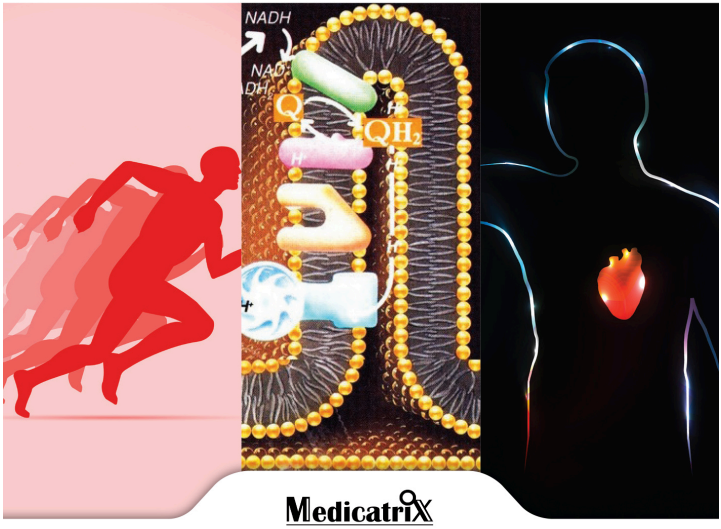
Gian Paolo **LITTARRU**

# Ubiquinol

## New insights into the most active form of Coenzyme $Q_{10}$




Medicatrix

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Printed in France (Nouvelle Imprimerie Laballery)

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ISBN 978–2–87211–149–7
Legal deposit October 2014/5053/I1

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# About the Author

Gian Paolo Littarru graduated as an M.D. at the Catholic University Medical School, Rome, in 1967. From 1969 to 1972, he was a postdoctoral fellow at the Institute for Biomedical Research, the University of Texas at Austin, under the direction of Prof. Karl Folkers. He was a professor in cellular biochemistry at the Catholic University in Rome until 1986 and then he moved to the Polytechnic University of the Marche, Ancona, where he taught medical chemistry and clinical biochemistry to medical students until his recent retirement. In 1997 he founded the International CoQ Association, of which he was the chairman until 2013. Professor Littarru's main research interest has always been Coenzyme Q. He became interested in this molecule back in 1968 when he first studied the structural specificity of Coenzyme Q in the respiratory chain of mitochondria from different animal species. He soon extended his research to humans. In the early '70's, in cooperation with cardiac surgeons and neurologists he accomplished the separation of mitochondria and the determination of $CoQ_{10}$ status in numerous heart and muscle biopsies. This biomedical research was then extended to the antioxidant properties of $CoQ_{10}$.

As Chairman of the International Coenzyme $Q_{10}$ Association the author has promoted cooperation in this field of research with several medical groups worldwide, thus contributing to a rapid broadening of the biochemical and clinical knowledge of the molecule known as Coenzyme $Q_{10}$ and of its most active form, Ubiquinol.

# 1st PART

# $CoQ_{10}$ Biology

# Introduction

The aim of this book is to introduce some of the basic biochemical facts and medical implications of Coenzyme Q, with particular emphasis on its reduced form, Ubiquinol.

The discovery of the $CoQ_{10}$ molecule came about as a result of an intensive program of research developed by Prof. D. E. Green, at the University of Wisconsin, to find out how mitochondria work. This occurred in 1957. It soon became evident that Coenzyme Q was essential to mitochondrial ATP formation,i.e.,to the most efficient mechanism which leads to the release of chemical energy housed in our nutrients. Several years later Prof. P. Mitchell was awarded the Nobel Prize, for his studies centered on the vital role of Coenzyme Q in oxidative phosphorylation. In order to understand the main role of Coenzyme Q, it is necessary to illustrate the cellular context in which Coenzyme Q is located. Therefore the mechanisms by which mitochondria harness the energy originally enclosed in biological fuels will be outlined in the initial chapters of this book.

The foresight of Prof. Karl Folkers, who in 1958 re-isolated Coenzyme $Q_{10}$, the human Coenzyme Q, from human heart and elucidated its structure, led him to predict the clinical importance of $CoQ_{10}$. At the same time, Prof. Yuichi Yamamura, a pioneer of $CoQ_{10}$ research in cardiology, developed the first clinical trial of $CoQ_{10}$ in patients with cardiovascular disease.

Those intuitions opened a new, variegated biomedical research field, and its knowledge base deepened along with the expanded understanding of the basic biochemistry of Coenzyme Q.

It soon became evident that some of the in vitro and in vivo properties of Coenzyme Q were the result of its antioxidant action, which are mainly related to the reduced form of Coenzyme $Q_{10}$, Ubiquinol. Today we have a broader picture of Coenzyme $Q_{10}$ properties, suggesting that some of $CoQ_{10}$ effects might be related also to its capabilities of modulating other mitochondrial functions and inducing gene expression.

During recent years, interest in oxidative stress and its implications in biology and medicine have grown unceasingly. Free radicals have been recognized as involved in many pathophysiological processes and in different diseases. Thus, the antioxidant role of $CoQ_{10}$ deserves attention.

After discussing the classical bioenergetic role of Coenzyme Q, I will attempt to illustrate the immense field where the struggle between oxidative damage and antioxidant defense takes place. The general principles will be described. Then I will focus on some pathophysiological processes related to free radical damage, in which experimental evidence strongly suggests a defensive, antioxidant role for Coenzyme Q.

I would like to conclude with the words of a late friend, Dr. Per Langsjoen, who left us a precious heredity represented by his valuable achievements in clinical research and by the humble enthusiasm of his approach to these new horizons: "I would prefer to speak of the clinical promise of $CoQ_{10}$ used as a crucial factor in cellular bioenergetics and in free radical quenching. I would also speak from the 'clinical trenches', which is my natural habitat."

From that point of view the overwhelming reality is not how much we know, but how little we know about many serious clinical problems (despite grandly assigning precise names to these conditions). Studies in this field, which embrace far more than just $CoQ_{10}$, represent a totally new dimension to man's understanding of the mysteries of health and poor health, which may well rank as the major advance

since the discovery of bacteria. In cardiology alone one can foresee a time when disease of the heart and circulation may lose its ranking as the number one cause of death. In medicine generally the rapid advance of knowledge is so broad in its implication that the entire field, in time, may profitably be re-evaluated on the basis of these new principles. "


Fig. 1 – Dr. Frederick Crane

A yellow compound was initially isolated in 1957 from beef heart mitochondria in the laboratories of the Enzyme Institute at the University of Wisconsin, directed by Dr. D. Green. The four postdoctors who accomplished this isolation or discovery were F.L. Crane, Y Hatefi, R.L. Lester and C. Widmer[1].

Dr. Karl Folkers received from Dr. Crane a few milligrams of the crystalline substance, which he took to the research laboratories of Merck, in Rahway, New Jersey. Together with his associates at Merck, Folkers re-isolated and determined the structure of that compound.

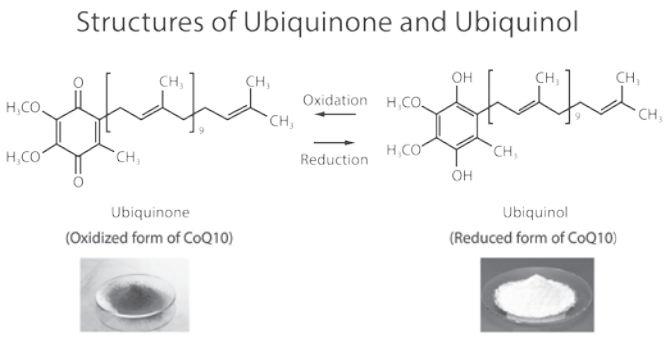


Fig. 2 – Structures of Ubiquinone and Ubiquinol.

