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Coenzyme Q_{10} supplementation in infertile men with idiopathic asthenozoospermia: an open, uncontrolled pilot study

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Objective: To clarify a potential therapeutic role of coenzyme Q_{10} (CoQ_{10}) in infertile men with idiopathic asthenozoospermia.

Design: Open, uncontrolled pilot study.

Patient(s): Infertile men with idiopathic asthenozoospermia.

Intervention(s): CoQ_{10} was administered orally; semen samples were collected at baseline and after 6 months of therapy.

Main Outcome Measure(s): Semen kinetic parameters, including computer-assisted sperm data and CoQ_{10} and phosphatidylcholine levels.

Result(s): CoQ_{10} levels increased significantly in seminal plasma and in sperm cells after treatment. Phosphatidylcholine levels also increased. A significant increase was also found in sperm cell motility as confirmed by computer-assisted analysis. A positive dependence (using the Cramer's index of association) was evident among the relative variations, baseline and after treatment, of seminal plasma or intracellular CoQ_{10} content and computer-determined kinetic parameters.

Conclusion(s): The exogenous administration of CoQ_{10} may play a positive role in the treatment of asthenozoospermia. This is probably the result of its role in mitochondrial bioenergetics and its antioxidant properties. (Fertil Steril® 2004;81:93–8. ©2004 by American Society for Reproductive Medicine.)

Key Words: Asthenozoospermia, coenzyme Q_{10} therapy, male infertility

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An excess of reactive oxygen species is known to impair sperm cell function and play a negative role in male factor fertility (1–7). Coenzyme Q_{10} (CoQ_{10}) is a component of the mitochondrial respiratory chain and plays a crucial role in energy metabolism (8, 9). Furthermore, it is an important liposoluble chain-breaking antioxidant associated with membranes and lipoproteins. It has long been known that CoQ_{10} biosynthesis is markedly active in testis (10) and high levels of its reduced form QH_2 (ubiquinol) are present in semen (11–13), which suggests a protective role as a scavenger in this biological system.

There is evidence that sperm cells with reduced motility also have a significant reduction in the phospholipid pool, as well as phosphatidylethanolamine and phosphatidylcholine (PC) content, probably related to a reduction in the antioxidant capacity of spermatozoa and seminal plasma (14).

We have recently demonstrated reduced levels of CoQ_{10} in the seminal plasma and sperm cells of infertile men with idiopathic and varicocele-associated asthenospermia (15). On this basis, we indicated CoQ_{10} as one of the compounds contributing to the total antioxidant buffer capacity of semen and its reduction as an impairment of the system in dealing with oxidative stress (16). Whether the exogenous administration of CoQ_{10} could lead to any modification of its content in semen or to any benefit on sperm cell function still remains an interesting open problem. To elucidate a potential therapeutic role, we analyzed semen parameters and CoQ_{10} and PC changes in idiopathic asthenozoospermic infertile patients undergoing CoQ_{10} dietary implementation.

MATERIALS AND METHODS

Patient Selection

Twenty-two patients (mean age, 31 years; range, 25–39 years) with idiopathic asthenozoospermia were enrolled in the study. The patients were selected at the Andrology Unit of the Division of Endocrinology, Umberto I Hospital, University of Ancona, Ancona, Italy. All subjects underwent medical screening, including history and clinical examination, and presented with a clinical history of primary infertility of at least 3 years. Testicular volume was evaluated in each patient using Prader's orchidometer. To accomplish a complete diagnosis, the following investigations were also performed: semen analysis; Mar-test (SperMar test, Diasint, Florence, Italy) for anti-spermatozoa antibodies (Ab); sperm culture and urethral specimens collection for *Chlamydia* and *Mycoplasma ureoliticum* detection; FSH, LH, T, E_2, and PRL assays, using commercial RIA kits; and testicular, prostatic, and seminal vesicle ultrasound and echo-color Doppler of venous spermatic plexus for anatomical abnormalities and varicocele detection.

There was no apparent female factor, since all partners (mean age, 28 years; range, 23–35 years) were ovulating regularly as formally proven by luteal phase progesterone (P) levels and no abnormal fallopian tube anatomy was detected after hysterosalpingography.

