratios of plasma CoQ10/total cholesterol and CoQ10/low-density lipoprotein cholesterol remained equal during treatment.

Conclusions: In children with FH, rosuvastatin causes a significant decrease in cellular PBMC CoQ10 status but does not affect mitochondrial adenosine triphosphate synthesis in children with FH. Further studies should address whether (rare) side effects of statin therapy could be explained by a deterioration in CoQ10 status.
INTRODUCTION

Statins are among the most widely prescribed drugs worldwide, due to their ability to prevent cardiovascular diseases in various populations at risk.\textsuperscript{1-4} Initiation of statin therapy during childhood is recommended in children with familial hypercholesterolemia (FH).\textsuperscript{5,6} Statins are remarkably well tolerated in both adults and children, although some side effects, including myalgia and fatigue, have been reported in a small proportion of patients.\textsuperscript{7,8}

Statins lower plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic cholesterol synthesis.\textsuperscript{9} HMG-CoA reductase is also the first committed and rate-limiting enzyme for the synthesis of a range of other compounds, including coenzyme Q10 (CoQ10).\textsuperscript{10} CoQ10 plays an important role as an electron carrier in mitochondrial respiratory chain–driven adenosine triphosphate (ATP) synthesis.\textsuperscript{11} Furthermore, the reduced form of CoQ10 (ubiquinol or CoQ10H\textsubscript{2}) serves as an important cellular antioxidant, protecting membranes and plasma lipoproteins against free radical–induced oxidation.\textsuperscript{12} Thus, inhibition of HMG-CoA reductase activity by statin treatment might result in a diminution of endogenous CoQ10 synthesis and failure of mitochondrial energy metabolism, as well as a decreased cellular antioxidant capacity. Interestingly, CoQ10 deficiency due to inherited defects in its biosynthesis has been associated with progressive muscle weakness and central nervous system dysfunction in early childhood.\textsuperscript{13-15}

Few studies have assessed the effect of statin treatment on cellular CoQ10 status or subsequent metabolic consequences.\textsuperscript{11} To assess the true consequences of statin therapy for CoQ10 synthesis and mitochondrial function, we measured the effect of rosuvastatin therapy on intracellular CoQ10 level and on respiratory chain–driven ATP synthesis in children with FH.

METHODS

The study was embedded in the PLUTO study,\textsuperscript{16} a multicenter trial set out to investigate the efficacy and safety of rosuvastatin therapy in pubertal children aged 10-17 years with heterozygous FH. It comprised two phases: a 12-week double-blind, placebo-controlled phase in which participants were randomized to placebo or 5, 10, or 20 mg
of rosvastatin, and a 40-week open-label phase in which the rosvastatin dose was
titrated to achieve a target LDL-C level of 110 mg/dL, with a maximum dose of 20 mg.

For this study on CoQ10, we invited all subjects included in one of the participating
centers (Academic Medical Center, Amsterdam) to participate. Because of the small
amount of subjects randomized per treatment group in this single-study center, we
chose to analyze baseline and end-of-study values, to increase the statistical power.
From those subjects who agreed to participate, additional blood samples were collected
after an overnight fast at baseline (treatment naïve) and at the end of the study, when all
subjects were treated with rosvastatin, after dose titration. In a random sample of 17
subjects, mitochondrial respiratory chain–driven ATP formation and CoQ10 level were
measured in peripheral blood mononuclear cells (PBMCs). All subjects were instructed
to adhere to a low-fat diet and maintain habitual physical activity throughout the entire
trial. The study was approved by the institutional ethics review board. All subjects and
their parents gave written informed consent for participation.

