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REVIEW

Bioenergetic and Antioxidant Properties of Coenzyme Q_{10}: Recent Developments

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Abstract For a number of years, coenzyme Q (CoQ_{10} in humans) was known for its key role in mitochondrial bioenergetics; later studies demonstrated its presence in other subcellular fractions and in plasma, and extensively investigated its antioxidant role. These two functions constitute the basis on which research supporting the clinical use of CoQ_{10} is founded. Also at the inner mitochondrial membrane level, coenzyme Q is recognized as an obligatory co-factor for the function of uncoupling proteins and a modulator of the transition pore. Furthermore, recent data reveal that CoQ_{10} affects expression of genes involved in human cell signalling, metabolism, and transport and some of the effects of exogenously administered CoQ_{10} may be due to this property. Coenzyme Q is the only lipid soluble antioxidant synthesized endogenously. In its reduced form, $CoQH_2$, ubiquinol, inhibits protein and DNA oxidation but it is the effect on lipid peroxidation that has been most deeply studied. Ubiquinol inhibits the peroxidation of cell membrane lipids and also that of lipoprotein lipids present in the circulation. Dietary supplementation with CoQ_{10} results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low-density lipoproteins to the initiation of lipid peroxidation. Moreover, CoQ_{10} has a direct anti-atherogenic effect, which has been demonstrated in apolipoprotein E-deficient mice fed with a high-fat diet. In this model, supplementation with CoQ_{10} at pharmacological doses was capable of decreasing the absolute concentration of lipid hydroperoxides in atherosclerotic lesions and of minimizing the size of atherosclerotic lesions in the whole aorta. Whether these protective effects are only due to the antioxidant properties of coenzyme Q remains to be established; recent data point out that CoQ_{10} could have a direct effect on endothelial function. In patients with stable moderate CHF, oral CoQ_{10} supplementation was shown to ameliorate cardiac contractility and endothelial dysfunction. Recent data from our laboratory showed a strong correlation between endothelium bound extra cellular SOD (ecSOD) and flow-dependent endothelial-mediated dilation, a functional parameter commonly used as a biomarker of vascular function. The study also highlighted that supplementation with CoQ_{10} that significantly affects endothelium-bound ecSOD activity. Furthermore, we showed a significant correlation between increase in endothelial bound ecSOD activity and improvement in FMD after CoQ_{10} supplementation. The effect was more pronounced in patients with low basal values of ecSOD. Finally, we summarize the findings, also from our laboratory, on the implications of CoQ_{10} in seminal fluid integrity and sperm cell motility.

Keywords Coenzyme Q10 · Mitochondrial bioenergetics · Cardiac contractility · Endothelial function · Lipoprotein peroxidation · Sperm cell motility

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Introduction

Coenzyme Q is a lipid with a wide distribution in nature and refers to a general structure composed of a nucleus, i.e. 2,3-dimethoxy-5-methylbenzoquinone, and, substituted at position 6 of this quinone, a side chain consisting of isoprene units (five carbons) all in trans configuration, and with one double bond. In human tissues by far the most abundant form of coenzyme Q is coenzyme Q_{10}, which has

10 isoprenoid units. For a number of years, coenzyme Q (CoQ_{10} in humans) was known for its key role in mitochondrial bioenergetics; later studies demonstrated its presence in other subcellular fractions and in plasma, and extensively investigated its antioxidant role. These two functions constitute the basis on which research supporting the clinical use of CoQ_{10} is founded. Also at the inner mitochondrial membrane level, coenzyme Q is recognized as an obligatory co-factor for the function of uncoupling proteins and a modulator of the transition pore [1]. Furthermore, recent data reveal that CoQ_{10} affects expression of genes involved in human cell signalling, metabolism and transport [2], and some of the effects of exogenously administered CoQ_{10} may be due to this property.