Eligibility Criteria

The following criteria were adopted for patient eligibility: [1] sperm count $>20 \times 10^6$/mL, sperm motility (forward motility, class a and b, according to World Health Organization (WHO) 1999 criteria) (17) $<50\%$ at two distinct sperm analyses, and normal sperm morphology $<50\%$; [2] seminal white blood cells (WBC) $<1 \times 10^6$/mL, negative sperm culture, and *Chlamydia* and *Mycoplasma ureoliticum* detection; [3] normal serum levels of gonadotropins, T, E_2, and PRL; [4] absence of infectious genital diseases, anatomical abnormalities of the genital tract including varicocele, and anti-spermatozoa Ab; [5] absence of systemic diseases or treatment with other drugs in the 3 months before enrollment in the present study; and [6] absence of smoking, alcohol, drug addiction, or occupational chemical exposure.

Study Design and Treatments

The enrolled patients underwent dietary implementation of CoQ_{10} (PharmaNord, Veyle, Denmark), 200 mg/day twice daily orally for 6 months. Clinical examination, semen analysis including computer-assisted sperm analysis (CASA), and CoQ_{10} and PC assays were performed at baseline and after 6 months of therapy. An additional semen analysis was performed 6 months after the termination of therapy (wash-out). This study was approved by the Institutional Review Board of the University of Ancona-Umberto I Hospital. All patients provided their written informed consent and completed the entire trial.

Safety Assessment

Safety assessment included medical history, physical examination, hematological screening, and serum chemistry at all visits and the monitoring of drug-related adverse events by recordation in patient diaries.

Seminal Fluid Analysis

Semen quality was assessed by the same biologist in terms of sperm concentration, motility, and morphology in accordance with WHO criteria (17). Briefly, seminal fluid was obtained by masturbation after 3–5 days of sexual abstinence. The samples were kept in the andrology lab at room temperature and processed within 1 hour from ejaculation. Sperm count was determined with the Makler chamber. Motile spermatozoa were assessed by phase contrast microscopy (10 μL of semen was delivered onto a glass slide and covered with a 22 mm $\times$ 22 mm coverslip) and graded as follows: class a and b, fast and weak forward motility; class c, nonprogressive motility; class d, immobile spermatozoa. Sperm morphology was evaluated on smears of seminal fluid, stained with the Giemsa method, and observed by oil immersion light microscopy. Conventional immunocytochemistry was used to assess WBC (17).

In addition, CASA for sperm cell motility was performed, as reported elsewhere (18). One semen aliquot (3 μL) was placed in a 20-μm depth chamber. Two 20-μm depth cell–VU chambers (Conception Technologies, La Jolla, CA) were loaded, and six different fields per chamber were randomly examined and at least 200 sperm for each field of the chamber were scored. Percentages of motile sperm and movement characteristics were analyzed using an automated analyzer at 37°C (CellTrack VP110, Motion Analysis Corporation, Palo Alto, CA). Sperm velocity and kinetic characteristics were evaluated only for motile sperm and expressed as mean values considering curvilinear velocity (VCL) and straight progressive velocity (VSL).

Preparation for High Pressure Liquid Chromatography Analysis

The liquefied semen samples were centrifuged at room temperature at 500 $\times$ *g* for 18 minutes. The sperm cell pellet was washed with 0.15 M NaCl. Two hundred fifty microliters of 1-propanol were added to 50 μL of a cellular suspension containing 10^6 sperm cells or to 50 μL of seminal plasma. After mixing for 1 minute, the samples were centrifuged for 5 minutes at 2,000 $\times$ *g* and the obtained supernatants were injected into the high pressure liquid chromatography (HPLC) apparatus. This extraction assured a total recovery of liposoluble antioxidants (19).

Analysis of PC was performed according to Frei et al. (20) with the modification described by Yamamoto et al. (21). After adding 1 mL of methanol to a cellular suspension containing about 10^6 sperm cells or to 50 μL of seminal plasma, samples were mixed for 1 minute; then 6 mL of hexane were added and samples were mixed again before

centrifugation (10 minutes at 500 *g*). Supernatant was collected and dried with a nitrogen stream. Dried sample residues were dissolved in 300 μL of ethanol.