The compound was named Coenzyme $Q_{10}$ ($CoQ_{10}$), since it had a coenzymatic activity in the enzyme systems of mitochondria. In general, coenzymes are substances that are necessary for the activity of an enzyme. $CoQ_{10}$ would soon be recognized as an essential coenzyme in the bioenergetics of respiration. From a chemical point of view it is a lipid. The letter Q is derived from its quinone group; the figure 10 defines the number of isoprenoid units in its side chain.

Dr. R.A. Morton, who also isolated and studied the compound, during the same years named it ubiquinone because of its widespread, ubiquitous presence diffusion in living organisms[2]. The ending or suffix "one" indicated the molecule's oxidized state. The reduced form was called ubiquinol.

Coenzyme $Q_{10}$ is the coenzyme of man and many mammals; other animal species have a Coenzyme Q with a shorter side chain having less than 10 isoprenoid units. Therefore, generally speaking, we will say Coenzyme Q, or ubiquinone. The term Coenzyme $Q_{10}$, or $CoQ_{10}$ will be used when dealing with the human Coenzyme Q, which is also available as a dietary supplement.

In the recent years Kaneka was able to produce Ubiquinol, the reduced form, and to make it available as a food supplement by developing a special procedure which stabilizes it in the reduced form.

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# Energy transformations in living organisms

Coenzyme Q was discovered in the inner membrane of mitochondria, the subcellular organelle where, through the intervention of oxygen, the chemical energy of certain organic molecules working as energy substrate is transformed into adenosine triphosphate (ATP). ATP is the form of chemical energy usable by our cells.

Mitochondrial membranes can be compared to complicated energy plants in which molecules are transformed into other molecules and, in the process, release some of their energy. We are in a continuous process of biological energy conversion. Thermodynamics, the study of energy conversion, deals with the reasons why certain reactions can happen spontaneously. Spontaneous events imply a net increase of disorder in the universe. Heat is energy in its most disordered form, a random movement of molecules. The flame of a stove increases the motion of the water molecules contained in a pan over the flame. When a cell releases heat, it increases the intensity of the movement of surrounding molecules. The burning of a wood or of an oil well leads to a huge, meaningless dissipation of heat, but the burning of gasoline makes cars run and airplanes fly, as well. Only under certain conditions, and only in part, can heat be transformed into work. This represents harnessing part of the energy produced in the biochemical world.

In biological systems part of the energy involved in complicated transformations is "transduced" into forms of energy other than heat. This energy transduction is associated, at the mitochondrial level, with a sophisticated series of enzymes and enzyme complexes: these are

essentially proteins which speed up the chemical reactions which make energy available in the living organism.

The fundamental aspect of this machinery is that reactions apparently destined to produce disorder, are coupled to other reactions that produce order, complexity and life. Energy is neither created nor destroyed: the release of heat and production of order require the intake of energy for life to continue. Regarding the source of this energy, for plants it is the sun's electromagnetic radiation. For animals, it is the chemical energy contained in the covalent bonds of the organic molecules that constitute their nourishment. In the very heart of both complex mechanisms there is the quinone structured molecule; plastoquinone in plants and Coenzyme Q in animals.

## Chemical fuels and energy

Energy for all our body needs derives from our biological fuels: sugars, fats and, to a lesser extent proteins. Animal cells universally can make a certain amount of ATP through a process that relies on a controlled partial demolition of glucose that does not require oxygen. This is anaerobic glycolysis. In this process only a small fraction of the total free energy potentially available from oxidation of the sugar is released. A much more consistent amount of energy is made available in **mitochondria**, by a process which consumes oxygen. Oxygen is a powerful oxidizing agent and considerable amounts of energy are released during oxidations. Combustion of wood, gasoline or other fuels are examples of strong oxidations.

To take away electrons means to oxidize. Thus, an element or a molecule which acquires electrons by taking electrons from another molecule is an oxidizing agent; the one that releases electrons is a reducing agent. We

are describing "**redox reactions**". Combustions are high energy oxidations performed by strong oxidants (highly electronegative atoms) which occur with a remarkable emission of light and heat. In the biological world, oxygen is the predominant oxidant and combustion reactions occur in an extremely controlled fashion without visible flames.

Another important feature of biological oxidation is that it takes place through a complex mechanism involving a molecule which conserves a considerable amount of the energy released by combustion. This molecule, which can convert energy into different kinds of work, is adenosine triphosphate (ATP).

Mitochondria can be considered to be the power plants of the cell. Energy is harnessed through the reactions taking place in the highly organized sequential steps of **oxidative phosphorylation** located in the inner mitochondrial membrane, which folds in cristae, a continuous zigzag pattern within an outer membrane. Oxidative phosphorylation is the process in which the major part of metabolic energy is released and transformed into a form of energy which can be universally used for many different cellular needs: the energy of ATP.

## Oxidative phosphorylation the mitochondrion: our main energy plant

The mitochondrial electron transport chain (or respiratory chain) could be compared to a rushing mountain creek, where electrons flow downhill towards oxygen. In the steepest parts of the slope enough energy is made available to feed a turbine. These are sophisticated engines which produce ATP, the molecular currency that powers biological movement – from eyeblink to strenuous exercise.

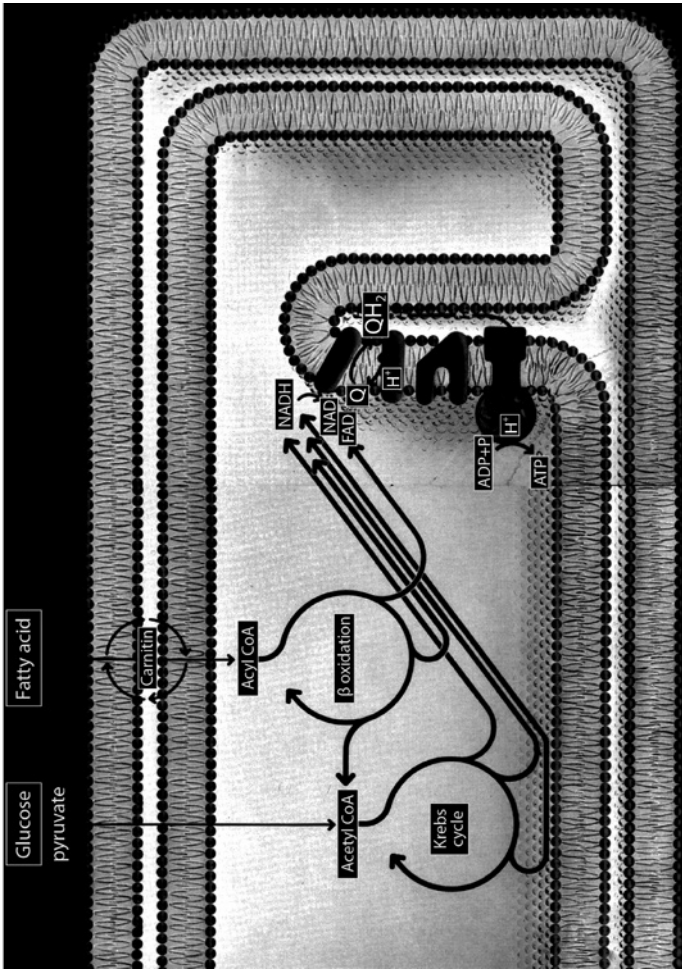


Fig. 3 – Outer and inner mitochondrial membrane

In the respiratory chain, hydrogen atoms are transported as such up to a certain point, then they are broken down into protons and electrons. The electrons pass through a

series of electron carriers along the chain until they reach oxygen which, by acquiring 2 electrons, becomes negatively charged ($O^{=}$). At last protons neutralize these negative charges and $H_2O$ is formed. The many proteins involved in the enzyme-catalyzed transport of protons and electrons to oxygen are organized in membrane bound respiratory complexes and Coenzyme Q plays an essential role in three of these complexes. It accepts protons and electrons from the upstream components, so becoming reduced (Ubiquinol) and then hands them on to the downstream components, returning to the oxidized form (Ubiquinone).