The primary outcome of the study was the absolute change in intracellular CoQ10 level
as measured in PBMCs. Secondary outcomes were the number of subjects with a PBMC
CoQ10 level outside the reference range of 37-103 pmol/mg protein, the correlation
between changes in PBMC CoQ10 and plasma creatine phosphokinase (CPK), alanine
aminotransferase (ALT), and aspartate aminotransferase (AST) levels and changes in
PBMC mitochondrial ATP synthesis, plasma CoQ10 level, and TC, LDL-C, and high-density
lipoprotein cholesterol (HDL-C) concentrations. In addition, plasma CoQ10 levels were
corrected for TC and LDL-C levels by calculating CoQ10/TC and CoQ10/LDL-C ratios.

For PBMC and plasma CoQ10 measurements, whole blood collected in a tube with EDTA
was separated into plasma and PBMCs directly after sampling using standard procedures,
as described previously. Plasma was frozen in liquid nitrogen and stored at -80°C. Isolated
PBMCs were washed with phosphate-buffered saline, and then suspended in 150 mL of
phosphate-buffered saline and stored at -80°C for later determination of CoQ10 level.

CoQ10 levels in plasma and in PBMC suspension were measured by high-performance
liquid chromatography analysis using ultraviolet detection at 275 nm, following the
method of Duncan et al. Protein concentration was determined according to method
of Lowry et al. For measurement of ATP synthesis, PBMCs were isolated from citrated
blood, as described earlier. Subsequently, 20 mL of a PBMC suspension was incubated in
a total volume of 100mL containing 150 mM KCl, 25 mM Tris (pH 7.4), 2 mM EDTA, 10
mM potassium phosphate (pH 7.4), 1 mM ADP, 40 μg/mL digitonin, 10 mM glutamate,
10 mM malate, and 1 mg/mL bovine serum albumin, as described previously. The reaction was allowed to proceed for 30 minutes at 25°C, followed by termination with perchloric acid (0.5 M). In the neutralized protein-free perchloric acid extracts, ATP was measured fluorimetrically as described previously. With this method, ATP formation has been shown to be fully suppressed by the addition of respiratory chain inhibitors, such as rotenone and antimycin, demonstrating that only mitochondrial respiratory chain–driven ATP formation is measured. LDL-C was estimated by the Friedewald formula.

We aimed to retrieve data for the primary outcome in at least 24 patients. This would result in 80% power to detect a difference in mean PBMC CoQ10 level of 30 nmol/L, assuming a SD of 50 nmol/L, and using a paired t test with a 0.05 two-sided significance level. Correlations between plasma CoQ10 and TC and LDL-C levels and between PBMC CoQ10 and CPK, ALT, and AST levels were assessed using Pearson correlation tests. Differences in outcome measures between baseline and the end of the study were compared using the paired-sample t test. Subsequently, dose response effects were determined for PBMC and plasma CoQ10 levels and TC, LDL-C, and HDL-C levels. For outcomes with similar baseline values for the different treatment groups, this was done by analysis of covariance, with the rosuvastatin dose administered at the end of the study as the fixed factor and baseline values as a covariant. When baseline characteristics were different, differences in the change from baseline to the end of the study were assessed by analysis of variance. For all statistical tests, a P value of <.05 was considered significant. All statistical analyses were performed using SPSS version 13.0.2.1 (SPSS Inc, Chicago, Illinois). Power calculations were performed using nQuery Advisor version 7.0 (Statistics Solutions Ltd, Cork, Ireland).

