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Coenzyme Q10 Concentrations in Normal and Pathological Human Seminal Fluid

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ABSTRACT: Coenzyme Q10 (CoQ10) levels were assayed in total seminal fluid or both in seminal fluid and seminal plasma in 77 subjects with normal or pathological findings at standard semen analysis. CoQ10 levels showed a significant correlation with sperm count and with sperm motility. An interesting exception was constituted by patients with varicocele, in whom the correlation with sperm concentration was preserved, whereas the correlation with sperm motility was lacking. Moreover, they showed an increased ratio of plasma CoQ to total seminal CoQ10 in comparison with the other subjects. These data suggest a pathophysiological meaning of CoQ10 in human seminal fluid and a possible molecular defect in varicocele patients. CoQ10 measurement could represent an important examination in infertile patients; moreover, from these results a rationale might arise for a possible treatment with exogenous CoQ10 in dyspermic patients.

Key words: Seminal fluid, sperm count, sperm motility, ubiquinone, varicocele.

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Coenzyme Q10 (CoQ10), a component of the mitochondrial respiratory chain, also known as ubiquinone for its wide diffusion throughout mammalian tissues, plays a key role in energy metabolism and has potent antioxidant properties for cellular membrane integrity. The biomedical importance of this molecule has been widely accepted. The antioxidant properties of CoQ10 were investigated (Ernster and Forsmark-Andrée, 1993), and the antioxidant role of this molecule in the protection of contractile fibers and their function, from impairment induced by free radical damage, was highlighted (Karlsson, 1987).

It has been demonstrated that the biosynthetic machinery for CoQ is present at remarkably high levels in rat testis (Kalèn et al, 1990); ubiquinone could cover important functions in seminal fluid, due to its metabolic and antioxidant properties. In fact, it is known that a large amount of mitochondria are present in spermatozoa, in which the motile activity requires a high energy expenditure (Fawcett, 1975). Further studies have also focused on the importance of reactive oxygen species in seminal plasma. Peroxidative stress can produce, in some cases, damage of sperm cells (Jones and Mann, 1977). It has also been demonstrated that the exogenous administration of CoQ10 ameliorates the results of membrane integrity tests (swelling test, Mazzilli et al, 1988). In light of these data, the determination of CoQ10 in human seminal fluid could reveal interesting results. Because to our knowledge studies regarding this parameter are not available in the literature, we have determined the concentrations of CoQ10 in human semen in men with normal or altered seminal fluids and correlated these values with standard semen analysis.

Materials and Methods

Seventy-seven subjects, 19–40 years of age, participated in this study. They underwent the analysis of semen for infertility, control after medical therapy for inflammation, or diagnosis for varicocele. The diagnosis of varicocele was established by the Doppler technique (Hirsh et al, 1980). Samples of semen were obtained by masturbation at an andrology center laboratory. They were collected on the occasions of medical tests not requested by the authors, performed for diagnostic purposes and not only for research. CoQ10 was assayed in total seminal fluid (in 60 patients), in seminal plasma (in 44 patients), or in both seminal fluid and seminal plasma (in 27 patients).

Aliquots of 0.5 ml for CoQ10 determination were immediately collected, stored in the dark (to avoid ubiquinone photodegradation), and frozen at −20°C until assayed. The samples (0.5

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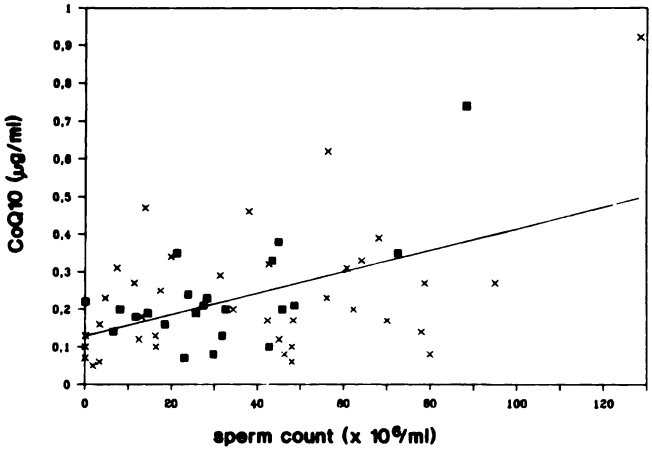


FIG. 1. Correlation between seminal CoQ10 values and sperm concentration (n = 60 subjects). Squares around the symbols denote patients with varicocele (n = 22). The trend regards the whole sample (including the patients with varicocele).

ml) of seminal plasma were obtained by centrifugation at 2,000 rpm for 15 minutes and immediate separation of supernatant plasma from the pellet of spermatozoa. They were then stored and frozen in the same way. A small quantity of seminal plasma was examined using a microscope to rule out the possibility that spermatozoa remained in the supernatant.