There are two mobile components that diffuse rapidly along the plane of the membrane: coenzyme $Q_{10}$ and cytochrome c. Electron transfer is mediated by random collisions between diffusing donors and acceptors along the enzyme complexes.

The oxidation of the reduced coenzymes liberates energy, which is utilized to feed a proton pump mechanism that concentrates $H^+$ in the intermembrane space.

One of the major molecular mechanisms basic to the proton flow involves Coenzyme Q, namely $CoQ_{10}$ in humans, which, being mobile in the hydrocarbon environment of the mitochondrial inner membrane, becomes protonated (ubiquinol, the proton-rich form) at one side of the membrane and deprotonated (ubiquinone) on the other side, leading to a build up of protons in the intermembrane space[1]. This translocation of protons generates a membrane potential ($\Delta V$, also traditionally indicated as $\Delta\Psi$) which determines a proton-motive force. Fig. 4 depicts ATP synthetase as being like a turbine, driven by the proton back flow and converting one form of energy into another.

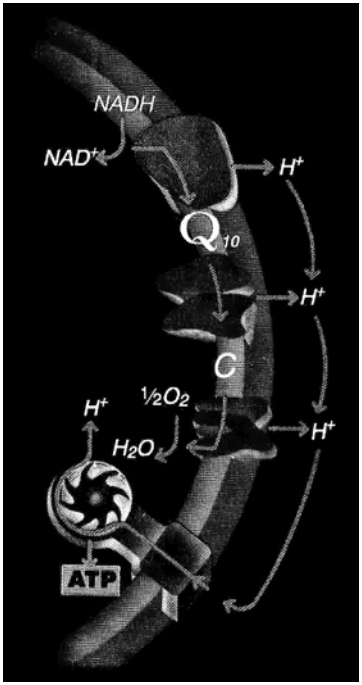


Fig. 4 – Mitochondrial energy production is like a turbine driven by a proton flow

## Location and function of Coenzyme Q in the respiratory chain

Coenzyme $Q_{10}$ is a molecule which carries hydrogen (or electrons + protons, i.e., $e^- + H^+$) by circulating either in the oxidized form as ubiquinone or in the unoxidized, proton-rich form as ubiquinol. As such, ubiquinol is a donating [$e^- + H^+$] center whereas ubiquinone is an accepting [$e^- + H^+$] center in catalytic proteins of the respiratory chain in the mitochondrial cristae membrane. Although Coenzyme $Q_{10}$ is

a moderately large molecule, it is highly mobile in the hydrocarbon rich environment of the phospholipid bilayer, in which different respiratory chain catalytic centers are situated[2].

Moreover, concentration of total Coenzyme $Q_{10}$ is critical for the velocity of the respiratory chain, and so is the Ubiquinol/$Q_{10}$ ratio[3].

This last concept helps us to understand the rationale that links together the classical bioenergetic proton-motive function of $CoQ_{10}$ and its antioxidant role. On the one hand, any condition of increased oxidative stress leading to enhanced involvement of Coenzyme $Q_{10}$ as an antioxidant might somehow decrease the critical availability of Coenzyme $Q_{10}$ itself for oxidative phosphorylation. On the other hand, it also helps to understand a possible mechanism of action of exogenous supplied $CoQ_{10}$ which, by overcoming a deficiency, could reestablish a higher energy flow and an enhanced energy transduction.

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# Endogenous and nutritional sources of Coenzyme $Q_{10}$

Coenzyme $Q_{10}$ is widely diffused in nature, thus it is present in many vegetal and animal tissues which are part of our normal diet. Coenzyme $Q_{10}$ is also actively synthesized by our cells. This is the reason it is not a vitamin according to the classical definition. So, our tissue levels of $CoQ_{10}$ depend both on an endogenous biosynthesis and on an exogenous supply. Metabolic demand and the turnover rate of $CoQ_{10}$ should also be taken into consideration when trying to establish a "nutritional status" of $CoQ_{10}$.

## Nutritional intake

The content in $CoQ_9$ and $CoQ_{10}$ of different types of food was evaluated in a paper by Kamei *et al*. $CoQ_9$ is usually present in cereals, while $CoQ_{10}$ is found in a variety of sources including meat, milk, fruit and vegetables, to name a few.. Dietary sources of $CoQ_{10}$ offer both the ubiquinol and ubiquinone forms[1]. For instance, both forms of $CoQ_{10}$ are found in soybeans (greater than 6 µg/g) with the ubiquinol in greater concentration than ubiquinone. $CoQ_{10}$ is also present in walnuts, almonds, in oils and fruits rich in oil and in green vegetables; spinach is particularly rich in $CoQ_{10}$. Some kinds of fish also have comparatively high amounts of $CoQ_{10}$. On a weight basis, sardines have more than twice as much $CoQ_{10}$ as beef; 1.6 kg of sardines contain 100 mg of $CoQ_{10}$. Milk and cheese have a lower content of Coenzyme $Q_{10}$. It is difficult to assess the relative importance of endogenous biosynthesis and exogenous intake; the latter plays a significant role as well. Data from Kishi *et al.* showed that in patients under total parenteral nutrition (TPN), plasma levels of $CoQ_{10}$ undergo

a remarkable reduction (50%) in just one week. This finding might be related to non-consumption of $CoQ_{10}$ and/or its precursors present in the diet or to stressors that resulted in the patient requiring TPN. In our lab we often found plasma levels of $CoQ_{10}$ close to or even lower than 0.1 µg/ml in traumatized patients. It is difficult to evaluate to what extent those data were related to total parenteral nutrition or to the serious clinical conditions of shocked patients. 0.1 µg/ml represents a very low value inasmuch as normal plasma levels are around 0.79±0.2 µg/ml.

