Effect of Coenzyme Q on Serum Levels of Creatine Phosphokinase in Preclinical Muscular Dystrophy*†

(treatment/bioenergetics)

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ABSTRACT   Coenzyme Q_{10} (CoQ_{10}) exists in human tissue, and is indispensable to mitochondrial enzymes of respiration. CoQ was administered to children with preclinical muscular dystrophy, CoQ enzymology was emphasized, and serum creatine phosphokinase, CPK, (ATP:creatine N-phosphotransferase, EC 2.7.3.2) was repeatedly monitored.

A 40-week treatment of an infant, 1–2 years of age, reduced serum CPK ($P < 0.001$; total CPK assays, 76). A 40-week treatment of a boy, 3–5 years of age, reduced serum CPK ($P < 0.01$); treatment through 80 weeks reduced CPK ($P < 0.001$; total CPK assays, 118).

This response of preclinical dystrophy to CoQ implies a deficiency of CoQ in skeletal muscle that was actually found previously by assay of the activity of the succinate dehydrogenase:coenzyme Q_{10} reductase of the rectus abdominis. The relationships among a CoQ deficiency in muscle, serum CPK, and use of CPK in muscle are uncertain; however, restoration of CoQ enzyme activity in muscle by oral administration of CoQ could lead to increased use of CPK in muscle to form phosphocreatine from creatine and ATP, with a corresponding decrease in serum levels of CPK. The great excess of CPK in serum comes from deteriorating muscle in which CPK is below normal.

Coenzyme Q_n (CoQ_n) consists of a homologous group of quinones, I. Coenzyme Q_{10} is dominant (1) in human tissue. Certain members of the group may biochemically substitute for the dominant CoQ (2). The name, coenzyme Q, is compatible with its vitamin activity which has been demonstrated in several animal species. CoQ is biosynthesized in mammalian tissue from tyrosine by mechanisms of a nutritional nature.
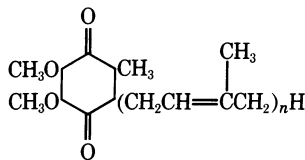
I $n = 1–10$; $n = 10$ for human tissue

Abbreviations: CoQ, coenzyme Q; CPK, creatine phosphokinase.

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The vitamin activity of CoQ has been extensively demonstrated in nongenetic nutritional deficiencies and also in dystrophic mice having a genetic defect. These dystrophic mice may have a coenzyme Q dependency state.

A possible relationship between CoQ and human muscular dystrophy was projected (3) on the basis of the existence of CoQ in muscle, and its indispensable role as an electron transfer moiety in the bioenergetics of the mitochondria which are essential to the vitality and health of muscle. Administration of CoQ to dystrophic patients has been in progress for about 7 years. We now correlate background and new results of the administration of CoQ to preclinical or very early stages of the fatal Duchenne muscular dystrophy.

Coenzyme Q in mitochondrial and other enzymes; importance to muscle function

Biochemical interests in CoQ concern pathways of electron transport. Chart 1, from Lehninger's *Biochemistry* (4) is representative to depict CoQ in respiration. It seems reasonable that a deficiency of CoQ in electron transport enzymes of muscle would impair vitality and health.

CoQ is frequently depicted to function at one site in respiration, but Lenaz *et al.* (2) demonstrated one site for succinoxidase and another for NADH oxidase. A third site was indicated when Salach (5) reported a role for CoQ in mitochondrial α-glycerophosphate oxidase.

Staudinger and Folkers (6) found CoQ_{10} in microsomes from rat liver and porcine adrenal glands. Rajagopalan *et al.* (7) described a cytoplasmic aldehyde oxidase containing CoQ_{10} in rabbit livers, and Weidenbach *et al.* (8) extended the data. Nyquist *et al.* (9) found CoQ in the Golgi apparatus from rat liver, and Fleischer *et al.* (10) found about the same amount of CoQ in Golgi and mitochondria from rat liver, and the presence of CoQ in rough endoplasmic reticulum fractions from bovine liver and pancreas.

A therapeutic benefit of CoQ to human dystrophy could be the resultant of single or multiple improvement of CoQ enzymes of mitochondria, Golgi apparatus, endoplasmic reticulum, or of other cellular components.

Source of energy for muscular function and the role of ATP

Actomyosin of the skeletal muscle cells converts the chemical energy of ATP into mechanical energy. Any impairment within the bioenergetics of respiration and phosphorylation, including a deficiency of CoQ, could reduce the dynamic

properties of muscle, which could then impair physical performance of an individual; such impairment could be at least a part of the genetic mechanism(s) causing muscular dystrophy.