Determination of CoQ_{10}

CoQ_{10} levels were assayed in sperm cells and seminal plasma using a Beckman Gold HPLC System (Beckman Instruments, San Ramon, CA) equipped with an electrochemical detector (EC, ESA 5100, Bedford, MA) as described elsewhere (15). The detector was supplied with a guard cell and an analytical cell with two electrodes in series; the potentials applied to the electrodes were, respectively, +0.6V, −0.45V, and +0.6V; chromatograms were recorded from the second analytical cell signal. One hundred microliters of samples were directly injected into the analytical column and analyzed; an analytical column was a Supelcosil reverse-phase C18, 15 cm × 0.46 cm ID, 3 μm (Supelco, Inc., Bellefonte, PA). Mobile phase, consisting of filtered 2 g/L lithium perclorate in methanol/ethanol mixture (40:60, v:v), was used at a flow rate of 1 mL/minute.

Determination of PC

Phosphatidylcholine was essentially determined according to Frei et al. (20). An analytical column was a Supelcosil LCSi, 25 cm × 0.46 cm ID, 5 μm (Supelco). A Jasco HPLC System (Jasco Corporation, Tokyo, Japan) provided with two PU-980 pumps, and a UV detector set at 205 nm wavelength was used for detention of total PC.

Statistical Analysis

Statistical analysis was performed using the SAS Statistical Package (SAS Institute Inc., Cary, NC). Results are reported as means ± SD. Differences among the samples were evaluated by Student's *t*-test, and the Kolmogorov-Smirnov and Shapiro-Wilks tests were used to appraise whether the data were random samples from a normal distribution. Finally, the Cramer's index of association was used to evaluate the degree of association among the variables.

RESULTS

CoQ_{10} and PC Determinations

CoQ_{10} levels increased in seminal plasma after treatment, the mean value rising significantly from 42.0 ± 5.1 ng/mL at baseline to 127.1 ± 1.9 ng/mL after 6 months of exogenous CoQ_{10} administration ($P<.005$). A significant increase of CoQ_{10} content was also detected in sperm cells (from 3.1 ± 0.4 to 6.5 ± 0.3 ng/10^6 cells, $P<.05$). Similarly, PC levels increased significantly both in seminal plasma and sperm cells after treatment (from 1.49 ± 0.50 to 5.84 ± 1.15 μM, $P<.05$; and from 6.83 ± 0.98 to 9.67 ± 1.23 nmol/10^6 cells, $P<.05$, respectively) (Table 1).

TABLE 1

Coenzyme Q_{10} (CoQ_{10}) and phosphatidylcholine (PC) levels in seminal plasma and sperm cells, baseline and after treatment.

	Baseline	After treatment
CoQ_{10}, seminal plasma (ng/mL)	42.0 ± 5.1	127.1 ± 1.9[a]
CoQ_{10}, sperm cells (ng/10^6 cells)	3.1 ± 0.4	6.5 ± 0.3[b]
PC, seminal plasma (μM)	1.49 ± 0.50	5.84 ± 1.15[b]
PC, sperm cells (nmol/10^6 cells)	6.83 ± 0.98	9.67 ± 1.23[b]

[a] After treatment vs. baseline; $P<.005$.
[b] After treatment vs. baseline; $P<.05$.

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Sperm Output

With regard to semen features, a significant difference was found in the forward (class a+b) motility of sperm cells after 6 months of CoQ_{10} dietary implementation (from 9.13% ± 2.50% to 16.34% ± 3.43%, $P<.05$) (Table 2). The improvement of motility was also confirmed after the computer-assisted determination of kinetic parameters. A significant increase of VCL (from 26.31 ± 1.50 to 46.43 ± 2.28 μm/second, $P<.05$) and VSL (from 15.20 ± 1.30 to 20.40 ± 2.17 μm/second, $P<.05$) was found after treatment. No significant differences were found in sperm cell concentration and morphology (Table 2).

Interestingly, although a direct correlation was not found (data not shown), a positive dependence (using the Cramer's index of association) was evident among the relative variations, baseline and after treatment, of seminal plasma or intracellular CoQ_{10} content and of CASA (VCL and VSL) kinetic parameters (Cramer's V = 0.4637, 0.3818, 0.3467, and 0.5148, respectively) (Table 3).