## Biosynthesis of $CoQ_{10}$

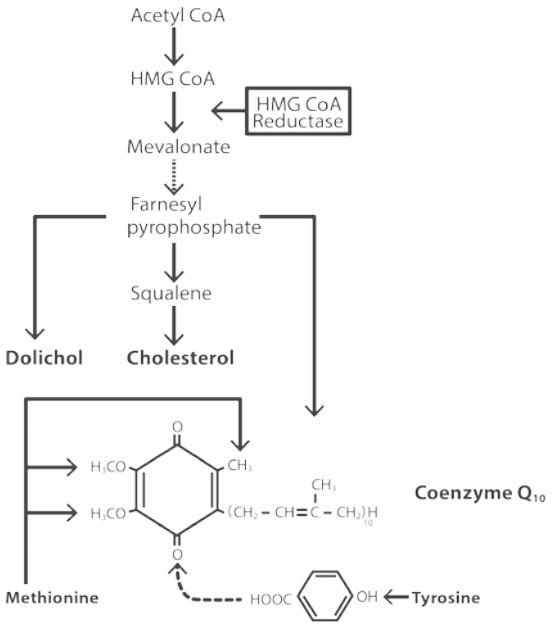


Fig. 5 – Schematic representation of the biosynthetic pathway leading to Cholesterol, Dolichol and Coenzyme Q

$CoQ_{10}$ is synthesized by our body and the biosynthetic pathway is common up to a certain point to cholesterol biosynthesis. In fact, cholesterol and Coenzyme Q are end-products of this important biosynthetic pathway which is under the control of a key-enzyme, hydroxy-methylglutaryl coenzyme A reductase (HMGCoA reductase). So, cholesterol and $CoQ_{10}$ share, up to a certain point, their biosynthetic pathway.

## $CoQ_{10}$ and inhibitors of HMG-CoA reductase (statins)

HMG-CoA reductase inhibitors, known as statins, represent a potent therapy in the anti-cholesterol strategy. Cholesterol lowering therapy plays an essential, well recognised role in the secondary prophylaxis of coronary heart disease, as shown for instance by the Scandinavian Simvastatin Survival Study [4S][2]. As $CoQ_{10}$ and cholesterol share the same biosynthetic pathway, it is reasonable to hypothesize that statin therapy may also lead to decreased levels of $CoQ_{10}$. In 1993 we reported the results of the first double blind study showing that both simvastatin and pravastatin produce a decrease in $CoQ_{10}$ plasma levels similar to that of cholesterol[3]. Other studies confirmed those data[4]. On the whole, statins are powerful, effective drugs, usually safe, with an apparently low frequency of side effects but which in some cases have been found to be very serious. In August 2001, a clear relationship between the use of cerivastatin and rabdomiolysis (destruction of muscle cells) with consequent death of some patients was established. Side effects of statins include muscle weakness, pain, and elevated plasma levels of CK and/or transaminases and these symptoms could, at least in part, be related to a certain degree of $CoQ_{10}$ deficiency. We can reasonably hypothesize that, in some conditions in which other $CoQ_{10}$-impoverishing causes exist, treatment with HMGCoA reductase inhibitors may seriously impair levels of $CoQ_{10}$ in plasma, and possibly in tissues.

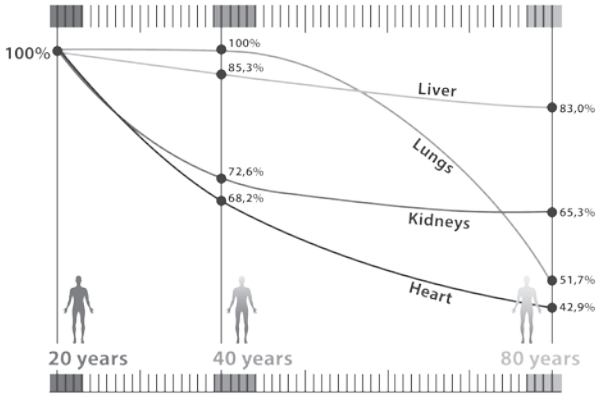


Fig. 6 – Age related decrease of Ubiquinol

A physiological decline in $CoQ_{10}$ occurs with age[5,6] and this might make the elderly more susceptible to statin-induced $CoQ_{10}$ depletion. Folkers *et al*. reported in a limited number of patients who had been on $CoQ_{10}$ therapy for congestive heart failure a sudden deterioration of their cardiac function when statins were added to the therapy. This worsening was overcome by increasing their daily dosage of $CoQ_{10}$. Besides the plasma $CoQ_{10}$ lowering effects, animal studies have also demonstrated tissue depletion in the course of statin treatment. A decrease in tissue coenzyme $Q_{10}$ during statin therapy may have adverse effects on cellular ATP production, as has been proven in dogs and guinea pigs. There are only a few studies on the effect of statin treatment, in humans on muscle $CoQ_{10}$ concentrations. In an early study by Laaksonen *et al*. decreases in serum ubiquinone concentrations in the course of simvastatin treatment did not result in $CoQ_{10}$ reduced levels in muscle tissue[7]. Those patients took 20 mg simvastatin per day for 4 weeks. In a more recent publication the same group reported the results of a study designed to assess the effect of high dose statin

treatment on cholesterol and ubiquinone metabolism and mitochondrial function in human skeletal muscle. Muscle ubiquinone concentration was indeed reduced significantly in the simvastatin group and in the muscle of several patients there was a remarkable deterioration of mitochondrial functions[8].

## $CoQ_{10}$/Ubiquinol supplementation and statin-induced myopathy

On the basis of these observations, it seems logical to hypothesize that supplementation with $CoQ_{10}$ might be an appropriate therapeutic tool to prevent and/or counteract adverse effects of statin treatment.

So far we only have few studies suggesting rapid improvement in statin induced myalgia and fatigue with supplemental $CoQ_{10}$.

In an article by Caso *et al*. published *in the American Journal of Cardiology*[12], thirty-two patients treated with statins under current Adult Treatment Panel III/National Cholesterol Education Program guidelines and reporting myopathic symptoms were enrolled in a double-blind study. Patients had been taking different statins, but a common feature was that they all developed myopathic symptoms defined as presence of muscle pain alone or accompanied by other symptoms, such as muscle weakness and fatigue. Patients were randomized and eighteen of them fell into the $CoQ_{10}$ group and received 100 mg of $CoQ_{10}$/day for one month while the others were treated with 400 IU of vitamin E/day for 30 days. The effect of supplementation with $CoQ_{10}$ on muscle pain was investigated before and after the intervention using the Brief Pain Inventory questionnaire, a widely used tool to assess pain and interference of pain with everyday life. Pain severity decreased

by 40% and pain interference with daily life activities by 38%, both figures being highly significant. No changes in pain severity or pain interference with daily activities was observed in the group treated with vitamin E. Results strengthen the hypothesis of a possible etiologic role of $CoQ_{10}$ depletion in the pathogenesis of myopathic symptoms in statin-treated patients. The authors also conclude that $CoQ_{10}$ supplementation may offer an alternative to stopping treatment with these drugs.

Zlatohlavek *et al.* also conducted a study, although not placebo-controlled, on the effect of Ubiquinol on minimizing muscular side effects in a group of patients treated with different statins and different doses[16]. In this group of 28 patients affected by statin myopathy Ubiquinol was given for 6 months, at a dose of 60mg/day. Plasma levels of $CoQ_{10}$ increased by almost 200%, own to the good bioavailability of Ubiquinol, pain decreased on average by 54% and muscle weakness by 44%.

Sometimes, in the course of statin treatment, cardiac problems can arise.

Silver and colleagues documented systematic impairment of diastolic ventricular function in stable outpatients being started on atorvastatin therapy for hyperlipidemia. The authors postulate that sensitive diastolic markers may represent early biomarkers for impairment of left ventricular function and found reversal of these abnormalities in the patients after supplemental $CoQ_{10}$ at 300 mg per day was added to their atorvastatin therapy[9].