Respiration is not a sole requirement for muscular contraction. A muscle can also perform mechanical work through high-energy phosphocreatine. In skeletal muscle, there may be over five times as much phosphocreatine as ATP. The reaction,

$$\text{phosphocreatine} + \text{ADP} \rightleftharpoons \text{creatine} + \text{ATP}$$

is catalyzed by creatine phosphokinase (CPK, ATP:creatine N-phosphotransferase, EC 2.7.3.2).

Serum CPK assays in dystrophy are elevated and are generally higher when the boy is younger and the disease less advanced. Patient J.B.; about 1 year of age, showed 7029 ± 1569 mU of serum CPK during the initial control period. [CPK was assayed by the Rosalki method (33). Numbers are the mean $\pm$ standard deviation.] A highly elevated serum CPK is generally correlated with highly active dystrophy. Treatment with CoQ, which reduces the serum CPK, could cause a corresponding improvement of abnormal bioenergetics. Since CPK is not being normally utilized in dystrophic muscle to convert phosphocreatine and ADP to ATP, reduction of CPK in the serum could mean increased utilization in muscle with improved formation of ATP and then improved potential for physical performance of the dystrophic individual.

Administration of coenzyme Q to older patients with muscular dystrophy

Sövik *et al.* (11) administered hexahydrocoenzyme Q_4 to four dystrophic boys, 6–11 years of age, but the dosage (50 mg to 1 g daily) now appears low. Decreases of serum CPK and aldolase for one boy were observed. Danowski *et al.* (12, 13) administered hexahydrocoenzyme Q_4 to 19 boys with pseudo-hypertrophic muscular dystrophy who were in early, intermediate, and late stages of dystrophy. Dosage was 250 mg/day for 8 months and than 1 g/day for 4 months. Evaluation of the 19 boys as a single group revealed no physical or biochemical improvement. This dosage is low when compared with the data presented herein. The serum CPK data for the youngest boys, 5, 6, and 7 years of age, showed some downward trend but there were too few control (2–4) and treatment values (3–7 at 250 mg and 0–2 at 1 g) for statistical evaluation.

Folkers *et al.* (14) selected patients for therapeutic trial by analysis of the succinate dehydrogenase:coenzyme Q_{10} reductase of the rectus abdominis muscle. This enzyme can be inactive in the muscle of older dystrophic patients. Two boys, 3 and 7 years of age, with active CoQ_{10} enzymes were selected, and serum CPK was extensively monitored. Serum CPK was reduced ($P < 0.01$) particularly for the 3-year old boy. These results led to treatment of preclinical dystrophy with CoQ.

Background knowledge of preclinical muscular dystrophy

The onset of dystrophy is frequently recorded when weakness clinically appears. Pearson (15) observed high levels of serum enzymes "long before clinical weakness becomes apparent," and "when a child was distinctly weakened, but still ambulant, at least 70–80% of fibers were affected and many were destroyed." Although regenerative processes were evident in a biopsy for a 3-year old child, they were not as extensive
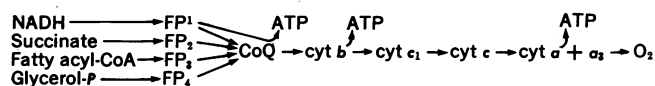

CHART 1. Pathway of electron transport or respiratory chain. *FP* is flavoprotein; *cyt* is cytochrome.

as for a 10-month old infant. Pearce *et al.* (16) concluded that the dystrophic process "is active even in early infancy and long before clinical weakness is manifest." Pearson (15) reported histological changes and elevated serum enzymes in a 4-month old infant and concluded that "before the clinical appearance of weakness, at least 50% of the muscle fibers are seriously involved in the dystrophic process." Zellweger (17) found that serum glutamate-oxaloacetate transaminase, CPK, lactate dehydrogenase, and aldolase were elevated in four boys, 1, 2, 4, and 5 years of age. Hudgson *et al.* (18) observed for dystrophic infants "there must be...a defect in some stage of the regenerative process which takes place in dystrophic muscle fibers and which renders regeneration ineffective."

CPK occurs primarily in cardiac and skeletal muscle, according to Thomson (19). High serum, CPK in dystrophy stems from these muscles. Reduction of serum CPK by treatment with CoQ could signify improved utilization and improved regeneration in muscle. It was, therefore, important to evaluate the treatment of preclinical dystrophy with CoQ.