To assess whether seminal plasma CoQ10 values were dependent on a release from damaged spermatozoa, seminal plasma lactate dehydrogenase (LDH) levels were also determined in 20 patients to highlight a possible correlation with ubiquinone. LDH was determined by an optimized ultraviolet (UV) method (Boehringer-Mannheim); concentrations were expressed as U/L.

CoQ10 was assayed by high-performance liquid chromatography (HPLC), employing a UV detector (275 nm), as previously described (Littarru et al, 1990). Semen (or seminal plasma), at 0.5 ml, supplemented with 2 ml of ethanol:isopropanol (95:5), 0.5 ml of 0.1 M sodium dodecyl sulfate, and 0.5 μg of coenzyme

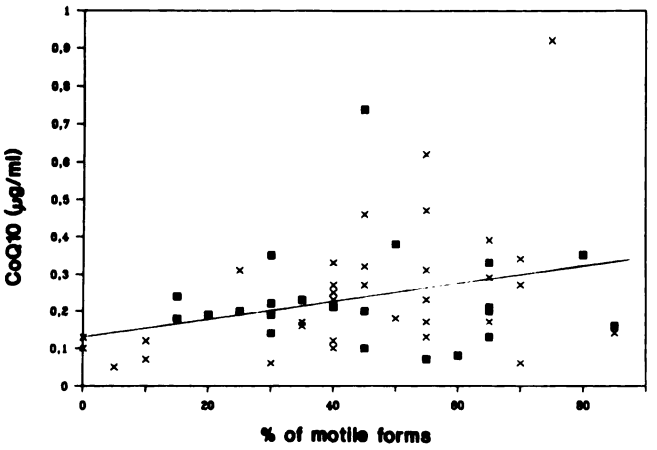


FIG. 2. Correlation between seminal CoQ10 values and total motility in the subjects tested (n = 60). Squares around the symbols denote patients with varicocele (n = 22). The trend regards the whole sample (including the patients with varicocele).

Q8 (CoQ8) as an internal standard, was extracted twice with 4 ml of n-hexane. Combined extracts were brought to dryness under N_2 (at 45–50°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 ± standard error of the mean [SEM]).

Standard semen analysis was performed according to World Health Organization criteria (Aitken et al, 1987). Statistical evaluation was performed by one-way analysis of variance (to assess differences among the groups) and linear regression analysis (to assess correlations between two different quantities within the same group).

Results

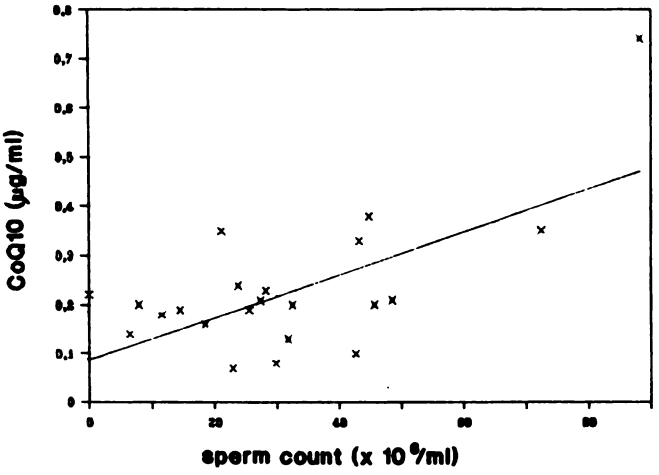
CoQ10 in Total Seminal Fluid

The sample (n = 60) included 21 patients with normozoospermia, 15 patients with azoospermia or oligozoospermia, 2 patients with germ-free genital tract inflammation, and 22 patients with varicocele (7 of them also showed oligo-azoospermia). CoQ10 levels were assayable in all human semen specimens by the same method as for blood CoQ10 determination (Littarru et al, 1990). Total seminal fluid CoQ10 concentrations ranged from 0.05 to 0.92 μg/ml (mean ± SEM: 0.23 ± 0.02 μg/ml). When considering the total sample, there was a significant correlation between CoQ10 values and sperm count (r = 0.504, $P < 0.0005$) (Fig. 1); moreover, there was a significant difference between the concentrations in patients with normozoospermia (n = 38, 20.0–128.5 × 10^6 spermatozoa/ml) and those in patients with azo- or oligozoospermia (n = 22, 0–19.9 × 10^6 spermatozoa/ml) (0.27 ± 0.03 μg/ml vs. 0.18 ± 0.02 μg/ml, $P < 0.05$).