CoQ_{10} and Mitochondrial Bioenergetics

The essential role of CoQ_{10} in mitochondrial bioenergetics was postulated many years ago [3]. According to this early view, the quinone was considered as a substrate in excess concentration over the prosthetic groups in the lipoprotein complexes of the respiratory chain. The kinetic analyses of Kroger and Klingenberg [4] showed that steady-state respiration in submitochondrial particles could be represented as a simple combination of two enzymes, the first one responsible for the reduction of coenzyme Q and the second one causing oxidation of ubiquinol. Experiments of direct titrations of CoQ-depleted mitochondria reconstituted with different CoQ supplements disclosed a Km of NADH oxidation close to a CoQ concentration of 4–10 mM in the lipid bilayer, whereas the Km for succinate oxidation was one order of magnitude lower [5]. As found by Lenaz, Km for CoQ_{10} of NADH oxidation in bovine heart mitochondria is within the range of its concentration in the membrane [5]. The physiological concentration, therefore, is not saturating and even a small increase in the CoQ_{10} concentration of mitochondrial membranes could lead to an increased respiratory rate. The control exerted by CoQ concentration over mitochondrial respiration could be of particular interest in situations of decreased CoQ levels and/or increased Km values for the quinone in some pathological states. In fact, this observation could represent the biochemical mechanism by which exogenous coenzyme Q_{10} ameliorates the bioenergetic impairment in a some mitochondrial myopathies and in cardiomyopathy [6 , 7].

Modern views indicate that respiratory complexes may have a supramolecular organization, i.e. stable super complexes [8]. The advantage of this super complex organization would be a more efficient electron transfer by channelling of the redox intermediates. Preliminary data suggest that alteration of the protein/phospholipid ratio and lipid peroxidation disaggregates the supercomplex organization with possible pathophysiological implications. Lenaz postulates [8] that in ageing, and in ischemic diseases, reactive oxygen species (ROS) produced by the mitochondrial respiratory chain induce a progressive peroxidation of mitochondrial phospholipids. This could lead to a dissociation of Complexes I–III aggregates and subsequent loss of facilitated electron channelling. Also according to this model, an increased concentration of coenzyme Q may, at least partially, counteract this deleterious effect of supercomplex disaggregation.

Antioxidant Effects on Lipids and Plasma Lipoproteins

The antioxidant role of CoQ_{10} as inhibitors of lipid peroxidation has been known for many years. Its reduced form, $CoQ_{10}H_2$, behaves as a phenolic antioxidant and its effects have been studied in lipid solutions, mycelles, subcellular organelles, cell cultures and in vivo. Coenzyme Q is the only lipid-soluble antioxidant synthesized endogenously. Ubiquinol inhibits the peroxidation of cell membrane lipids and also that of lipoprotein lipids present in the circulation. In particular, $CoQ_{10}H_2$, ubiquinol-10, has been investigated in human low-density lipoproteins (LDL). This field has been extensively explored, since the early studies of Stocker [9]. This author hypothesized and then demonstrated that ubiquinol-10, besides reacting directly with peroxyl radicals, effectively reduces alpha-tocopheroxyl radical to alpha-tocopherol and in this way eliminates a potentially prooxidant radical and regenerates the active form of vitamin E. Studies carried out on LDL peroxidability in relation to its ubiquinol content clearly indicate that in the early stage of oxidation processes ubiquinol is the most active antioxidant [9]. Supplementation with exogenous CoQ_{10} leads to an increase in the ubiquinol content of LDL and a decrease of their peroxidability [10].

The heaviest fraction of LDL, namely LDL3, is also the most peroxidizable, and epidemiological studies have demonstrated that the abundance of this subfraction is correlated with the incidence of ischemic heart disease. In 1995, we demonstrated that LDL3 also have a lower content of CoQ_{10} compared to LDL1 and LDL2; moreover, administration of CoQ_{10} to normal volunteers increased the CoQ_{10} content, particularly in the LDL3 subfraction, and decreased their peroxidizability [11].

Antiatherogenic Effect of CoQ_{10}

CoQ_{10} has also a direct anti-atherogenic effect, which has been demonstrated in apolipoprotein E deficient mice fed with a high fat diet. In this model supplementation with

CoQ_{10} at pharmacological doses was capable of decreasing the absolute concentration of lipid hydroperoxides in atherosclerotic lesions and of minimizing the size of atherosclerotic lesions in the whole aorta [12]. Whether these protective effects are only due to the antioxidant properties of coenzyme Q remains to be established; recent data point out that CoQ_{10} could have a direct effect on endothelial function. In the past it has already been shown that CoQ_{10} has a significant hypotensive effect in humans [13]. More recent studies have demonstrated an effect on endothelial function, which contributes to functional impairment of the arteries and often precedes the appearance of the atherosclerotic lesion. Endothelial dysfunction is an important component of CHF (chronic heart failure) and may depend either on reduced nitric oxide synthesis, or increased nitric oxide inactivation, or both. Increased oxidative stress has been shown to augment the inactivation of nitric oxide to peroxy-nitrite, which is itself an oxidizing species.