Langsjoen reported a study on a group of 50 patients who had been on statin drug therapy for an average of 28 months and showed one or more statin-related adverse effect[9]. All patients were supplemented with coenzyme $Q_{10}$ and followed for an average of 22.4 months. There was a remarkable improvement in the decrease in fatigue,

myalgia, dyspnea, memory loss and peripheral neuropathy. No evidence was found of any adverse consequences upon statin drug discontinuation. With 84% patients having been followed for more than a year, no cases of myocardial infarction or stroke occurred. This study has a serious limitation inasmuch as two simultaneous interventions were made, i.e. statin discontinuation and supplementation with $CoQ_{10}$.

The correct way to address this issue could be to set up a study with a sufficient number of patients treated with statins where the ones showing side effects would be randomized and treated with placebo or $CoQ_{10}$. Even better, a large group of patients could start with statins and $CoQ_{10}$; the effects of statins and the occurrence of side effects would be monitored. Although statin therapy has been shown to have benefits, the long-term response in ischemic heart disease may have been blunted due to the $CoQ_{10}$ depleting effect.

The whole issue was reviewed by Marcoff and Thompson. They conclude that there is insufficient evidence to prove the etiologic role of $CoQ_{10}$ deficiency in statin-associated myopathy and that large, well-designed clinical trials are required to address this issue[13].

Recently, a qualified research group of Hartford Hospital CT, also led by Prof. Thompson, further inquired the mechanisms of statin-induced myalgia[14]. About 800 patients undergoing statin therapy (half of them with myalgia and half without) were genotyped to evaluate 31 candidate genes from the literature for their association with statin-induced common myalgia. Among the tested genes the strongest association between genetic variant and myalgia was found for COQ2, a gene encoding a key enzyme in the biosynthesis of $CoQ_{10}$. From a practical point of view, this means that not all patients are equally sensitive to statin

side effects. We might better understand which patients undergo a $CoQ_{10}$ deficiency during statin treatment and which patients may benefit from $CoQ_{10}$ treatment.

In the cardiology section we will refer to the recent results obtained by treating with Ubiquinol patients presenting statin-related side effects.

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# Plasma levels of Coenzyme $Q_{10}$

Coenzyme $Q_{10}$ is also present in human blood, where it is transported by plasma lipoproteins, mainly LDL. Coenzyme $Q_{10}$ blood levels have definite metabolic and diagnostic implications.

$CoQ_{10}$ is present in each blood component, as it has been deeply investigated[1]. White blood cells and platelets possess $CoQ_{10}$, which is not surprising since they have mitochondria. Its presence has a metabolic significance within the bioenergetics of these cells. One of the early observations correlated with the presence of $CoQ_{10}$ also in red blood cells was that it likely plays a protective role as an antioxidant. Supplementing human red blood cells with exogenous $CoQ_{10}$ enables these cells to become more resistant to thermal autoxidation and their ATPase activity is better preserved. Plasma $CoQ_{10}$ is routinely assayed in some laboratories, both in basal conditions and after oral supplementation with $CoQ_{10}$, in order to verify the attainment of therapeutic level and to establish dose–response relationships.

Plasma $CoQ_{10}$ levels do not necessarily correlate with tissue $CoQ_{10}$ and the $CoQ_{10}$ status of the body[2]. Plasma concentrations of $CoQ_{10}$ are relevant for two reasons. They probably reflect the metabolic demand of tissues and play an intrinsic antioxidant role protecting LDL cholesterol from oxidative modifications. The oxidation of LDL modifies the inert cholesterol into an atherogenic role, thereby promoting the fatty plaques in the arteries. (This role will be more extensively discussed in another chapter of this book.) Low plasma levels of $CoQ_{10}$ are a constant finding in hyperthyroidism. In fact, in thyroid disease, also when pharmacologically induced, plasma $CoQ_{10}$ levels unequivocally reflect the clinical status of the patient. Through experiments conducted on guinea pigs,

it was verified that hyperthyroidism and physical exercise are two conditions which stimulate tissue biosynthesis of $CoQ_{10}$, probably within a more general increase in metabolic demand, also leading to an accelerated uptake of $CoQ_{10}$ by tissues. $CoQ_{10}$ is mainly associated with plasma, rather than with cellular components of blood. Almost 60% of $CoQ_{10}$ appears to be associated with LDL, 26% with HDL and 14% with other lipoproteins[1]. As virtually all $CoQ_{10}$ transported in the blood is bound to cholesterols I believe that $CoQ_{10}$ content should be expressed both as an absolute concentration (µg/ml or µM) and as a $CoQ_{10}$/Chol ratio (nmoles$CoQ_{10}$/ mmolesChol).

$CoQ_{10}$ is mainly transported by LDL, where about 95% of it is in the reduced form, i.e., Ubiquinol. These "normalized" values might better reflect the $CoQ_{10}$ status of plasma and be useful to highlight some differences. In fact hypercholesterolemic patients, having higher levels of LDL, also generally have higher levels of $CoQ_{10}$. Therefore, an apparently normal level of $CoQ_{10}$ might really be low, if those patients had elevated LDL[4,5,6,7].

## Plasma coenzyme $Q_{10}$ level as independent predictor of mortality in chronic heart failure

A few years ago the group of Dr. Florkowski, in Christchurch New Zealand, published a study in which plasma $CoQ_{10}$ levels were correlated with mortality in a group of patients with symptomatic Chronic heart Failure (CHF)[8]. Patients were followed-up for a median of 2.69 (range 0.12 to 5.75) years; monitoring was performed at least every 3 months. Lower $CoQ_{10}$ and $CoQ_{10}$ to lipid ratios were predictors of poorer survival. Mutivariate analysis allowing for effects of standard predictors of survival, including age at admission, gender, previous myocardial infarction, renal function and

NT-proBNP (an indicator of heart failure), showed that $CoQ_{10}$ was an independent predictor of survival. The ratio of $CoQ_{10}$ to total cholesterol was also a significant predictor of survival. The strength of association between (low) $CoQ_{10}$ and mortality was greater than that observed for NT-proBNP, a recognized marker of heart failure. According to the authors, it is therefore plausible that $CoQ_{10}$ deficiency might be an important pathogenic mechanism associated with worse outcomes in CHF, and this lends further support for controlled intervention studies of $CoQ_{10}$ supplementation.

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# Free radicals, oxidative damage and antioxidant defense

Electrons occupy regions of space named orbitals. Each orbital can contains one or two electrons: an unpaired electron is one that is alone in one orbital. **A free radical is usually defined as any species, capable of independent existence, containing one or more unpaired electrons.** The presence of one or more unpaired electrons is conventionally indicated by a dot, which therefore designates the radical character, e.g. R• (R dot or R radical). A radical can give an electron to another molecule or might take one electron from it thus transferring its radical character. Also when a radical adds on to a non-radical, a new radical is formed.

The feature that is becoming clear is that **a radical generates another radical**, leading to a chain reaction. A special case of **propagation of the radical chain**, which is of particular relevance in vivo, is the one that happens through the so called hydrogen abstraction. The hydrogen atom is made of a single proton and a single electron. Thus, a radical that removes a hydrogen atom from a molecule leaves an unpaired electron on the atom to which the hydrogen was originally linked.

- **Electron withdrawal**

I would like to remind readers that to take away an electron means to oxidize, and a molecule that looses one electron is therefore oxidized. This can happen by means of radiation or through oxidation by another radical:

- **Electron donation**

To give one electron means to reduce.

