Coenzyme Q10: Another Biochemical Alteration Linked to Infertility in Varicocele Patients?

Antonio Mancini, Domenico Milardi, Gianluigi Conte, Antonio Bianchi, Giancarlo Balercia, Laura De Marinis, and Gian Paolo Littarru

Previously we demonstrated that coenzyme Q10 (CoQ10) is present in human seminal fluid and shows a direct correlation with seminal parameters except in patients with varicocele (VAR). We have now evaluated CoQ10 distribution in VAR, versus control subjects, in order to discover metabolic abnormalities within this condition. We studied 32 patients with VAR (11 with oligoasthenozoospermia, 13 with asthenozoospermia, and 8 with normozoospermia), and, as controls, the following groups of subjects, matched with VAR patients according to seminal parameters: 16 patients with idiopathic oligozoospermia, 11 patients with isolated asthenozoospermia, and 14 normal fertile men. CoQ10 was assayed in total seminal fluid, plasma, or cell pellet by high-performance liquid chromatography (HPLC). We found a significantly higher proportion of CoQ10 in seminal plasma in VAR; cellular CoQ10 showed an inverse correlation with sperm concentration and motility in VAR, at variance with controls. As seminal plasma ubiquinone reflects an interchange between intracellular and extracellular compartments, the different distribution in VAR patients could represent a greater sensitivity to peroxidative damage and could suggest reduced utilization for energy, which in turn could cause a defective motility even in patients with a normal cell count. These data suggest a pathophysiological role of CoQ10 in seminal plasma and a possible molecular defect in VAR.

COENZYME Q10 (CoQ10), also known as ubiquinone for its wide diffusion throughout mammalian tissues, is a lipidic molecule involved in the proton and electron transport in the respiratory chain of mitochondria and in the defense of the cell from reactive oxygen species (ROS) and free radical damage. CoQ10 is transported in blood by plasma lipoproteins and its levels can be easily detected in serum and tissue homogenates.[1] In the cell, ubiquinone is located in the membranes and exerts important effects against oxidative damage of polyunsatured fatty acids of the lipid bilayer. CoQ10 is the only lipid-soluble antioxidant that animal cells can synthesize de novo and regenerate in the reduced form by enzimatic mechanisms.

Further studies have focused on the importance of ROS in seminal plasma. The role of ROS in male infertility was detected through a study of their formation in an unselected population of male patients consulting for infertility. A significant negative correlation between the level of ROS formation and semen volume was reported, thus revealing the presence of a form of protection inside the seminal vesicles that contribute to the majority of the semen volume; the level of ROS formation was also inversely correlated to the percentage of global or progressive motile cells, thus suggesting that damaged spermatozoa are mainly responsible for the generation of ROS. The centrifugation of spermatozoa by Percoll gradient indicates that seminal plasma does not produce ROS.[2]

On the other hand, as a component of the respiratory chain, CoQ10 plays a role in oxidative phosphorylation leading to adenosine triphosphate (ATP) production. Plasma CoQ10 levels are considered an index of metabolic demand of various tissues in different physiological and pathological conditions.[3] In particular, an increased metabolic demand, in conditions such as hyperthyroidism or physical training, leading to enhanced extraction from the blood compartment, is associated with low plasma levels.

We have previously demonstrated that ubiquinone is present at remarkable levels in human seminal fluid and shows a direct correlation with the main parameters of standard semen analysis (sperm count and motility). An interesting exception was observed in patients with varicocele (VAR), in whom the correlation of ubiquinone with sperm motility was lacking and a higher proportion of CoQ10 was found in seminal plasma.[4]

We have now evaluated the levels of CoQ10, focusing the attention on the distribution of CoQ10 between plasma and spermatozoa and the cellular content of CoQ10 in patients affected by VAR, correlating these data with cell concentration and motility in such a condition, in comparison with control subjects (idiopathic oligoasthenospermia or isolated asthenospermia and normal men), matched according to seminal data.