Watts et al. [14] showed in 2002 that CoQ_{10} supplementation improves endothelial function in dyslipidemic patients with Type II diabetes.

CoQ_{10} and Ischemic Heart Disease: Bioenergetic Effect or Improvement of Endothelial Function?

We also recently investigated, whether in patients with stable moderate congestive heart failure (CHF), oral CoQ_{10} supplementation could ameliorate endothelial dysfunction and functional impairment of the cardiac muscle [15]. Endothelial dysfunction was evaluated by assessment of the brachial artery vasomotor function (flow-mediated dilation [FMD]). The FMD was measured after release of a sphygmomanometer inflated at 240 mmHg for 4.5 min at the wrist. The sheer stress resulting from readmission of blood flow activates endothelial NO synthase and the release of nitric oxide induces a vasodilatation measurable by an ultrasound technique. In the same patients, maximal oxygen uptake (peak VO_2) was assessed by cardiopulmonary exercise test and myocardial contractility by the sensitive dobutamine stress echocardiography test. Oral CoQ_{10} supplementation significantly improved the endothelium-dependent relaxation of the brachial artery, left ventricle contractility and peak VO_2.

The result of this study could be interpreted in the light of the bioenergetic, as well as the antioxidant, properties of coenzyme Q. We found a significant improvement in left ventricle contractility in dysfunctional segments located in non-infarcted areas served by stenotic arteries, where hibernation and/or chronic stunning is likely to occur. The amelioration of contractile function after CoQ_{10} suggests that chronic post-ischemic stunned cells improve or normalize their metabolism and function. Animal studies had suggested that CoQ_{10} protects stunned myocardium in an open-chest swine model [16]. Myocardial stunning is clearly related to ischemia-reperfusion damage. ATP is depleted during ischemia and experimental evidence indicates increased oxidative stress in stunned myocardium. Ex vivo work in a rabbit heart model of ischemia and reperfusion showed a relative maintenance of tissue stores of ATP, a relative preservation of ATP-generating capacity of mitochondria and a relative absence of calcium overload in CoQ_{10}-pretreated rabbits [17]. We might reasonably hypothesize that in our clinical study, administration of 300 mg of CoQ_{10} per day was capable of increasing, even slightly, myocardial ATP content, therefore, improving electron transport rate and ATP production. Although the increase in tissue CoQ_{10} content upon oral administration is generally lower than the remarkable elevations in plasma, because the mitochondrial membrane concentration of CoQ_{10} is probably close to the Km value for the NADH oxidation systems, even a small increase could generate an increase in respiratory rate [5], especially in a myocardium with a certain degree of CoQ_{10} deficiency. In a study conducted by Sohal and co-workers [18], CoQ supplementation resulted in an increase in total CoQ content in the heart and the skeletal muscle homogenates. After 4 weeks of supplementation, total CoQ content showed a significant increase in homogenates of heart and skeletal muscle (23% and 45%, respectively; $P < .05$). In a more recent paper, the same group showed that supplementation of mice with CoQ_{10} significantly increased CoQ_9 and CoQ_{10} content in homogenates and mitochondria of liver, heart, kidney, skeletal muscle and brain [19]. This issue was also addressed by Rosenfeldt et al. in rats and in human myocardial tissue [20]. Hearts isolated from senescent (35 months) rats were tested in vitro in a model, where they were subjected to rapid electrical pacing. After pacing, the senescent hearts, when compared to young, showed reduced recovery of pre-stress work performance. CoQ_{10} pre-treatment of rats from which the senescent hearts were isolated improved the heart recovery of the aged myocardial muscle. In another experiment [20], the same authors showed that trabeculae isolated from older patients (>70 years of age) during heart surgery showed reduced recovery of developed force after simulated ischemia compared to younger counterparts (<70 years). CoQ_{10} content was decreased in the myocardium from aged patients. A few years later, the study was extended to test the effect of CoQ_{10} therapy before cardiac surgery on mitochondrial function [21]. Patients undergoing elective cardiac surgery were randomized to receive oral CoQ_{10} (300 mg/die) or placebo for 2 weeks preoperatively. Trabeculae from right atrial appendages were excised and mitochondria isolated and studied. Patients treated with CoQ_{10} had increased ubiquinone levels in isolated

mitochondria; mitochondrial respiration (ADP/O ratio) was more efficient and malondialdehyde content was lower in the specimen from CoQ_{10}-treated patients. Myocardial tolerance to oxidative stress was also improved.

