CINRG PILOT TRIAL OF COENZYME Q10 IN STEROID-TREATED DUCHENNE MUSCULAR DYSTROPHY

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ABSTRACT: Introduction: Corticosteroid treatment slows disease progression and is the standard of care for Duchenne muscular dystrophy (DMD). Coenzyme Q10 (CoQ10) is a potent antioxidant that may improve function in dystrophin-deficient muscle. Methods: We performed an open-label, 'add-on' pilot study of CoQ10 in thirteen 5–10-year-old DMD patients on steroids. The primary outcome measure was the total quantitative muscle testing (QMT) score. Results: Twelve of 16 children (mean age 8.03 ± 1.64 years) completed the trial. Target serum levels of CoQ10 ($\geq$2.5 μg/ml) were shown to be subject- and administration-dependent. Nine of 12 subjects showed an increase in total QMT score. Overall, CoQ10 treatment resulted in an 8.5% increase in muscle strength ($P = 0.03$). Conclusions: Addition of CoQ10 to prednisone therapy in DMD patients resulted in an increase in muscle strength. These results warrant a larger, controlled trial of CoQ10 in DMD.

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Duchenne muscular dystrophy (DMD) is the most common and severe form of muscular dystrophy, affecting 1 in 3500 males. Corticosteroids are the standard of care and slow muscle disease progression.[1] However, it is likely that a "cocktail" of drugs targeting important pathophysiological pathways in DMD (inflammation, metabolic insufficiency, fibrosis, apoptosis, and others) could synergistically provide better disease control than steroids alone.[2] This approach was validated in the dystrophin-deficient *mdx* mouse model.[1,3]

One potential additional therapy in DMD is coenzyme Q10 (CoQ10). CoQ10 is a hydrophobic molecule bound to the inner mitochondrial membrane that functions as an electron acceptor molecule for complexes I (nicotinamide adenine dinucleotide, or NADH) and II (succinate dehydrogenase, or SDH) of the respiratory chain. Exogenous incorporation of CoQ10 into mitochondria can double the oxidative activity of NADH, thus providing metabolic support to muscle.[4] CoQ10 is also a potent antioxidant that is able to reduce the excessive oxidative radicals and calcium overload in DMD muscles.[5,6] In addition, CoQ10 modulates the mitochondrial transition pore and could decrease mitochondrial calcium accumulation.[7] Preclinical studies in exercised *mdx* mice showed that CoQ10 treatment preserved strength by 42% compared with controls (Granchelli, 2000, personal communication).[8]

We conducted this pilot trial to determine whether the addition of CoQ10 to a stable steroid regimen could further preserve or increase muscle strength in DMD. A secondary objective was to find an effective and safe dose of CoQ10 in the pediatric population studied.

METHODS

Trial Design. We conducted a prospective, open-label, pilot study of oral CoQ10 (30- and 100-mg tablets; Myoquinon Q10; IND #62,369; Pharma-Nord, ApS) supplementation in ambulant DMD children between 5 and 10 years of age with an established DMD diagnosis who were on a stable regimen of prednisone for >6 months. Each child served as his own control. Exclusion criteria included severe systemic diseases, and recent or current use of CoQ10, glutamine, creatine, or other herbal supplements.

Participants were recruited from two centers (Children's National Medical Center, Washington, DC, and Washington University, St. Louis, Missouri), with institutional review board approval at each site in accordance with the Helsinki Declaration. After obtaining assent from participants and consent from their parents, participants underwent two screening evaluations. Those able to repeat elbow flexion quantitative muscle testing (QMT) within 15% of their initial performance were enrolled in the study. This inclusion criterion assures that patients enrolled in the study can reliably perform the required testing.[9,10]

Table 1. Average values of primary and secondary outcomes at baseline, end of lead-in period, and treatment month 6 showing increases in muscle strength testing with significant improvements seen in QMT total score at treatment month 6 compared with lead-in 3 ($P = 0.03$).