MATERIALS AND METHODS

From the outpatient clinic of the male infertility factor, 32 subjects, aged 19 to 40 years and affected by VAR, were recruited for this study, after they had given an informed consent confirming to the guidelines of the Helsinki Declaration. The diagnosis of VAR was established by clinical examination and confirmed by Doppler technique.[5] On the basis on semen analysis,[6] subjects were classified as oligoasthenozoospermic (n = 11), asthenozoospermic (n = 13), or normozoospermic (n = 8). As controls, we studied a group of 41 non-VAR subjects: 16 patients with idiopathic oligoasthenozoospermia, aged 25 to 45 years, 11 with idiopathic isolated asthenozoospermia, aged 26 to 40 years, and 14 normal fertile subjects, aged 22 to 40 years. These different control group subjects were recruited to perform a comparison with VAR, matching with the corresponding seminal picture. CoQ10 was assayed in total seminal fluid (total CoQ10), in seminal plasma (plasma CoQ10), and in cell pellet (cellular CoQ10). Seminal fluid was collected and immediately stored in the dark to avoid ubiquinone photodegradation and 1 mL of seminal fluid was imediately stored at −80°C until assayed. The remaining sample was centrifugated for 10 minutes

From the Institute of Endocrinology, Catholic University of the Sacred Heart, Rome; Internal Medicine Division, Hospital of Galatina, Lecce; Division of Endocrinology and Institute of Biochemistry, School of Medicine, University of Ancona, Ancona, Italy.

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Address reprint requests to Antonio Mancini, MD, Institute of Endocrinology, Catholic University, Largo A. Gemelli 8, 00168 Rome, Italy.

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Table 1. Seminal Parameters in VAR Patients and Non-VAR Controls

	VAR		Controls	
	Sperm Count ($\times 10^6$/mL)	Motility (% progressive motile forms)	Sperm Count ($\times 10^6$/mL)	Motility (% progressive motile forms)
Oligoasthenozoospermic	11.7 ± 1.7	25.5 ± 3.4	10.2 ± 1.8	29.7 ± 4.7
Asthenozoospermic	37.1 ± 3.3	32.1 ± 2.8	47.1 ± 5.1	41.8 ± 1.0
Normozoospermic	39.9 ± 5.7	58.7 ± 2.3	53.6 ± 5.4	63.1 ± 2.2

at 2,000*g*; seminal plasma was immediately removed. Microscopical examination of seminal plasma confirmed that almost all spermatozoa had been removed. Aliquots of 0.5 mL for CoQ10 determination were stored at −80°C until assayed. The spermatozoa, suspended in 1 mL of normal saline, were frozen in the dark (−80°C) until assayed. CoQ10 levels were determined by high-performance liquid chromatography (HPLC), as previously described for measurement in total seminal fluid and seminal plasma.[7] CoQ10 concentrations in the spermatozoa were expressed as nanograms per 10^6 cells.

CoQ10 was assayed by HPLC, using a UV detector (275 nm). The sample of 0.5 mL (semen or seminal plasma), supplemented with 2 mL of ethanol:isopropanol (95:5), 0.5 mL of 0.1-mol/L sodium dodecyl sulfate, and 0.5 μg of coenzyme Q8 (CoQ8) as an internal standard, was extracted twice with 4 mL of n-hexane. Combined extracts were brought to dryness under N2 (at 45 to 50°C) and redissolved in 100 μL of ethanol. An aliquot of 20 μL was injected into the HPLC apparatus, whose conditions were as follows: column, ultrasphere octodecylsilane, 250 × 4.6 mm; mobile phase, ethanol-methanol (70:30); detector, UV 275 nm. The levels were expressed as concentrations (μg/mL, mean ± SEM).

The distribution of the data was evaluated by Kolmogorov-Smirnov test. As the data were not normally distributed, comparison among groups was performed using Kruskal-Wallis test; comparison between the 2 populations of subjects (VAR *v* controls) was performed using the Mann-Whitney test. Linear regression analysis was used when correlating different parameters within groups. Software used for statistical analysis was Arcus Quickstat (Software Publishing, Biomedical Version 1.2, Cambridge, UK)

RESULTS

On the basis of seminal paramethers (Table 1), 11 VAR patients were classified as oligoasthenozoospermic, 13 as asthenospermic, and eight as normozoospermic. Total CoQ10 in VAR patients was not significantly different from control subjects (mean ± SEM, 0.18 ± 0.01 *v* 0.22 ± 0.02 μg/mL). Mean values of CoQ10 in different subsets of patients, divided according to seminal parameters, are reported in Table 2. No significant differences were observed when comparing VAR versus controls and different groups within VAR and control populations.