Other literature indicates that the administration of CoQ_{10} increases cellular ATP production. In a study conducted by Marriage et al., 12 patients with proven defects of oxidative phosphorylation were treated with a combination of CoQ_{10} (5 mg/kg body weight), carnitine, vitamin B complex, vitamin C and vitamin K [22]. At baseline, all 12 patients had decreased ATP synthesis in lymphocytes, with all substrates tested, when compared to control subjects. Overall there was a 34% reduction in ATP synthesis when no substrate was added; after 12 months of treatment, the ATP synthesis reflecting the oxidation of endogenous substrate was only reduced by 12% relative to mean control values. In order to identify the potential active ingredient(s) in the cofactor mixture, the authors analysed the ability of the individual compounds to influence ATP synthesis rates using lymphocytes from control subjects. Only CoQ_{10} supplementation of control lymphocytes could lead to an increase in ATP synthesis. Interestingly, also a marked increase in non-mitochondrial ATP synthesis was observed, which is compatible with additional functions of CoQ_{10} as a component of extra mitochondrial redox reduction. Some patients in this study showed an increase in energy levels and improvement in exercise tolerance.

Statins and CoQ_{10}: Possible Interactions

On the basis of the common biosynthetic pathway of CoQ_{10} and cholesterol, statins, which are potent inhibitors of cholesterol biosynthesis, also adversely affect CoQ_{10} levels. A decrease of CoQ_{10} levels has been widely demonstrated in plasma of patients treated with statins and in plasma and tissues of laboratory animals [23]. CoQ_{10} deficiency has been postulated as one of the possible causes of the relatively uncommon side effects seen in the course of statin treatment. In this review, I would like to mention a study in the light of the relationship between CoQ_{10} levels and ATP production. In a trial conducted in 2005 by Päivä and co-workers [24], 48 patients with hypercholesterolemia were randomly assigned to receive different statins or placebo for 8 weeks. Plasma samples and muscle biopsy specimens were obtained at baseline and at the end of the follow-up. The muscle ubiquinone concentration was reduced significantly in the group treated with 80 mg/die with simvastatin. Respiratory chain enzyme activities were decreased in six patients with markedly reduced muscle ubiquinone compared to matched subjects selected from the placebo and the lower-statin dosage groups. Citrate synthase was also decreased in mitochondria from those six patients, so it is possible that a reduction in mitochondrial number explains the decrease in both muscle ubiquinone and mitochondrial enzyme activity.

CoQ_{10} an Extracellular SOD

Extracellular superoxide dismutase (ecSOD) is a particular SOD that plays an important role in protecting the endothelium from oxidative stress; it can be also assayed in plasma, where it is released from the extracellular matrix by means of a functional test. Vascular ecSOD activity is substantially reduced in patients with coronary artery disease. In a recent study conducted in our laboratory [25], 38 patients affected by ischemic heart disease were randomized into two groups: one group received CoQ_{10} orally at a dose of 300 mg/day for one month, while the other group received placebo. ecSOD, endothelium-dependent relaxation, as well as peak VO_2 increases in the CoQ_{10}-treated group were statistically higher versus the variations in the placebo group. In particular, improvements elicited by CoQ_{10} supplementation were remarkable in subjects presenting low initial endothelium-bound ecSOD and thus more prone to oxidative stress. Improvements in the ED relaxation and endothelium-bound ecSOD activity might be related to CoQ_{10}'s capability of enhancing endothelial functionality by counteracting nitric oxide (NO) oxidation. Enhancement of peak VO_2 is likely due to the bioenergetic effect of CoQ_{10} also on the basis of the observed improvement in O_2 pulse. More NO availability could also lead to an increased ecSOD gene-expression [26], which could in turn contribute to preserve NO from inactivation by O_2.

Coenzyme Q and Reduction of Ferrylmyoglobin

Another antioxidant mechanism of coenzyme Q might have practical implications in the ischemia-reperfusion damage. It is known that interaction of myoglobin with hydrogen peroxide leads to the formation of ferrylmyoglobin and/or its radical form: these transformation products of myoglobin are regarded as powerful oxidizing agents capable of attacking important cellular constituents. Formation of ferrylmyoglobin is an event that might have considerable practical importance, since skeletal muscle and the myocardium are rich in myoglobin, and the formation of hydrogen peroxide arising from ischemia-reperfusion might trigger myoglobin oxidation to ferrylmyoglobin, with further damage. In our experiments [27], the addition of H_2O_2 to a metmyoglobin solution induced the rapid formation of a compound with the spectral characteristics of ferrylmyoglobin. The addition of ubiquinol 1 (we used reduced CoQ_{10}