Formation of free radicals in the biologic environment leads us to consider the deep involvement of oxygen in radical reaction and oxidative damage. In cellular systems, the most efficient mechanisms of energy extraction from biological fuels are based on the combustion of sugars and fats by means of the highly oxidative power of molecular oxygen. These processes are carefully controlled and rely on the sophisticated system of the mitochondrial respiratory chain.

In the mitochondrial production of ATP, oxygen itself is reduced in such a way that two electrons (and two protons) are accepted by each oxygen atom leading to the formation of a water molecule: it is therefore named the tetravalent reduction of oxygen, since four electrons are taken up by molecular oxygen. A small percentage of electrons leak away from the mainstream respiratory chain and lead to the "monoelectronic, univalent molecular oxygen" reaction, which generates **superoxide anion** ($O_2^-$).

From this radical species other, more reactive, radicals are generated. Free radicals are not always bad: in fact, numerous adaptative mechanisms in the cells rely on a complex signalling network triggered by radicals. In some cases free radicals produced by particular cell lines constitute the basis for an efficient defensive system against bacteria, such as the bactericidal mechanisms of neutrophils, a type of white blood cells. We should also keep in mind that these bullets not only kill the bug but they might also damage the surrounding tissues. There is a subtle balance, in our body, between the level of free radicals and the magnitude of the antioxidant defense: an imbalance toward the former is called oxidative stress. A high level of free radicals might depend on an accelerated production by the body, on exposure to high levels of free radicals deriving from the environment, or a dietary regimen without adequate intake of antioxidants without adequate intake of antioxidants.

## Lipid peroxidation and membrane damage

As I mentioned above, a radical strives to complete its half-filled- electronic orbital with electrons taken away from high electron density structures with which it might collide. When this happens in a biological membrane a new radical is formed, which propagates and amplifies the radical reaction within the membrane. When sufficiently high oxygen tension is present, a **peroxyl radical is formed: these are some features of the so called lipid peroxidation.** We should imagine a dynamic situation in which propagation of the radical chain reaction is continuously opposed by the lipid soluble membrane antioxidants **Coenzyme Q and vitamin E**. Hydroperoxides are removed by enzymatic systems, such as glutathione peroxidase. Antioxidant mechanisms will be discussed in the following pages.

Lipid peroxidation of cell membranes leads to a loss of cell membrane fluidity, cell membrane and tissue permeability changes, and general derangement of the lipid-protein interaction. All these alterations cause harmful repercussions on membrane-associated enzymes, ion channels, transport proteins and receptors.

**Free radicals also attack proteins and nucleic acids. We will only mention this fact, but in the following pages we will highlight the protective effect of Coenzyme $Q_{10}$, in particular of Ubiquinol, on DNA oxidation.**

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# Anti-oxidant refreshment biochemistry for medical professional

## Antioxidant mechanisms

Our organisms are protected against radical-induced oxidative damage by various antioxidant defense strategies which are committed to counteract the oxidative attack in its early moments, i.e., formation of the priming radicals as well as during the initiation and chain propagation process. Furthermore, part of the antioxidant defense is the ability to remove damaged structures and repair them. Finally, adaptation can also be included among the antioxidant mechanisms[1].

## Preventive mechanisms

When an appropriate concentration of metals is available, the presence of fatty acids or of phospholipid hydroperoxides can lead to the generation of the highly reactive hydroxyl radical, which would initiate the chain reaction. A first antioxidant defense line relies on mechanisms which reduce $H_2O_2$ and other peroxides and sequester metal ions responsible for their decomposition. Peroxidases are enzymes which catalyze the reduction of hydroperoxides using different substrates as reducing agents. Two well known peroxidases are catalase, which reduces $H_2O_2$ by using another molecule of $H_2O_2$ as a reducing agent and glutathione peroxidase, which uses two molecules of glutathione as hydrogen donors.

As mentioned above, since transition metals are necessary for the formation of the hydroxyl radical, an effective sequestration of metals, mainly iron, represents a valid antioxidant mechanism within the category of preventive mechanism.

**Carotenoids**, such as **beta-carotene** and lycopene also constitute an important antioxidant primary defense line in the

lipid environment: by scavenging singlet oxygen they prevent the formation of lipid peroxides. The superoxide anion ($O_2^{\cdot-}$), although not particularly reactive, is in some cases responsible for direct attack on target molecules. More relevance is given to its contribution to iron-mediated oxidative stress. $O_2^{\cdot-}$ can reduce $Fe^{+++}$ to $Fe^{++}$, the "dangerous iron," and can also release it from ferritin where it is stored. Superoxide dismutase (SOD) can be classified as a member of the preventive antioxidants as it removes $O_2^{\cdot-}$.

Dismutation is a reaction where the same species acts as a oxidizing and reducing agents. In case of the superoxide anion, one molecule is reduced to $H_2O_2$ by the other one which is oxidized to $O_2$. The whole reaction also needs two protons:

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$$

In eukaryotic cells, two intracellular superoxide dismutases exist: Cu, ZnSOD, the major intracellular SOD and the MnSOD, located primarily in the mitochondrial matrix. In 1982, a third SOD was discovered by Marklund and coworkers and termed extracellular superoxide dismutase (ecSOD) as it is the predominant SOD in the extracellular fluids[2,3]. The loss or the decreased levels or the dysfunction of one of the three SODs has been associated with free radical mediated diseases. This concept will be further illustrated in the chapter on $CoQ_{10}$ and ecSOD.

## Chain breaking mechanisms

The second line of defense against oxidative insult relies on molecules which react with free radicals and somehow neutralize their radical character by transforming them into non radicals. They are usually defined as "radical scavengers". This action interrupts the chain propagation reaction. SOD is sometimes classified as radical scavenger for it abolishes, through dismutation, the radical character of superoxide anion. Most

radical scavengers have relatively low molecular weight; some of them are hydrophilic and some lipophilic, and this endows the different cellular environments with appropriated antioxidant defense. Among the hydrosoluble ones we will mention **albumin, ascorbic acid, uric acid, bilirubin** and the **thiols**.

Among the lipophilic ones **Vitamin E and Ubiquinol** are the most studied. The real antioxidant efficiency of all these molecules is related to their concentration, their intrinsic reactivity and their mobility within the microenvironment in which they are located. All these factors contribute to their capability of intercepting and nullifying radicals. They generally act as hydrogen donors with respect to radicals. In other words, radicals can very easily act as hydrogen abstractors when they collide with a radical scavenger, which now itself becomes a radical. The radical form of the scavenger is generally a more stable radical when compared with the free radical from which it was generated, so the chain propagation is interrupted. More importantly, it can be reconverted to its reduced, antioxidant form, by appropriate reducing agents, as it will be described for vitamin E. When considering lipid peroxidation, a classic chain-breaking mechanism is the one performed by tocopherols, especially alpha-tocopherol (vitamin E), which donates the hydrogen of its phenolic group to the peroxyl radical, thus reducing it to hydroperoxide. When this happens the active form of Vitamin E is consumed, and the so called Tocopheryl radical formed in the process. The latter can be reconverted to the active form of vitamin E by Ubiquinol. Therefore **Ubiquinol acts as chain breaking antioxidant and also regenerates Vitamin E to its active form.** The Ubiquinol_Vitamin E combination represents a powerful antioxidant duet in the lipid environment.