Measurements	Baseline [mean (SD)]	LI3 [mean (SD)]	TM6 [mean (SD)]	P-values		
				Baseline vs. LI3	LI3 vs. TM6	Baseline vs. TM6
QMT total score (lbs.)	11.6 (2.0)	11.7 (1.8)	12.7 (2.4)	0.75	0.03	0.06
QMT arm score (lbs.)	9.0 (2.0)	9.2 (2.3)	10.0 (2.6)	0.60	0.10	0.09
QMT leg score (lbs.)	13.6 (2.5)	13.8 (2.7)	14.9 (3.2)	0.72	0.11	0.11
QMT grip score (lbs.)	12.6 (3.2)	12.5 (1.7)	13.7 (2.9)	0.94	0.16	0.16
MMT score	237.4 (10.9)	233.8 (12.1)	244.5 (20.9)	0.49	0.35	0.48
Timed walk (s)	5.3 (1.7)	5.3 (1.8)	5.8 (2.9)	0.58	0.24	0.22
Timed climb 4 steps (s)	4.3 (2.9)	4.1 (3.0)	4.5 (4.3)	0.83	0.43	0.74
Timed standing from lying position (s)	4.3 (1.8)	4.2 (1.1)	5.7 (3.8)	0.68	0.12	0.09

LI3, lead-in 3; MMT, manual muscle testing; QMT, quantitative muscle testing; TM6, treatment month 6.

Dose Optimization. Normal serum levels of CoQ10 have been established in adults and pediatric healthy individuals.[11,12] Exogenous supplementation of CoQ10 results in varied elevation of blood levels with those >2.5 μg/ml being considered therapeutic for several disease states.[11,12] We used a dose-escalation design with high-performance liquid chromatography (HPLC) assessment of serum CoQ10 levels with a 2.5-μg/ml target.

Participants underwent 3 months of lead-in evaluation while at their pre-established dose of prednisone, and then began a CoQ10 dose-finding period at a starting dose of 90 mg/day CoQ10 and a dose escalation of 30 mg/day until they achieved a serum CoQ10 level of 2.5 μg/ml. With the initial 3 patients we were unable to rapidly achieve the desired levels, so we modified the protocol to increase the initial dose to 400 mg with dose escalations of 100 mg. In addition, the drug was given with a small amount of fatty snack (ice cream). Once the minimum level was achieved, participants began the 6-month evaluation period, with repeat drug level monitoring at each visit. Dose adjustments were performed as needed, and reductions were only performed if there were side effects considered to be related to the drug. We performed pill count and review of medication diaries as a method to monitor for compliance and possible medication interactions.

Outcome Measures. The primary outcome measure was the total QMT score.[13,14] Maximal isometric muscle strength, measured by the Richmond Quantitative Measurement System (RQMS) of bilateral elbow and knee flexors and extensors, and grip testing was performed.[13] A total QMT score was calculated as the mean of the 10 RQMS scores (grip R/L, elbow extension R/L, elbow flexion R/L, knee extension R/L, and knee flexion R/L). QMT was chosen due to the need for a highly sensitive and reliable method of strength measure-

ment in this small pilot trial. Secondary outcomes were arm, leg, and grip QMT scores; manual muscle testing (MMT) score; timed function tests (time to climb 4 steps, to stand up from a supine position on the floor, and to walk/run 10 meters); left ventricular ejection fraction as measured by two-dimensional (2D) echocardiography (Phillips Sonos 5500); and electrocardiography.[13,15] Safety evaluations included clinical and laboratory monitoring for cell blood count, basic chemistries, and liver function tests.

Statistical Considerations. Total QMT score and secondary outcomes were measured at baseline, end of the lead-in period, and monthly thereafter during the 6 months of treatment (T1–T6). Data were analyzed using paired t-tests (two-sided) on the changes in scores and timed measurements between the end of the treatment cycle and lead-in. $P \leq 0.05$ was considered statistically significant. Missing data at lead-in 3 and at treatment month 6 were replaced by using the last observations carried forward for the QMT muscle scores. This imputation was done for 6 patients. The data in log scale were also analyzed using paired t-tests as references. Linear mixed-effects models were used to investigate the linear trend in the total QMT scores.[16,17]

RESULTS

Sixteen children were screened. Three children did not meet the QMT reliability inclusion criterion. Thirteen children 5–10 years old, mean age 8.03 ± 1.64 years, were enrolled in the lead-in phase, but 1 obese child was removed from the study due to failure to achieve target CoQ10 levels despite several months of dose escalation to up to 800 mg/day. Twelve participants completed the trial.