because of its good water solubility) provoked a rapid and progressive reduction of ferrylmyoglobin to metmyoglobin and oxymyoglobin. When reduced Coenzyme Q was added together with H_20_2, formation of ferrylmyoglobin was considerably slowed. In transforming MB IV to MB III and $MB0_2$, reduced Coenzyme Q is oxidized. It is worthwhile to remember that cells have several mechanisms capable of "regenerating" reduced Coenzyme Q, besides the one constituted by the Coenzyme Q reductases of the mitochondrial respiratory chain. Availability of reduced Coenzyme Q is, therefore, capable of feeding a mechanism allowing the heme protein to act as a device that removes hydrogen peroxide and possibly other peroxides, thus neutralizing their harmful character.

Protective Effects on DNA Oxidation

Several years ago, we also investigated the role of coenzyme Q_{10} in the prevention of DNA oxidative damage. A first experiment was conducted on freshly isolated human blood lymphocytes, pre-incubated in vitro with liposomes loaded with ubiquinone-10 or ubiquinol-10 [28]. Exposure of control lymphocytes to 100 μM H_2O_2 resulted in a detectable increase in DNA strand breaks, measured by the comet assay technique. DNA strand breaks occurred after a significant decrease in the cellular content of ubiquinone-10. Incubation of cells with ubiquinol-10 enriched liposomes increased the total cellular CoQ_{10} from 14.9 ±1.8 to 61.0 ± 5.5 pmol/10^6 cells. The proportion of CoQ_{10} present in the reduced form also increased. Exposure of ubiquinol-10 supplemented lymphocytes to 100 μM H_2O_2 also resulted in a rapid decrease in cellular content of ubiquinol–10. Despite this loss, however, a relatively large proportion of the cell's CoQ_{10} remained in the reduced, antioxidant-active form. Enrichment with ubiquinol-10 protected lymphocyctes from H_2O_2-induced DNA strand breaks. We hypothesized that in such a system ubiquinol-10 acts as an early target for H_2O_2 derived antioxidants: this finding is similar to the situation in LDL, where ubiquinol-10 is the first lipid soluble antioxidant to be consumed in the early stages of lipid peroxidation [9]. Lymphocytes incubated with liposomes enriched with the oxidized form of CoQ_{10} (ubiquinone-10) increased their content of total CoQ_{10}, but only about 8% was present in the reduced form. Surprisingly, however, these ubiquinone-10 enriched cells were also more resistant to H_2O_2-induced DNA damage and loss in viability when compared with control cells.

In a subsequent study, in vivo supplementation with CoQ_{10} was shown to enhance the recovery of human lymphocytes from oxidative DNA damage [29]. The oral intake of 100 or 300 mg/day of CoQ_{10} for two consecutive weeks increased the endogenous CoQ_{10} cellular content by 45% and 144%, respectively. DNA of CoQ_{10}-enriched lymphocytes was less damaged by the exposure to oxygen, and the extent of DNA strand break formation was inversely related to the concentration of CoQ_{10} in plasma and cells. The activity of DNA repair enzymes was also assessed, by incubating lymphocyte extracts containing DNA repair enzymes with a substrate consisting of oxidized purine basis previously exposed to Ro-19-8022: upon irradiation with a visible light source, Ro-19-8022 produces singlet oxygen. DNA repair enzymes introduce breaks at sites of oxidized purines and enzyme activity is reflected by the number of DNA breaks. CoQ_{10} supplementation enhanced DNA repair activity, which was markedly higher in cellular extracts from CoQ_{10}-enriched lymphocytes, 148 ± 25 arbitrary units (au.) vs. 55 ±_15 au. and 94 ± 21 au., $P < 0.05$, respectively. The effects of CoQ_{10} might be related to its protective effect against oxidation and a stimulation of the activity of repair enzymes. Changes in the redox state of the transcriptional factors have been proposed as a mechanism regulating gene expression. In a recent study [2], we showed that in a human cell line incubated in vitro with CoQ_{10}, there was an increased expression of several hundred genes involved in cell signalling, metabolism and transport.

We are currently evaluating the results of a clinical trial conducted in patients affected by Down syndrome treated with CoQ_{10}. The aim of this study is to measure the extent of DNA damage, and the effect of CoQ_{10} administration, by means of a new, optimized, single-cell gel electrophoresis technique [30].