RESULTS

In total, 35 subjects proved eligible for participation in the PLUTO study at our study site. Of these, 1 patient did not consent to participate in the present study, and 2 patients were excluded because no samples were obtained at baseline. We did not have end-of-study values in 3 patients who withdrew consent during the study, 1 due to lack of time, 1 due to mild nausea after 21 weeks of treatment with 5 mg rosuvastatin, and 1 due to complaints of fatigue after 33 weeks of treatment with 5 mg rosuvastatin. Thus, a sample of 29 patients remained available for CoQ10 analyses. In 17 of these patients, we assessed mitochondrial respiratory chain–driven ATP synthesis in PBMCs. Analysis of PBMC CoQ10 failed for two patients, and measurement of plasma CoQ10 failed in one patient.
During the randomized placebo-controlled phase, 5 patients were treated with placebo, 9 were treated with rosuvastatin 5 mg, 7 were treated with 10 mg, and 8 were treated with 20 mg. Dose titration in the open-label phase resulted in 5 patients being treated with rosuvastatin 5 mg, 4 being treated with 10 mg, and 20 being treated with 20 mg. In the open-label phase, subjects had been treated with their last and maximum rosuvastatin dose for a mean period of 29 weeks (range, 7-40 weeks). Fifteen subjects were male, and 14 were female. The mean (± SD) age at randomization was 14.4 ± 1.9 years.

**Table. Changes in intracellular and plasma CoQ10 levels, ATP synthesis, and lipoproteins.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coenzyme Q10 (n=28)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMC (pmol/l)</td>
<td>89.36 ±59.00</td>
<td>-29.04 ±64.04, -32% p=0.02</td>
</tr>
<tr>
<td>Plasma (umol/l)</td>
<td>668.21 ±280.03</td>
<td>-299.65 ±241.97, -45% p&lt;0.01</td>
</tr>
<tr>
<td>CoQ10/TC ratio</td>
<td>85.69 ±30.38</td>
<td>-8.87 ±38.73, -10% p=0.25</td>
</tr>
<tr>
<td>CoQ10/LDL-C ratio</td>
<td>111.88 ±41.78</td>
<td>13.88 ±64.74, 12% p=0.28</td>
</tr>
<tr>
<td>ATP synthesis (n=17)</td>
<td>13.04 ±5.38</td>
<td>0.88 ±6.81, 7% p=0.60</td>
</tr>
<tr>
<td>(umol/(min.mg))</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lipoproteins (n=29)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>304 ±67</td>
<td>-120 ±51, -40% p&lt;0.01</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>51 ±12</td>
<td>1 ±5.2% p=0.90</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>236 ±61</td>
<td>-120 ±47, -51% p&lt;0.01</td>
</tr>
</tbody>
</table>

Data are shown as mean ±SD, % change. ATP means adenosine triphosphate; CoQ10 coenzyme Q10; PBMC peripheral blood mononuclear cell; TC total cholesterol; LDL-C low-density lipoprotein cholesterol; HDL-C high-density lipoprotein cholesterol.

**PBMC CoQ10**

There was a significant drop in mean PBMC CoQ10 level (of 29 ± 64 pmol/mg [P=.02], from 89 ± 59 pmol/mg to 63 ± 21 pmol/mg), during rosuvastatin treatment (Table and Figure 1). Baseline PBMC CoQ10 levels did not differ among the dose-titrated treatment
groups. There was a significant difference in end-of-study CoQ10 levels, corrected for baseline values, between the rosuvastatin 5 mg and 10 mg groups (P = .02). This difference was not present between other treatment groups, however, and no trend for a dose-related effect of rosuvastatin on PBMC CoQ10 level was observed. The decrease in PBMC CoQ10 level during the study was 27 ± 73 pmol/mg in subjects titrated to rosuvastatin 5 mg, 37 ± 51 pmol/mg in those titrated to rosuvastatin 10 mg, and 85 ± 67 pmol/mg in those titrated to rosuvastatin 20 mg. At baseline, 6 patients (20%) had a PBMC CoQ10 level below the reference range of 37-133 pmol/mg and 7 (24%) had a level above this range. At the end of the study, 3 patients (10%) had a level below this range, and none had a level above this range. We found no correlation between changes in PBMC CoQ10 level and changes in CPK, ALT, or AST level. There was no significant difference in changes between male and female participants.

**Figure 1. CoQ10 levels in PBMC's at baseline and end of study.**

**PBMC Mitochondrial ATP Synthesis**

The results displayed in the Table and Figure 2 demonstrate no change in the capacity of mitochondrial respiratory chain-driven ATP synthesis from baseline [mean 13 ± 5 nmol/(min-mg)] to the end of the study [14 ± 7 nmol/ (min-mg)] (P = .60).