What improvement may be expected from treatment of muscular dystrophy and how may improvement be established

Pearson's data (15) on a 10-month old infant and a 3-year old child indicate that any treatment could be increasingly less effective the older the child from an age of about 1 year.

Dowben (20) stated that substantiation of benefit from the treatment of a chronic disease, such as dystrophy, may be difficult unless a specific laboratory assay shows improvement, and that "a dramatic improvement in muscle strength of dystrophic patients is not expected with any therapeutic agent, because more and more muscle is replaced by fat and connective tissue as the disease progresses."

J.B. showed control levels of serum CPK of 7029 ± 1569 mU at about 1 year, and S.R. showed 4184 ± 739 mU at about 3 years. Normal values are less than about 50 mU. It may be unreasonable to expect to therapeutically reduce serum CPK to normal, because some muscle fibers beyond regeneration may be present even from infancy and increase in number with age. Pearson's estimate of at least 70–80% afflicted fibers, with many destroyed, in a weakened but ambulant child underscores the unlikelihood of reducing serum CPK to normal except possibly by starting therapy at birth. Dowben (20) stated therapy should be started when high serum levels of aldolase or CPK are found even though there is no weakness.

Evaluation of the administration of CoQ to J.B. and S.R. is necessarily based on biochemistry and not physical tests, because of their ages. Pearson and Dowben commented on the unreliability of tests of strength in children.

Results of administration of coenzyme Q to patients having preclinical dystrophy

Reduction of Serum Levels of Creatine Phosphokinase. J.B. was born Jan. 13, 1970 from the mother's seventh pregnancy.

TABLE 1. *Statistical analyses on CPK of patient J.B.*

Period	Duration of treatment (days)	No. of assays	CPK (mU)[d] Mean ± SD
Initial control period	56	18	7029 ± 1569
CoQ$_7$-treated period at 20 mg and 27 mg/kg	38	12	8551 ± 1651
1st H$_6$CoQ$_4$-treated period at 100 mg/kg[a]	87	32	6797 ± 2494
2nd H$_6$CoQ$_4$-treated period at 165 mg/kg[b]	44	14	5359 ± 1144
Latter part of 1st H$_6$CoQ$_4$-treated period	42	12	4735 ± 1644
Combined latter part of 1st H$_6$CoQ$_4$-treated period and 2nd H$_6$CoQ$_4$-treated period	86	26	5054 ± 1539

t values and probability

	Degrees of freedom	*t* value	Probability*P*
1st H$_6$CoQ$_4$-treated period at 100 mg/kg vs. initial control period	48	0.35	N.S.[c]
2nd H$_6$CoQ$_4$-treated period at 165 mg/kg vs. initial control period	30	3.34	<0.01
Latter part of 1st H$_6$CoQ$_4$-treated period vs. initial control period	28	3.84	<0.001
Combined latter part of 1st H$_6$CoQ$_4$-treated period and 2nd H$_6$CoQ$_4$-treated period vs. initial control period	42	4.15	<0.001

[a] 500 mg in corn oil three times a day.
[b] 500 mg in corn oil five times a day.
[c] N.S. No biological significance ($P > 0.05$).
[d] By the method of Oliver (32) and Rosalki (33).

Three brothers have dystrophy. He has a Duchenne muscular dystrophy. Collection of blood specimens was systematized. Positive controls of serum CPK were always analyzed.

During a control period of 56 days; the mean of 18 assays of serum CPK was 7029 ± 1569 mU (Table 1).

The initial dose of CoQ$_7$ was kept low for safety (20 mg/kg and then 27 mg/kg of CoQ$_7$ for 38 days); 12 assays of serum CPK showed 8551 ± 1651 mU or no response, as expected at this dosage.

Hexahydrocoenzyme Q$_4$ at a dosage comparable to that which was effective for S.R. (Table 2), or 100 mg/kg for 87 days elicited no reduction of serum CPK during the first half of this period but did during the last half; 32 determinations of the 87-day period showed 6797 ± 2494 mU.

Significant reduction of serum CPK appeared after 10 weeks of treatment with 100 mg/kg of H$_6$COQ$_4$. If the values obtained during this lag period are not considered, 12 values are significantly different from the control values ($P < 0.001$). Tissue regeneration may require time before the CPK decreases.