Implications of Coenzyme Q_{10} in Male Infertility

A growing body of evidence indicates that damage inflicted to spermatozoa by ROS plays a key pathogenetic role, implicating oxidative stress as a mediator of sperm dysfunction in the etiology of male infertility. In order to counteract the potentially hazardous effects of oxidative stress, spermatozoa and seminal plasma are endowed with several protective antioxidant systems. Some studies have shown that infertile men have an impaired seminal plasma non-enzymatic antioxidant capacity, suggesting that decreased total antioxidant capacity may exert a pathogenetic role in male infertility. The first determination of endogenous CoQ_{10} levels in seminal fluid was performed by our group [31]. Several years later, we showed [32] a significant correlation between the reduced form of coenzyme Q10 and sperm count in seminal plasma and an inverse correlation between ubiquinol and hydroperoxide levels, both in seminal plasma and seminal fluid. We also found an inverse correlation between ubiquinol/ubiquinone

ratio and the percentage of abnormal sperm cells. Moreover, a lower ubiquinol/ubiquinone ratio was shown in sperm cells from idiopathic asthenozoospermic patients (IDA) and in seminal plasma from IDA and varicocele-associated asthenozoospermic patients compared to controls. The above-mentioned studies constitute a rationale which eventually led us to treat infertile subjects with exogenous CoQ_{10}. The effect of CoQ_{10} on sperm motility in vitro had previously been reported by Lewin and Lavon [33].

In an attempt to elucidate a potential therapeutic role, we administered CoQ_{10} to a group of idiopathic asthenozoospermic infertile patients [34]. About 22 patients (mean age: 31 years, range: 25–39 years) affected by idiopathic asthenozoospermia, were enrolled in the study. All subjects presented a clinical history of primary infertility of at least 3 years. No female-related factor was apparently involved in sterility. Eligible patients had sperm count $>20 \times 10^6$/ml, sperm motility (forward motility, class a and b, according to WHO 1999 criteria) <50% at two distinct sperm analyses, and normal sperm morphology >30%.

The enrolled patients were administered CoQ_{10} 200 mg/day, divided into two doses, for 6 months. Semen analysis, including computer-assisted sperm analysis and motility (C.A.S.A.), CoQ_{10}, and phosphatidylcholine assays, were performed at baseline and after 6 months of therapy. A semen analysis was further performed after 6 months from interruption of therapy (washout). The results showed an increase of CoQ_{10} levels in seminal plasma after treatment, the mean value rising significantly from 42.0 ± 5.1 at baseline to 127.1 ± 1.9 ng/ml after 6 months of exogenous CoQ_{10} administration ($P < 0.005$). 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; $P < 0.05$). As far as semen features are concerned, a significant difference was found in forward (class a + b) motility of sperm cells after 6 months of CoQ_{10} dietary implementation.

A significant increase of curvilinear velocity VCL (from 26.31 ± 1.50 to 46.43 ± 2.28 μm/s, $P < 0.05$), and straight progressive velocity VSL (from 15.20 ± 1.30 to 20.40 ± 2.17 μm/s, $P < 0.05$) was found after treatment. No significant differences were found in sperm cell concentration and morphology. 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 C.A.S.A. (VCL and VSL) kinetic parameters (Cramer's $V = 0.4637$; 0.3818; 0.3467; 0.5148, respectively). Sperm forward motility was significant reduced after 6 months of wash-out (from 16.34 ± 3.43 to 9.50 ± 2.28%, $P < 0.001$), while no significant differences were found in sperm cells concentration and morphology. The wives of 3 out of 22 patients (13.6%) achieved spontaneous pregnancy within 3 months from the discontinuation of therapy (2.4% pregnancy rate per cycle). The data of the present study show a significant improvement of kinetic features of sperm cells after 6 months of administration of CoQ_{10}, both on the basis of manual and computer-assisted evaluation. Furthermore, these results constitute the first demonstration that exogenous administration of CoQ_{10} increases its levels in seminal plasma and in spermatozoa.

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 than baseline value) are commonly found in blood plasma after chronic administration of the quinone. As 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 passive or involves an active mechanism. Statistical analysis did not reveal any significant functional relationship among the therapy-induced variations of CoQ_{10} and kinetic parameters of spermatozoa, probably because of the small number of samples. Nevertheless, the good degree of association among these variables, according to Cramer's V index of association, supports the hypothesis of a pathogenetic role of CoQ_{10} in asthenozoospermia according to previously reported data [35]. The improvement of spontaneous pregnancy rate also suggests a benefit of this therapeutic approach.

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