## Repair mechanisms

The third defensive line is comprised of mechanisms which remove the molecules damaged by the oxidative attack and

replace the compromised structures with new ones. Within the membrane's lipid-rich environment, for example, specific phospholipases exist which remove oxidized fatty acids from phospholipids. In this reparative system, hydroperoxides become more susceptible to peroxidases, and lysophospholipids (a phospholipid from which a fatty acid has been detached) are reacylated. Oxidatively damaged proteins are recognized and degraded by proteases. Important DNA repairing mechanisms also exist and are briefly mentioned in the chapter dealing with Oxidative Damage on DNA.

## Adaptation mechanisms

Free radicals formed in the cellular environment and reactive oxygen species also work as a signal capable of inducing the synthesis and the transport of the appropriate antioxidant to its site of action. Physical training, for instance, is a stimulus which induces the synthesis of catalase, other peroxidases and Coenzyme Q. The membranes of trained animals can better withstand peroxidative insult.

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# Antioxidant properties of Coenzyme $Q_{10}$

Besides the well recognized, vital role of Coenzyme Q in energy transduction and oxidative phosphorylation, there is considerable evidence that Coenzyme Q functions as a lipid soluble antioxidant in biological membranes. This evidence has been produced by numerous experimental models, both in vivo and in vitro[1-13].

Ubiquinol acts by slowing down the chain propagation reaction according to the general mechanism of hydrogen donation to the radicals, as discussed when we mentioned the chain breaking lipid soluble antioxidants.

The other well known mechanism by which ubiquinol exerts its antioxidant properties is the one first explored by Packer, Kagan *et al*. These authors demonstrated that Ubiquinol, the reduced Coenzyme Q, regenerates alpha -tocopherol, the active form of vitamin E, by reducing the tocopheryl radical[11].

Ernster stressed the fact that Coenzyme Q is the only lipid soluble antioxidant that animal cells can biosynthesize 'de novo" and for which appropriate enzymatic mechanisms exist to regenerate the reduced form[10]. Several years ago our group demonstrated that Ubiquinol exerts its antioxidant properties also by inactivating ferrymyoglobin, a species capable of triggering the oxidative attack at muscular and cardiac level[12].

A field which typically illustrates the importance of the antioxidant properties of Ubiquinol is represented by plasma lipoproteins. This issue will be explored on page 51.

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# Oxidative damage to DNA

DNA is constantly exposed to endogenously generated reactive oxygen species (ROS) which produce oxidative modifications[1]. Exogenous chemical and physical agents also attack the DNA molecule, leading to other routes of possible oxidative damage. Our cells also possess efficient DNA repair systems, but if these are not sufficient mutations can occur, a crucial step in carcinogenesis. The repair defence is supposed to be sufficient in a "normal" environment in which ROS are efficiently counteracted by antioxidants, both enzymatic and non-enzymatic ones. When the exposure to oxidizing agents is excessive or antioxidant defenses are insufficient, the overwhelming insult can result in increased levels of unrepaired DNA.

## Assessing the extent of DNA oxidation

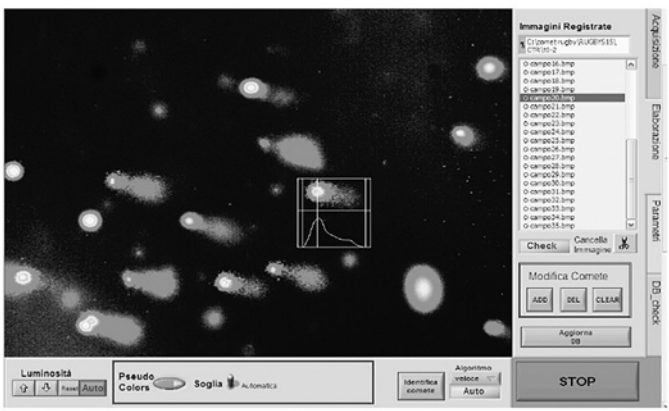


Fig. 7 – Comet image at the microscope

HPLC methods are often used to quantify products of DNA oxidation. Another approach is single cell gel electrophoresis (SCGE) also known as the comet assay. During electrophoresis in the same alkali condition, DNA fragments induced by DNA-damaging agents migrate to the anode side of the chamber, revealing, after appropriate staining, the characteristic comet image in which the tail represents damaged DNA streaming away from the head made of more intact DNA.

Comet data can be collected either by visual scoring or by using computer automated image analysis.

## Coenzyme $Q_{10}$ and DNA damage

The antioxidant role of $CoQ_{10}$ was mainly investigated in the lipid environment, with special attention given to its capability of counteracting lipid peroxidation, but it is also able to prevent protein and DNA oxidation. In our initial studies regarding this topic we demonstrated that $CoQ_{10}$ protects DNA from oxidation caused by $H_2O_2$ in an in-vitro model utilizing isolated human peripheral lymphocytes (cells were preincubated with liposomes loaded with ubiquinone or ubiquinol)[2]. Subsequent studies revealed that human lymphocytes from volunteers supplemented in vivo with $CoQ_{10}$ were also protected against oxidative DNA damage and protection was proportional to the concentration of $CoQ_{10}$ in plasma and cells[3]. In the same study it was also highlighted that the activity of DNA repairing enzymes was positively affected by $CoQ_{10}$ supplementation.

More recently our group conducted a study in order to verify whether oral $CoQ_{10}$ administration could provide a means to reduce DNA oxidative damage in pediatric patients affected by Down syndrome[4,5].

Characteristic hallmarks of this pathology are mental retardation, Alzheimer-like dementia and premature ageing. Oxidative stress is known to play a major role in this syndrome both due to genetic and epigenetic factors, suggesting that oxidative imbalance contributes to the clinical manifestation of DS. In particular, the implications of oxidative DNA damage in Down syndrome have been linked with neurodegeneration associated with the syndrome. The effects of oxidative stress in Down syndrome are documented in very early stages even from embryonic life.

Peripheral blood lymphocytes from DS patients did show a significantly increased DNA damage quantified by means of the comet assay. In the study performed in our lab, 30 patients affected by Down syndrome, were enrolled and randomly assigned to the intervention group (16 patients) or to the placebo one (14 patients). The main endpoints of the study were variations of the comet indexes of DNA damage (tail length, tail intensity and tail moment). Patients were supplemented with 4 mg/kg/ day of $CoQ_{10}$ or an equivalent amount of placebo constituted by identical softgel capsules containing the phospholipid vehicle of $CoQ_{10}$.

Basal plasma $CoQ_{10}$ levels were similar in DS patients and healthy children, but a significantly lower $CoQ_{10}$ level was observed in lymphocytes and platelets from DS. When the patient was considered as a unit of observation, no significant differences in DNA damage were found between patients treated with $CoQ_{10}$ or placebo. Considering the cell as an observation unit, the analysis of the data did show a significantly protective effect of $CoQ_{10}$ treatment in relation to tail length and tail migration, whereas tail intensity and tail moment were not affected. A statistical approach taking into consideration all cells from patients related with $CoQ_{10}$ versus all cells from

patients who received the placebo showed a significant effect of $CoQ_{10}$ in minimizing DNA damage. These observations could indicate that $CoQ_{10}$ treatment, in our experimental conditions, was not able to revert DNA damage in heavily compromised cells, however it was able to minimise DNA damage in the main population of lightly damaged cells probably delaying the transition to a more advanced stage of DNA damage. In fact, $CoQ_{10}$ treatment was effective in decreasing by 12-16% the percentage of cells showing low levels of DNA damage, making them fall into the category of undamaged cells. This could indicate a potential application in preventive therapies aimed at delaying DNA damage and the progression of such damage in several pathological conditions.