Table 1 gives the average values of the primary and secondary outcome measures at baseline, end
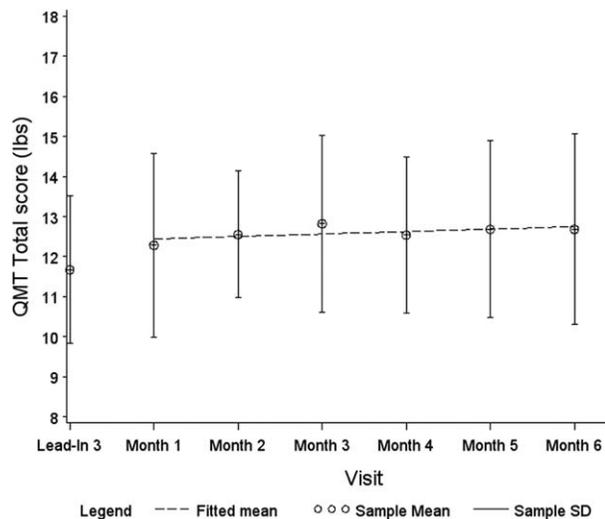
FIGURE 1. Plots of mean ± standard deviation of the observed data at each of the time-points from lead-in 3 to treatment month 6 and fitted trend lines obtained in the trend analysis via mixed-effects models. Lead-in 3 is the period immediately prior to the treatment cycles. Please note the linear trend does not apply to the period from lead-in 3 to treatment month 1, because the time from the end of lead-in 3 to the start of treatment month 1 varies from patient to patient due to the dose escalation required to reach the desired level.

of lead-in period, and end of month 6 for all subjects who completed the study. There was a significant ($P = 0.03$) increase in the average total QMT score from LI3 to the end of treatment (from 11.7 to 12.7 lbs., or 8.5%). Analysis of individual QMT score changes shows an average 1.01-lb. increase in the QMT score from LI3 to treatment month 6 with a standard error of 1.4 lbs. This was reproduced with the analysis in log scale ($P = 0.046$).

Figure 1 shows the observed and fitted values of the total QMT score throughout the treatment period. The total QMT score increased by 0.06 lb./month during the treatment period (slope $P = 0.28$). This trend supports the beneficial effect of CoQ10 on muscle strength.

Of the 12 subjects who completed the study, 9 (age 5.2–10.2 years) showed an increase in total QMT score from baseline. Among them, 6 subjects showed at least a 10% increase in QMT total scores, 1 increased by 8%, 1 increased by 6%, and 1 increased by 2% in QMT total score (Fig. 2). The 3 patients who did not show an improvement were between 7.5 and 8.7 years of age. The percent increase results are generally consistent within the QMT arm (11%), leg (8%), and handgrip (9%) scores. Correlation analysis showed that there was no significant correlation between the individual response and baseline disease severity in total QMT or any other strength parameters. In addition, the spread of the age of the patients who showed some improvement included only 2 children under 7 years of age. This suggests that the

effect measured was not due to a "honeymoon" period.

There were no significant changes in cardiac parameters during the trial. At the start of the trial, the ejection fraction (62 ± 4%), left ventricular internal diameter (3.8 ± 0.2 cm), and posterior wall thickness (0.6 ± 0.1 cm) during diastole and electrocardiograhy were normal and did not change significantly at the end of the trial.

There was no correlation between baseline CQ10 serum levels and disease severity as measured by the baseline total QMT score. There were also no significant correlations between age at baseline or disease severity and response to CoQ10 treatment.

There was significant variability between subjects in achieving target serum concentrations of CoQ10. To achieve the same level, children required doses ranging between 90 and 510 mg/day. The average dose per kilogram of weight at the end of the study was 27.8 mg/kg, ranging from 8.7 to 48.4 mg/kg. Initial CoQ10 serum levels varied between 0.6 and 3.6 μg/ml, when the initial dose was 90 mg, and between 1.3 and 8.1 μg/ml, when the initial dose was changed to 400 mg. Serum levels increased more rapidly and remained more constant when CoQ10 was co-delivered with a small fatty snack, presumably due to increased bioavailability of the CoQ10. Serum levels were maintained between 2.6 and 9.2 μg/ml during the 6-month treatment phase for all subjects. There was no correlation between baseline total QMT score and dose required to maintain the therapeutic level.