A significant correlation was also found between CoQ10 value and the percent of total motile forms (r = 0.261, $P < 0.05$) (Fig. 2). Different CoQ10 concentrations were also observed in subjects with normal (>40%, n = 35) or altered (<40%, n = 25) sperm motility (0.28 ± 0.03 μg/ml vs. 0.19 ± 0.02 μg/ml, $P < 0.05$).

CoQ10 in Seminal Plasma

Seminal plasma values of CoQ10 were determined in 44 patients, 13 of whom were affected by varicocele. In 27 patients (10 with varicocele), CoQ10 levels were determined both in total seminal fluid and in separate aliquots of seminal plasma. We observed a mean value of 0.12 ± 0.01 μg/ml in plasma samples ($P < 0.0005$ when compared with total seminal fluid concentrations) and a mean proportion of 51.63 ± 4.97% of CoQ10 in seminal plas-



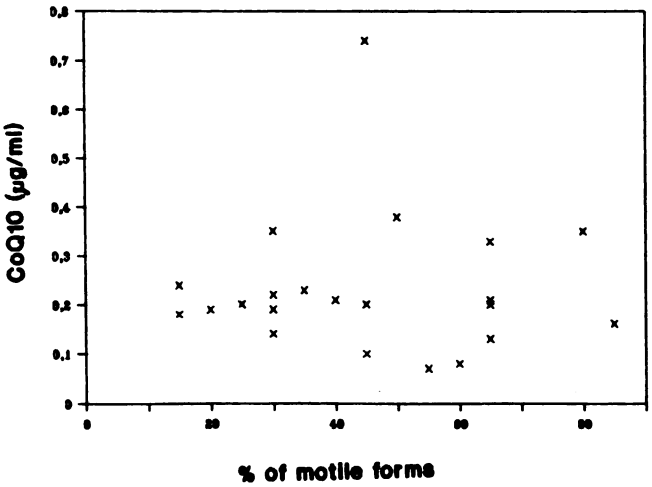


FIG. 3. Correlation between seminal CoQ10 values and sperm concentration and between seminal CoQ10 values and total motility in varicocele patients (n = 22).

ma. No correlation was found between CoQ10 and plasma LDH concentrations ($r = -0.085$, $P = 0.841$). LDH values ranged from 860 to 6,600 U/L (mean ± SEM: 2,792 ± 587).

Studies in Non-varicocele Patients

More interesting results were obtained when considering the different pathologies that led the patients to be studied. In the patients who did not show any clinical or instrumental (Doppler) evidence for varicocele (n = 38), a good correlation was shown between seminal CoQ10 (0.24 ± 0.03 µg/ml, range 0.05–0.92 µg/ml) and sperm count (0.5–128.5 × 10^6 spermatozoa/ml, r = 0.490, $P < 0.005$) as well as sperm motility (0–75%, r = 0.428, $P < 0.01$); moreover, only 41.21 ± 5.64% of total ubiquinone was found in seminal plasma.

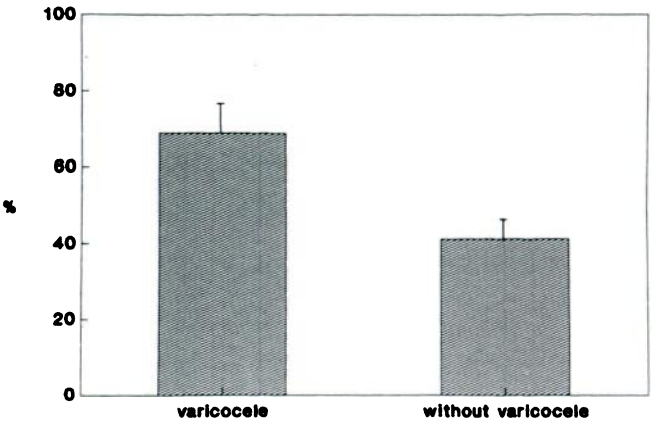


FIG. 4. Percent distribution in plasma vs. total seminal fluid of CoQ10 in patients with varicocele (n = 10) and the other patients (n = 17).