J.B., 1–2 years of age, had about the same weight as S.R., 4–5 years of age. When the dosage for J.B. was increased to 165 mg/kg for 44 days, the 14 mean of determinations was 5359 ± 1144 mU ($P < 0.01$ versus control). Combining these 14 and the previous 12, CPK was significantly reduced ($P < 0.001$ versus control).

J.B. was overweight. S.R. was thin; clinical variation of Duchenne dystrophy is known. The time-lag in response of J.B. to hexahydrocoenzyme Q$_4$ may correlate with the response of S.R.; during the last half of the second treatment with hexahydrocoenzyme Q$_4$ (105 mg/kg), serum CPK was 2888 ± 757 mU ($P < 0.001$).

S.R. was born Dec. 19, 1967. His diagnosis was Duchenne dystrophy. Folkers *et al.* (14) reported reduction of serum CPK for S.R. when hexahydrocoenzyme Q$_4$ and coenzyme

Q$_7$ were separately administered (Table 2); seven determinations during 49 days averaged 4184 ± 739 mU for the initial control.

During 77 days, dosage of hexahydrocoenzyme Q$_4$ was increased from 28 to 245 mg/kg and 11 determinations averaged 3437 ± 1124 mU (not significantly different from control values).

The first placebo period was 56 days during which seven determinations averaged 3829 ± 1451 mU (agrees with 4184 ± 739 for the control).

During the first treatment period with CoQ$_7$ (21 mg/kg) for 28 days, four determinations averaged 2767 ± 970 mU ($P < 0.03$ versus control, but treatment was brief).

During the second placebo period of 82 days 14 determinations averaged 4000 ± 1563 mU; ($P < 0.05$ versus the first treatment period). Serum CPK increased during this placebo period.

After this second placebo period, and during a second treatment with 21 mg/kg of CoQ$_7$ for 148 days, 26 determinations averaged 4161 ± 1470 mU (not significant versus the initial control or the second placebo period or the combined control and placebo period). This dosage was too low.

A second treatment with hexahydrocoenzyme Q$_4$ at 105 mg/kg for 150 days resulted in a mean value for 43 determinations of 3249 ± 1001 mU ($P < 0.05$, $P < 0.05$, and $P < 0.01$, respectively, versus the initial control, the second placebo, and the combined control and placebo periods). Reduction of serum CPK during this second treatment with hexahydrocoenzyme Q$_4$ took place during the last half of the 150 days ($P < 0.001$ versus the initial control).

Since J.B. was also given 165 mg/kg, S.R. was then given 175 mg/kg of hexahydrocoenzyme Q$_4$ for a third treatment of 21 days; six determination averaged 2341 ± 952 mU ($P < 0.01$, $P < 0.05$, and $P < 0.01$, versus the initial control, second placebo, and combined control and placebo periods, respectively).

TABLE 2. *Statistical analysis on CPK of patient S.R.*

Period	Duration of treatment (days)	No. of assays	CPK $(mU)^e$ mean ± SD
Initial control period	49	7	4184 ± 739
1st H_6CoQ_4-treated period at variable dosages[a]	77	11	3437 ± 1124
1st placebo period	56	7	3829 ± 1541
1st CoQ_7-treated period at 21 mg/kg	28	4	2767 ± 970
2nd placebo period	82	14	4000 ± 1563
2nd CoQ_7-treated period at 21 mg/kg	148	26	4161 ± 1470
2nd H_6CoQ_4-treated period at 105 mg/kg[b]	150	43	3249 ± 1001
3rd H_6CoQ_4-treated period at 175 mg/kg[c]	21	6	2341 ± 952
Combined control and placebo periods (initial control period + 1st and 2nd placebo periods)	187	28	4003 ± 1357
Latter half of 2nd treated prriod	77	22	2888 ± 757

t values and probability

Period	Degrees of freedom	*t* value	Probability *P*
1st H_6CoQ_4-treated period			
vs. initial control period	16	1.54	N.S.[d]
vs. combined control and placebo periods	37	1.22	N.S.
1st CoQ_7-treated period at 21 mg/kg			
vs. initial control period	9	2.74	<0.03
vs. combined control and placebo periods	30	1.74	N.S.
2nd CoQ_7-treated period at 21 mg/kg			
vs. initial control period	31	0.04	N.S.
vs. 2nd placebo period	38	0.32	N.S.
vs. combined control and placebo periods	52	0.41	N.S.
2nd H_6CoQ_4-treated period at 105 mg/kg			
vs. initial control period	48	2.35	<0.05
vs. 2nd placebo period	55	2.10	<0.05
vs. combined control and placebo periods	69	2.69	<0.01
3rd H_6CoQ_4-treated period at 175 mg/kg			
vs. initial control period	11	3.93	<0.01
vs. 2nd placebo period	18	2.43	<0.05
vs. combined control and placebo periods	32	2.87	<0.01
Latter half of 2nd treated period vs. initial control period	27	3.97	<0.001

[a] Dosage of H_6CoQ_4 was increased gradually from 28 mg to 245 mg/kg during this period.