There were no serious adverse events. One patient developed a headache of moderate intensity associated with high blood levels of CoQ10
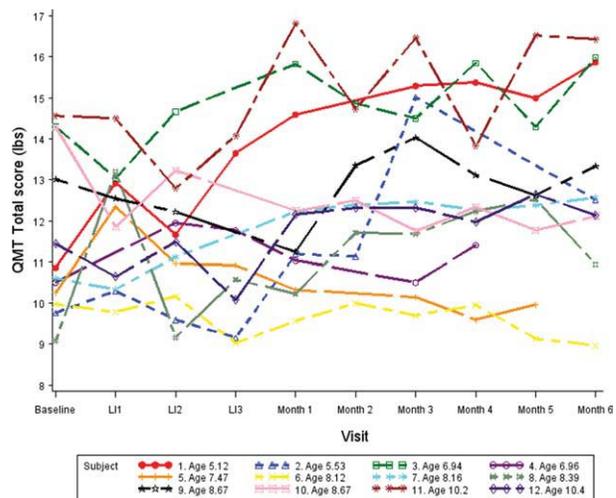

FIGURE 2. Individual QMT total score (lbs.) for 12 patients from baseline to treatment month 6, showing an overall increase in the muscle strength of 9 subjects.

(7.37 μg/ml, dose 400 mg, 20 mg/kg). The event resolved with a decrease of the dose to 200 mg/day (serum level 3.66 μg/ml).

DISCUSSION

This limited study shows the addition of CoQ10 to prednisone treatment in DMD results in an average improvement of QMT total score of 1.0 lb. (8.5%) from LI3 ($P = 0.03$), above and beyond steroid-related improvements. Secondary outcomes, including MMT score and individual QMT scores, all showed improvements. These results suggest that further testing of CoQ10 as an additional therapy in DMD is warranted.

Two previous studies have shown the efficacy of CoQ10 in dystrophinopathies, although quantitative muscle strength was not an outcome measure in either study.[6,18,19] QMT is a very sensitive measure of strength; however, the clinical significance of an 8.5% increase in total score is not yet established. In this study, this magnitude of increase in muscle strength did not relate to an improvement in function. In a DMD prednisone study recently completed by the Cooperative International Neuromuscular Research Group (CINRG), obvious clinical and functional benefits were seen when the QMT total score improved by 15% (paper accepted for publication). A more important point is that there was no further decrease in muscle scores. The participants' average age was almost 9 years and they were absolutely outside a "honeymoon period" and in the deterioration state of the disease, even on prednisone. A recent study by our group showed that children aged 7 years and older show deterioration in QMT scores (unpublished data). So, even stabilization of QMT scores during this 6-month trial could be a beneficial effect of CoQ10. A longer trial duration and larger sample size could help evaluate the effects of this magnitude of strength improvement on function and other less sensitive strength measurements (MMT).

CoQ10 treatment for 6 months was well tolerated in this patient cohort. There was significant variability in the dose required to achieve therapeutic blood levels. From this study, we suggest that the starting dose of CoQ10 should be 400 mg/day, and it should be increased by 100 mg/day according to serum levels. The 400-mg dose was well tolerated and rapidly led to therapeutic levels in most participants. Of importance, we note that CoQ10 absorption was improved when administered with a small fatty snack, usually ice cream. Future administrations of CoQ10 should consider this or a similar method to minimize dosing and optimize serum levels.

We were unable to obtain therapeutic serum levels in 1 obese child. Although the specific reason for this is not known, cholesterol levels can alter plasma levels of CoQ10.[20] Although we performed clinically relevant biochemical tests during treatment that did not show any abnormalities, cholesterol levels were not part of this study and may have played a role with difficult patients. We would recommend cholesterol levels be included in the chemistry panels of a larger, controlled future study.

Although we monitored cardiac function in this study, there was an absence of cardiac abnormalities in our study population. This was likely related to the age of our population and also the concurrent use of steroids, which were shown to preserve cardiac function in a previous study.[21] The effects of CoQ10 on cardiac function in DMD patients still need further investigation.

This study was limited by the small cohort of patients, lack of randomization, and short duration of therapy. However, the effect size found in this pilot trial can be used for power analysis in the design of future studies.

In conclusion, in this study we found an increase in muscle strength in steroid-treated DMD patients given CoQ10 for 6 months. The results are encouraging and indicate a potential role for CoQ10 therapy in young DMD patients, and further controlled, randomized trials are warranted.

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