In addition, plasma CoQ10 concentrations in VAR did not significantly differ from controls (mean ± SEM, 0.10 ± 0.01 *v* 0.10 ± 0.01 μg/mL). However, the proportion of CoQ10 in seminal plasma was significantly higher in VAR patients. The ratio of plasma/total CoQ10 was 61.33% ± 2.5% in VAR and 41.26% ± 2.3% in controls ($P = .01$). Mean levels of plasma CoQ10 in different subsets of patients, divided according to seminal parameters, are reported in Table 2. Again, no significant differences were observed when comparing VAR versus controls and different groups within VAR and control populations. On the contrary, the ratio of plasma/total CoQ10 was significantly greater in all VAR subgroups when compared with the respective control subgroups (mean ± SEM, oligoasthenozoospermic VAR, 60.5% ± 0.6% *v* oligoasthenozoospermic controls 42.6% ± 3.8%, $P < .05$; asthenozoospermic VAR 56.62% ± 3.8% *v* asthenozoospermic controls 43.13% ± 3.4%, $P < .05$; normozoospermic VAR 70.12% ± 7.3% *v* normozoospermic controls 38.29% ± 4.7%, $P < .05$).

Cellular CoQ10 was lower in VAR versus controls, although not significantly (mean ± SEM, 2.54 ± 0.25 *v* 3.86 ± 0.56 ng/10^6). Mean values of CoQ10 in different subsets of patients, divided according to seminal parameters, are reported in Table 2. No significant differences were observed when comparing VAR versus controls. Cellular CoQ10 was significantly higher in oligoasthenozoospermic VAR versus asthenozoospermic and normozoospermic VAR and in oligoasthenozoospermic controls versus asthenozoospermic and normozoospermic controls.

Correlation analysis showed that total CoQ10 was significantly correlated with cell concentrations both in VAR and in controls (Fig 1), but correlated with the percentage of motile cells only in controls (Fig 2).

Plasma CoQ10 did not correlate with sperm concentration in VAR or in controls ($r = 0.2$, $P = .1$ and $r = .02$, $P = .8$, respectively) or with the percentage of motile cells ($r = .2$, $P = .1$ and $r = -.05$, $P = .7$, respectively).

Cellular CoQ10 showed a significant inverse correlation with sperm cell concentrations (Fig 3) and with percentage of sperm

Table 2. Total CoQ10, Plasma CoQ10, and Cellular CoQ10 (mean ± SEM) in Different Subsets of Patients and Non-VAR Controls, Divided According to Seminal Parameters

	VAR			Controls		
	Total CoQ10 (μg/mL)	Plasma CoQ10 (μg/mL)	Cellular CoQ10 (ng/10^6 cells)	Total CoQ10 (μg/mL)	Plasma CoQ10 (μg/mL)	Cellular CoQ10 (ng/10^6 cells)
Oligoasthenozoospermic	0.15 ± 0.01	0.09 ± 0.01	3.60 ± 0.22	0.19 ± 0.03	0.09 ± 0.03	5.40 ± 0.93
Asthenozoospermic	0.18 ± 0.02	0.09 ± 0.01	2.27 ± 0.44*	0.23 ± 0.03	0.12 ± 0.01	2.66 ± 0.80*
Normozoospermic	0.21 ± 0.04	0.16 ± 0.03	1.50 ± 0.35*	0.24 ± 0.03	0.10 ± 0.02	3.02 ± 0.97*

*$P < .02$ *v* oligoasthenozoospermic subjects.

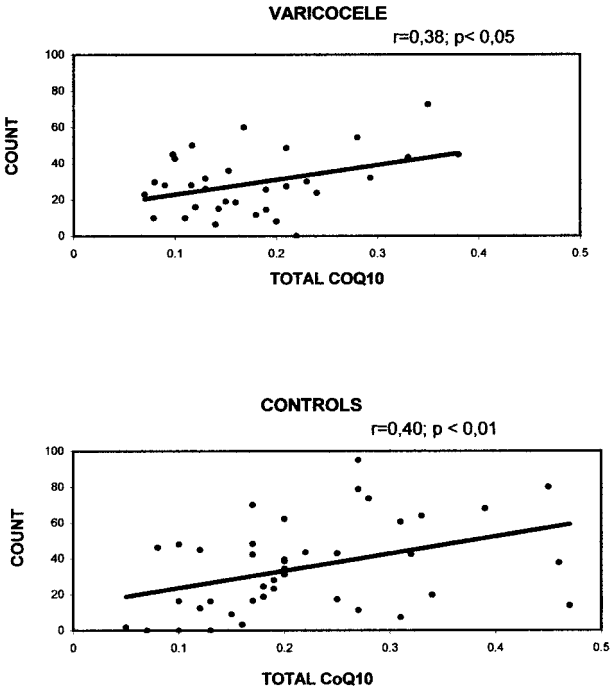

Fig 1. Correlation between total CoQ10 (μg/mL) and sperm count ($\times 10^6$/mL) in VAR patients and control non-VAR subjects.