**Plasma CoQ10 Level**

Mean plasma CoQ10 level, uncorrected for plasma lipid level, decreased significantly, from 668 ± 280 nmol/l to 354 ± 154nmol/L (P < .01) (Table and Figure 3). Analyses of covariance revealed a significant difference between those treated with rosuvastatin 10 mg and those on rosuvastatin 20 mg at the end of the study (P = .03), but not between the other rosuvastatin dosage groups. The decline in plasma CoQ10 was 263 ± 269 nmol/L in the 5 mg group, 272 ± 126 nmol/L in the 10 mg group, and 316 ± 262 nmol/L in the 20
mg group. The rosuvastatin dose at the end of the study was not significantly associated with uncorrected end-of-study plasma CoQ10 level (P = .05). There was no significant difference in changes in plasma CoQ10 level between male and female participants.

**Figure 2. PBMC ATP synthesis at baseline and end of study.**

**Figure 3. Plasma CoQ10 levels at baseline (BL) and end of study (EOS), uncorrected and after correction for TC and LDL-C.**

**Plasma Lipid Levels**

Plasma concentrations of both TC and LDL-C decreased significantly over the study period (P < .01). No change in HDL-C level was observed (P = .90) (Table). There was a clear dose-response effect for both TC and LDL-C levels (P < .01).
CoQ10/Plasma Lipid Ratio
Plasma TC and CoQ10 levels at baseline and at the end of the study were significantly correlated ($P = 0.05$ and $0.04$, respectively). There was a trend for correlation between plasma LDL-C and CoQ10 levels ($P = 0.08$ and $0.13$, respectively). No significant changes occurred in the plasma CoQ10/TC and CoQ10/LDL-C ratios from baseline to the end of the trial (Table and Figure 3).

Discussion
This study reveals evidence of a decrease in intracellular CoQ10 concentration following treatment with the HMG-CoA reductase inhibitor rosuvastatin. Nonetheless, the observed 32% decrease in PBMC CoQ10 level did not perturb mitochondrial respiratory chain-driven ATP synthesis in pediatric FH patients.

Patients with inborn errors of CoQ10 metabolism and resulting impaired oxidative phosphorylation have been reported with residual CoQ10 tissue levels ≤25% compared with controls.$^{13,22}$ This implies that cellular CoQ10 levels must fall below a certain threshold level before mitochondrial ATP synthesis becomes impaired, and that in our study, the deficit in CoQ10 status was insufficient to perturb ATP synthesis. Our finding of no increase in the number of children with a PBMC CoQ10 level below the reference range after rosuvastatin treatment is in agreement with this hypothesis. It has been suggested that the drop in intracellular CoQ10 level after statin treatment reflects a treatment-induced decrease in mitochondrial volume or enrichment.$^{23}$ This hypothesis is contradicted, however, by our finding that mitochondrial ATP synthesis was similar at baseline and after rosuvastatin treatment, despite a significant drop in PBMC CoQ10 level.

The decrease in uncorrected plasma CoQ10 level during statin therapy observed in this study is consistent with previous reports.$^{24}$ However, when plasma CoQ10 level is standardized to either TC or LDL-C level, the drop in plasma CoQ10 is nullified. This strongly suggests that the declining plasma CoQ10 status after rosuvastatin treatment solely reflects decreases in plasma lipoproteins, the major carriers of CoQ10 in the circulation,$^{25}$ which is in line with most previous studies investigating the influence of statin therapy on plasma CoQ10 level.$^{24}$