Studies in Varicocele Patients

In patients affected by varicocele, the correlation between CoQ10 and sperm count was still observed (n = 22, 0.05–88.5 × 10^6 spermatozoa/ml), with mean values of 0.23 ± 0.03 µg/ml (r = 0.666, $P < 0.0005$) (range 0.07–0.74 µg/ml). On the contrary, the correlation between CoQ10 and sperm motility (15–80%) was completely lacking (r = 0.008, P = 0.971) (Fig. 3). The distribution of CoQ10 between plasma and cells was significantly different; we observed 68.98 ± 7.11% of total CoQ10 in seminal plasma ($P < 0.01$ vs. the other subjects, Fig. 4).

Finally, two more exceptions were found: 1) elevated CoQ10 values (0.47 and 0.46 µg/ml) were present in the samples from an oligospermic patient (13.8 × 10^6 spermatozoa/ml) and from a normospermic patient (37.8 × 10^6 spermatozoa/ml), both characterized by the presence of numerous white blood cells, whose content of CoQ10 is known to be high; 2) only one patient exhibited a low seminal CoQ10 value in the presence of normospermia and without any evident pathology that could explain this phenomenon.

Discussion

Our data indicate that endogenous CoQ10 is assayable in human semen. They show a good correlation with sperm count and motility, except in subjects with varicocele, in whom a significant correlation with sperm count is still present, but the correlation with spermatozoa motility is completely lacking (r = 0.008 vs. 0.428 in non-varicocele patients). Moreover, in these subjects a significantly higher proportion of total CoQ10 is present in seminal plasma when compared with the other subjects. Due to its hydrophobic structure, CoQ10 is normally located in cellular membranes, and, at blood level, it is transported by

plasma lipoproteins, where it exerts an important antioxidant function (Mohr et al, 1992). Because its concentration in seminal plasma does not correlate with LDH levels in the patients studied, it is unlikely that the amounts of CoQ10 in plasma are due to spermatozoa damage and subsequent release of ubiquinone from the cells. Microscope examination of plasma also ruled out the presence of significantly large amounts of spermatozoa that, if persistent after centrifugation, could have explained the high levels of CoQ10 observed in seminal plasma.

An alternative hypothesis is that seminal plasma CoQ10 levels reflect an interchange between cellular and extracellular compartments. They could therefore have a pathophysiological meaning similar to serum ubiquinone values (Littarru et al, 1991). We can reasonably hypothesize that an abnormal distribution of CoQ10 in the seminal plasma and cell compartment, with a relative prevalence in plasma, could reflect an impaired utilization by the cell machinery. In this sense, the lack of correlation of total seminal CoQ10 concentrations with sperm motility (although the correlation with sperm count was preserved), together with the higher seminal plasma CoQ10/total seminal CoQ10 ratio observed in varicocele patients, could have clinical relevance, especially in light of the altered seminal characteristics that are known to be found in these subjects (Turner, 1983). A relative deficiency or utilization of CoQ in sperm cells could therefore be hypothesized in varicocele patients. This datum could contribute to the respiratory chain defect reported in spermatozoa of these subjects, in which O_2 consumption is known to be reduced (De Carlo et al, 1990). Moreover, the seminal plasma CoQ10 concentration could be related to an antioxidant activity in the semen, also at extracellular levels. It is possible that a molecular defect is involved in the sperm alterations of varicocele patients, leading to a higher sensitivity to a peroxidative insult.

Finally, the phlogosis could contribute to elevate seminal fluid CoQ10 in oligospermic subjects; a deficiency of CoQ10 could be hypothesized in absence of other predisposing causes. CoQ10 is a component of the respiratory chain and, due to its function in energy metabolism, could play an essential role in motile activity of spermatozoa. Due to its antioxidant activity, it could also be involved in the protection of sperm cell membranes from oxidative insult. Because oral treatment with CoQ10 has shown significant improvements in pathologies characterized by a deficiency of ubiquinone (Langsjoen et al, 1990), these hypotheses, if confirmed, could have clinical relevance. Recently, the effects of exogenous administration of CoQ10 in dyspermic subjects were reported (Mazzilli et al, 1990); taken as a whole, these data could represent the rationale for a possible medical treatment in a selected group of infertile patients, in which a deficiency of endogenous CoQ10 is demonstrated.

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