[b] 500 mg in corn oil three times a day.

[c] 500 mg in corn oil five times a day.

[d] N.S. No biological significance $(P > 0.05)$.

[e] See footnote *d* of Table 1.

Molar relationship of dosages of H_6CoQ_4 and CoQ_7 for reduction of CPK

H_6CoQ_4, CoQ_7, and CoQ_{10} have the same redox functionality, and molar equivalents could elict the same response. The dosage of 105 mg/kg (1.5 g/day) of H_6CoQ_4 for S.R. would require 150 mg/kg (2.15 g/day) and 197 mg/kg (2.81 g/day) of CoQ_7 and CoQ_{10}, respectively.

The dosage of coenzyme Q which reduces serum CPK in Duchenne dystrophy

The dosage of hexahydrocoenzyme Q_4 which lowers serum CPK in J.B. and S.R. is high. Nutritional deficiencies caused by dietary lack of vitamins are treated with low dosages of vitamins which are generally lower than the total body levels. Vitamin dependency states due to genetic defects require large dosages of the vitamins to elicit responses.

Homocystinuria (21), a vitamin B_6 dependency state, is treated with 200–500 mg/day of vitamin B_6. Methylmalonic aciduria (22), a vitamin B_{12} dependency state, is treated with 1000 μg. "Pseudo–deficiency rickets" (23), a vitamin D dependency state, is treated with 25,000–100,000 IU of vitamin D in contrast to 400 IU for a primary deficiency. Muscular dystrophy involves genetic defects. The high dosage of CoQ, necessary for response, indicates a possible coenzyme Q dependency state, and is not necessarily and entirely due to the degree of absorption.

Significance of reduction of serum CPK in Duchenne dystrophy

Reduction of serum CPK, assayed repeatedly to circumvent fluctuation, appeared due to the administration of CoQ. While the mechanism causing reduction in serum CPK is uncertain, it could result from correcting the deficiency of coenzyme Q_{10} in skeletal muscle which is the primary site of CPK and its function. Succinate: CoQ_{10} enzyme activities of biopsies from dystrophic patients showed a deficiency of coenzyme Q_{10} in the rectus abdominis (Folkers *et al.*, ref. 14).

Hexahydrocoenzyme Q_4 or coenzyme Q_7 can biochemically substitute for coenzyme Q_{10} in muscle.

Pearson (15) stated "it is quite likely...in dystrophy... that a biochemical lesion of the contractile or energy-producing mechanisms is probably involved." Thomson (19) has stated "that CPK is by far the best measure of therapeutic efficacy." Munsat *et al.* (24) "found CPK to be clearly the most valuable enzyme to measure" of all the altered serum enzymes in neuromuscular disorders.

Improvement of the activity of CoQ_{10} enzymes in the respiratory chain, close to one coupling site and influential on two other coupling sites, could improve bioenergetics and ATP formation, and thereby improve formation of phosphocreatine from creatine and ATP by increased utilization of CPK. Karpati and Sherwin (25) found the CPK content of atrophic and hypertrophic fibers was low.

Deficiency of coenzyme Q in mice having a genetic muscular dystrophy and treatment with coenzyme Q

Mice (129/*Rd-dydy*) with genetic dystrophy were physically improved (26) when treated with hexahydrocoenzyme Q_4. When Scholler *et al.* (27) increased dosage life-span was increased 4-fold. Zuckerman *et al.* (28) observed an 8-fold increase in muscle strength when these mice were treated with hexahydrocoenzyme Q_4.

Nilsson *et al.* (29) found an actual deficiency of CoQ_9 in these mice. Littarru *et al.* (30) found a deficiency ($P < 0.01$) in the activities of the succinate:coenzyme Q_9 enzyme of the hearts and hind leg muscles of dystrophic mice. Treatment with CoQ_7 prolonged survival and improved physical performance (Scholler *et al.*, ref. 31). Ho and Folkers (32) reported data showing these mice could have a coenzyme Q dependency state.

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