Sperm forward motility was significantly reduced after 6 months of wash-out (from 16.34% ± 3.43% to 9.50% ± 2.28%, $P<.001$), while no significant differences were found in sperm cell concentration and morphology (Table 2).

In an attempt to verify whether different responses were evident as functions of age, the relative variations (before and after treatment) of CoQ_{10} and PC content in seminal plasma and sperm cells, as well as forward motility, were analyzed, but no dependence was found (data not shown).

Spontaneous Pregnancy

Three out of 22 patients (13.6%) achieved spontaneous pregnancy within 3 months of the discontinuation of therapy (a 2.4% pregnancy rate per cycle).

Safety Assessment

CoQ_{10} oral administration was generally well tolerated, and no laboratory abnormalities were observed.

DISCUSSION

Defective sperm function has been strictly associated with the overproduction of reactive oxygen species (ROS) by

TABLE 2

Semen parameters (concentration, motility, and morphology), including computer-assisted sperm analysis kinetic features baseline, after 6 months of coenzyme Q_{10} dietary implementation and 6 months of wash-out.

	Baseline	After treatment	After wash-out
Sperm concentration (10^6/mL)	27.60 ± 7.41	25.92 ± 5.63, NS	26.18 ± 4.04, NS
Forward motility (%)	9.13 ± 2.50	16.34 ± 3.43[a]	9.50 ± 2.28[b]
Teratozoospermia (%)	69.76 ± 4.63	67.82 ± 7.44, NS	66.23 ± 5.34, NS
Curvilinear velocity (μm/second)	26.31 ± 1.50	46.43 ± 2.28[a]	
Straight progressive velocity (μm/second)	15.20 ± 1.30	20.40 ± 2.17[a]	

Note: NS = not significant.

[a] After treatment vs. baseline; $P<.05$.

[b] After treatment vs. after wash-out; $P<.0001$.

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abnormal spermatozoa and leukocytes. The consequent impairment of sperm motility and membrane integrity induced by lipid peroxidation plays a critical role in male factor infertility (1–7). Furthermore, a negative correlation between ROS excess and incidence of spontaneous pregnancy has been shown (22). On the other hand, some data support a reduced total scavenging capacity of seminal plasma in infertile men with abnormal semen parameters (16, 23, 24).

The role of CoQ_{10} as a lipophylic antioxidant has been well established in plasma lipoproteins (12, 25), and its possible involvement in male factor infertility was suggested (11, 13). Moreover, our group recently found a significant reduction of CoQ_{10} levels in the seminal plasma and sperm cells of infertile men with idiopathic and varicocele-associated asthenozoospermia (15), which suggests a pathogenetic role through the impairment of total antioxidant reserve.

Some antioxidant molecules (vitamins, gluthatione, carnitine) have been used therapeutically in patients with semen quality impairment and infertility (26–30); their real effectiveness remains uncertain at present, mainly because of the indiscriminate selection of patients undergoing treatment, as pointed out by several investigators (31–35). Recent data from a double-blind controlled study in selected cases of male factor infertility (36) and an open study of a selected group of infertile men with prostato-vesiculo-epididymitis (37) suggest that a therapeutic approach with carnitine could reduce male factor infertility.

The data of the present study show a significant improvement of sperm cell kinetic features after 6 months of administration of CoQ_{10}, on the basis of both manual and computer-assisted evaluation. Furthermore, our results are the first to demonstrate that exogenous administration of CoQ_{10} leads to increased levels in seminal plasma and in sperm cells.

The increment was relevant, especially in seminal plasma where post-treatment levels were 3 times higher than basal ones. Similar increases of CoQ_{10} concentration (2–3 times higher compared with baseline value) are commonly found in blood plasma after chronic administration of the quinone (39). Because CoQ_{10} is a highly lipophylic molecule, we could reasonably hypothesize that it can diffuse through the phospholipid bilayer of cellular membranes, but we presently do not know whether transport from blood plasma to testicular and accessory male genital glands is a passive one or one that involves an active mechanism.