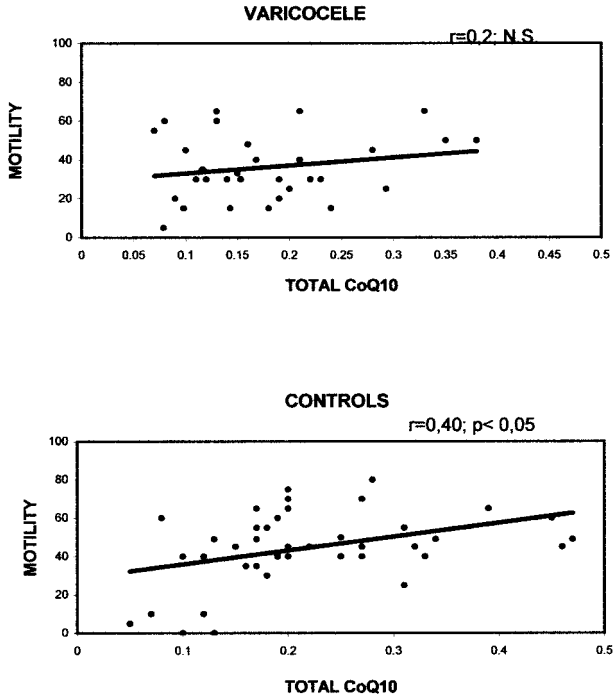

Fig 2. Correlation between total CoQ10 (μg/mL) and percentage of progressive motile forms in VAR patients and control non-VAR subjects.

motility (Fig 4) in VAR patients, while no significant correlation was observed in controls.

DISCUSSION

Our results, coupled with previously published data,[4] show that CoQ10 content and distribution is different in varicocele patients when compared with normal subjects. First, while in normal subjects total CoQ10 directly correlates with cell concentration and with motility, suggesting a physiological role of the molecule in semen, in VAR the correlation with motility is lost, suggesting a possible alterated employment in this condition. Second, the cellular concentration of CoQ10 has a trend toward lower values in VAR, with a significantly higher percent concentration in plasma versus controls. Since, as in other biological fluids, cellular and extracellular compartments freely exchange CoQ10, this indicates an alterated compartment distribution. This, in turn, could indicate a greater susceptibility to oxidative damage, due to the antioxidant function of CoQ10, and simultaneously a worse use for energetic purpose. Finally, the cellular concentration of CoQ10 inversely correlates with the sperm concentration and with percentage of motile forms only in VAR, suggesting a reduced utilization of the coenzyme in the asthenozoospermic VAR population.

These observations strenghten the possibility of a physiological role of CoQ10 in seminal fluid. As far as the antioxidant role is concerned, it is known that ROS produced by the mammalian spermatozoon are implicated in the physiological process, leading to hyperactivated motility and acrosome reaction.[8] Peroxidative stress can, in some cases, damage sperm cells.[9] These cells are extremely sensitive to ROS-induced damage, due to the wide surface of their membranes, poor cytoplasmic mechanism of defense, and, in the female genital tract, lack of protection supplied by the seminal plasma.[10,11]