Our study population comprised children with FH, aged 10-17 years. We consider this population of specific interest for a study on the effect of statin treatment on CoQ10 status. First, because these patients are generally clinically healthy, they have no history of atherosclerotic cardiovascular disease or other conditions associated with CoQ10 depletion, such as cardiac failure,$^{26}$ which limits confounding by comorbidity. Second,
children with FH will have a very long exposure to statin treatment, given that current guidelines recommend initiating statin treatment in children with FH at as young as 8 years of age.\textsuperscript{5} We found that a decrease in PBMC CoQ10 level could be observed after a minimum statin exposure of 40 weeks. Although a deterioration in CoQ10 status did not perturb ATP synthesis, the effects of longer-term treatment are uncertain. Furthermore, in view of the reported association between skeletal muscle and PBMC CoQ10 status,\textsuperscript{17} the diminished PBMC CoQ10 status after rosvastatin therapy also might be reflected in the skeletal muscle of these patients. Few studies have assessed the CoQ10 status of patients experiencing myotoxicity after statin therapy. Lamperti et al\textsuperscript{27} reported a mild decrease in skeletal muscle CoQ10 status (2 SD below the normal mean in 3 patients and >1 SD below normal in 7 patients) in 10 out of 18 patients who experienced statin-related myopathy.\textsuperscript{27} In addition, a decrease in skeletal muscle CoQ10 status together with a deficiency in cytochrome oxidase activity was reported in 2 patients with severe myotoxicity after statin treatment.\textsuperscript{28} The existing data on the use of CoQ10 supplementation in the treatment of statin-induced myopathy is scarce and contradictory. Caso et al\textsuperscript{29} reported an association between supplementation with CoQ10 and significantly reduced myopathic pain in patients receiving statin therapy. In contrast, a study assessing the effect of CoQ10 supplementation on statin tolerance and myalgia in patients presenting with statin-related myalgia found no beneficial effects of supplementation on myalgia scores or statin tolerance.\textsuperscript{30} The precise role of the involvement of CoQ10 deficiency in the pathophysiology of statin-related myotoxicity remains uncertain, and further studies are warranted to address this issue. A recent study suggested that a deficit in serum 25 (OH) vitamin D level also may be involved in the myotoxicity associated with statin treatment;\textsuperscript{31} vitamin D level was not assessed in the present study, however.

Although we found a significant overall change in PBMC CoQ10 level, we could not identify a dose-related effect. This might have been due to our study’s lack of statistical power. At the end of the study, only 5 subjects were treated with rosvastatin 5 mg and 4 were treated with rosvastatin 10 mg, compared with 20 subjects on rosvastatin 20 mg. Also, the limited size of our study did not allow for comparison between rosvastatin-treated and placebo-treated subjects regarding CoQ10 level and ATP synthesis in the first randomized study phase. From a methodological perspective, this would have been ideal to correct for unknown confounders. A well-known confounder for plasma CoQ10 level is diet;\textsuperscript{32} however, in this trial, subjects were explicitly instructed to maintain a regular diet.

Unfortunately, we were unable to measure CoQ10 levels and ATP synthesis in the 3 subjects who discontinued the study. These patients, 2 of whom experienced side
effects, might have responded differently to the study drug with respect to our outcomes compared with those who continued rosuvastatin treatment. Another disadvantage of the study design is that the duration of treatment with various doses of rosuvastatin differed between subjects. Nevertheless, all subjects were treated with some dose of rosuvastatin for at least 40 weeks and received their maximum dose for at least 7 weeks, with a mean treatment duration with the maximum dose of 29 weeks.

Treatment with other statins also might be expected to cause a decrease in intracellular CoQ10 status. However, there are important pharmacological differences between statins, such as regarding lipophilicity. Rosuvastatin is relatively hydrophilic and thus may have a limited capacity to pass cell membranes and influence cellular metabolism compared with more lipophilic statins.24

We do not know the extent to which our findings are applicable for treatment with other statins. Future studies should investigate a possible role of statin-related CoQ10 depletion in clinical adverse outcomes experienced by patients on statin therapy.
REFERENCES