Statistical analyses have failed to prove any significant functional relationship among the therapy-induced variations of CoQ_{10} and the kinetic parameters of spermatozoa, probably because of the low number of samples. Nevertheless, the good degree of association among these variables, according to the Cramer's *V* index of association, supports the hypothesis of a pathogenetic role of CoQ_{10} in asthenozoospermia based on previously reported data (15). The improvement in spontaneous pregnancy rates also suggests a benefit of this therapeutic approach.

The positive effect of exogenous administration could be explained on the basis of the well-known involvement of CoQ_{10} in mitochondrial bioenergetics and of its widely recognized antioxidant properties. Regarding the first point, it is well-known that the mitochondrial concentration of CoQ_{10} in mammals is close to its KM, as far as NADH oxidation is concerned, and therefore is not kinetically saturating (38). In these conditions, one might reasonably hypothesize that a small increase in mitocondrial CoQ_{10} leads to a relevant rise in respiratory velocity. The resulting improvement of oxidative phosphorylation might well affect sperm cells. Since low PC levels in semen were found to be related to a reduction of the phospholipid pool and to low antioxidant capacity (14), the increased PC content in semen after treatment might reasonably involve the restoration of scavenger equilibrium. Another possible reason for this finding is that increased levels of CoQ_{10} also need an appropriate, high concentration of a lipid carrier.

In conclusion, the administration of CoQ_{10} may play a positive role in treatment of asthenozoospermia, probably related both to its role in the mitochondrial respiratory chain and to its antioxidant role. The increased concentration of

TABLE 3

Two-way contingency tables.

Percent variation after and before treatment between VCL (μm/sec) and cellular CoQ_{10}

VCL CoQ_{10}	(−10%; 0%)	(0%; 10%)	(10%; 20%)	(20%; 30%)	(30%; +)	Total
(50%; 100%)	1	1	3	2	1	8
(100%; 150%)	1	0	3	3	2	9
(150%; 200%)	0	0	0	2	0	2
(200%; +)	0	2	1	0	0	3
Total	2	3	7	7	3	22

Cramer's $V = 0.4637$

Percent variation after and before treatment between VSL (μm/sec) and cellular CoQ_{10}

VSL CoQ_{10}	(0%; 10%)	(10%; 0%)	(20%; 30%)	(30%; 40%)	(40%; 50%)	(50%; +)	Total
(50%; 100%)	0	1	1	2	2	2	8
(100%; 150%)	1	1	2	2	2	1	9
(150%; 200%)	0	0	0	0	0	2	2
(200%; +)	0	0	1	1	0	1	3
Total	1	2	4	5	4	6	22

Cramer's $V = 0.3818$

Percent variation after and before treatment between VCL (μm/sec) and plasmatic CoQ_{10}

VCL CoQ_{10}	(−10%; 0%)	(0%; 10%)	(10%; 20%)	(20%; 30%)	(30%; +)	Total
(0%; 100%)	0	1	2	1	0	4
(100%; 200%)	1	0	2	1	0	4
(200%; 300%)	1	2	1	3	2	9
(300%; 400%)	0	0	1	0	0	1
(400%; +)	0	0	1	2	1	4
Total	2	3	7	7	3	22

Cramer's $V = 0.3467$

Percent variation after and before treatment between VSL (μm/sec) and plasmatic CoQ_{10}

VSL CoQ_{10}	(0%; 10%)	(10%; 20%)	(20%; 30%)	(30%; 40%)	(40%; 50%)	(50%; +)	Total
(0%; 100%)	0	0	0	2	1	1	4
(100%; 200%)	1	0	0	1	2	0	4
(200%; 300%)	0	2	2	1	0	4	9
(300%; 400%)	0	0	0	1	0	0	1
(400%; +)	0	0	2	0	1	1	4
Total	1	2	4	5	4	6	22

Cramer's $V = 0.5148$

Note: CoQ_{10} = coenzyme Q_{10}; VCL = curvilinear velocity; VSL = straight progressive velocity.

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CoQ_{10} in seminal plasma and sperm cells, the improvement of semen kinetic features after treatment, and the evidence of a direct correlation between CoQ_{10} concentrations and sperm motility strongly support a cause/effect relationship.

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