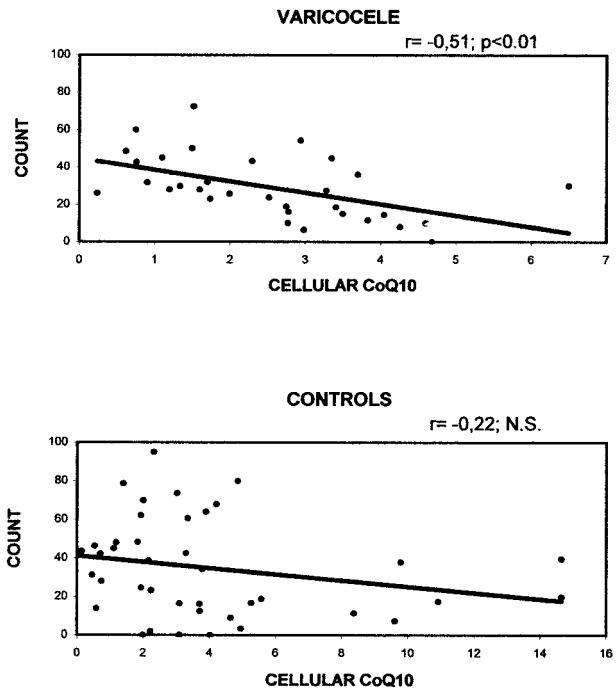

Fig 3. Correlation between cellular CoQ10 (ng/10^6 cells) and sperm count (10^6/mL) in VAR patients and control non-VAR subjects.

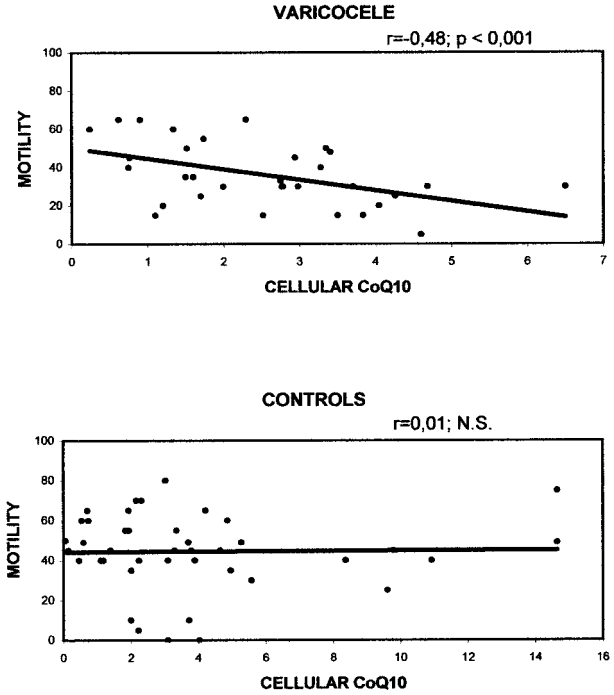

Fig 4. Correlation between cellular CoQ10 (ng/10^6 cells) and percentage of progressive motile forms in VAR patients and control non-VAR subjects.

The damage caused by ROS can be considered one of the contributory causes that intervenes in the genesis of the infertility of a couple without causing anatomic or functional clinically evident damage, and thus is not detected by the various strategies commonly used in andrologic diagnosis.[12] Recent studies have confirmed an increased ROS generation and a reduced total antioxidant capacity in VAR patients.[13]

According to previous results,[4] we have observed a different distribution of ubiquinone between intracellular and extracellular compartments in patients with or without VAR. If seminal plasma ubiquinone reflects an interchange between intracellular and extracellular compartments,[14] the different distribution in VAR patients could cause a greater sensitivity to peroxidative damage and could be related to the hypofertility observed in VAR patients.

Data in agreement with this hypothesis come from a study of Weese et al,[14] who observed a higher ROS generation in the spermatozoa of VAR patients and a restoration of the lower values observed in fertile, non-VAR subjects after varicocelectomy. Even if it is debated whether a surgical repair of VAR is indicated in the presence of infertility, with or without an alteration of seminal parameters, it is clear that a long-term, high-grade VAR can produce irreversible testicular damage.[15] The higher sensitivity of spermatozoa of VAR patients to the oxidative damage could explain the impaired fertilization ability of these subjects.

On the other hand, the energetic role of CoQ10 is documented by in vivo and in vitro studies, in different pathologies.[16-18] We have shown an inverse correlation between cellular CoQ10 and motility parameters in VAR, but not in controls. This suggests reduced utilization, which in turn could cause a defect of motility even in patients with a normal cell count. This hypothesis is in agreement with data showing altered oxygen consumption, decreased lactate dehydrogenase,[19] and reduced oxidative phosphorylation[20] in VAR patients.

One can speculate that a saving of CoQ10 for energetic purposes is finalistically shifted to the increased need of antioxidant systems. Further studies can clarify the relationships between the two functions of this key molecule. Finally, the clinical relevance of these observations could better our understanding of infertility associated with VAR.

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