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# Plasma lipoproteins, oxidative damage and atherosclerosis

## Structure and function of plasma lipoproteins

Fats are by definition insoluble in water, yet a lipid interchange exists between different organs for many metabolic reasons. This interchange, of course, occurs through the blood stream, so that special ways of transport are utilized by our organism. The way lipid-soluble substances (for example $CoQ_{10}$) are transported in the water environment of the blood is through lipoproteins. Different classes of lipoproteins can be classified and separated on the basis of different relative amounts of lipids and proteins. Lipids are lighter than proteins, so density decreases with increasing percentages of lipids. LDL (low density lipoproteins) and VLDL (very low density lipoproteins) have relatively higher content of lipid material as compared with HDL (high density lipoproteins). LDL, which will be taken into consideration in these pages as the principal atherogenic.

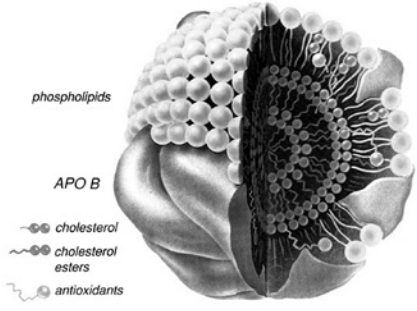


Fig 8 – Schematic representation of low density lipoprotein (LDL) structure. LDL have a spheroidal shape, with a radius of 25-30 nm. The outer layer is made of phospholipids and free cholesterol, while triglycerides and cholesterol esters are in the inner part. Apoproteins stay on the surface, even though they penetrate to a certain extent towards the inner part.

Lipoproteins, are schematically illustrated in Fig. 8. The LDL particle has a spheroidal structure ,where the external part is very compatible with water, and their inner part is lipophilic, i.e. it well accommodates cholesterol and other fats. In fact the surface layer is made of phospholipids, which are amphypathic, i.e., have a chemical structure made of a head which can interact with the aqueous environment of plasma, and a tail which has a high affinity for lipids.

## Lipoprotein oxidation and protection by Ubiquinol

Since their isolation was made possible, it soon became evident that lipoproteins, especially LDL, are easily oxidized and that LDL oxidation products are cytotoxic[1,2]. LDL are often rich in polyunsaturated fatty acids, such as linoleic and arachidonic acid, which can be easily peroxidized. Dietary supplementation with oleic acid, which is monounsaturated significantly lowers peroxidizability of LDL[3].

LDL contain well defined amounts of antioxidants and their oxidative modification is the result of a delicate balance between oxidative attack and efficacy of the antioxidant mechanisms. It was commonly assumed, until some years ago, that ascorbic acid and alpha-tocopherol were the first antioxidants to intervene and to be consumed while exerting their antioxidant action, followed by lycopene and by carotene. In the early 1990s Professor Roland Stocker and his group discovered that **Ubiquinol is the most reactive antioxidant in LDL and is also capable of regenerating the active form of vitamin E**[4]. Furthermore it was soon evident that oral supplementation with $CoQ_{10}$, even for a few days, makes LDL more resistant to oxidation. In the following years different researchers, including our group, confirmed those data). This phenomenon is depicted in Fig. 9. Safeguarding LDL represents one of the protective effects

of Ubiquinol on our arteries. High levels of LDL, as well as smoking and hypertension, are primary risk factors among those contributing to cardiovascular disease.

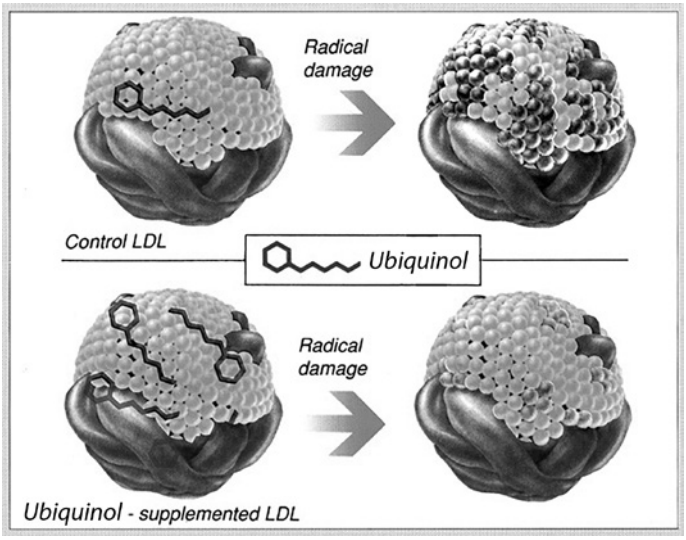


Fig. 9 – Ubiquinol makes LDL more resistant to oxidation

Experimental evidence has been produced indicating that **oxidatively modified LDL** become atherogenic[2]. It was found that endothelial cells are involved in the oxidative attack against LDL as described above. Oxidative attack on LDL deeply affects the protein moiety as well. As a consequence of these changes, LDL are no longer "recognized" by the normal receptors and are taken up more readily by the scavenger receptors of macrophages. A schematic representation of these events is depicted in Fig. 10. LDL leave the blood stream, penetrate the endothelial cell lining and reach the subendothelial space, where they undergo oxidative attack by the endothelial cells. Oxidatively modified LDL are capable of triggering further events, including platelet activation, and exert a chemo-

tactic attraction on circulating monocytes, which migrate to the subendothelial space where they become macrophages. These cells are able to take up more rapidly oxidatively modified LDL and this uptake involves a special receptor, called the "scavenger receptor". These events lead to an accumulation of lipids, mainly cholesterol and cholesterol esters, in the macrophages, which are going to become lipid-laden foam cells. Foam cells may be considered the essence of the atheromatous lesions.

Dr. Kontush confirmed that vulnerability of LDL is directly correlated with their content in polyunsaturated fatty acids and clearly demonstrated that, on the other hand, it is inversely correlated with the content in Ubiquinol[5]. Our group showed that most of Ubiquinol present in blood is transported by LDL. In fact high plasma levels of Ubiquinol mean high level of Ubiquinol in LDL and extensive work has been carried out by our group, showing that there is a strong correlation, in LDL, between Ubiquinol content and resistance to oxidative attack.

Showing that Ubiquinol protects LDL from oxidation strongly suggests that its presence is relevant in counteracting atherosclerosis, but it is not a direct proof of its antiatherogenic effect. In fact this proof effect was obtained in two animal models.

A true antiatherosclerotic effect of $CoQ_{10}$ was shown by Stocker *et al.* in the apo lipoprotein E-knockout mice (ApoE-/-)[6]. 0.5% $CoQ_{10}$ alone significantly decreased atherosclerosis at the aortic root and arch, but not the descending thoracic aorta, whereas vitamin E (0.2%) alone was anti- atherogenic in the aortic root only.

R.B. Singh also reported the results of a similar study conducted in a model of an experimental atherosclerosis in the rabbit[7].

The treatment with coenzyme Q led to significantly smaller artery plaque sizes. Aortic plaque frequency, aortic plaque ulceration, coronary plaque ulceration, aortic plaque thrombosis, coronary plaque thrombosis or haemorrhage were significantly lower in the CoQ group than in the placebo group, indicating that $CoQ_{10}$ treatment can improve the quality of the atherosclerotic plaque.

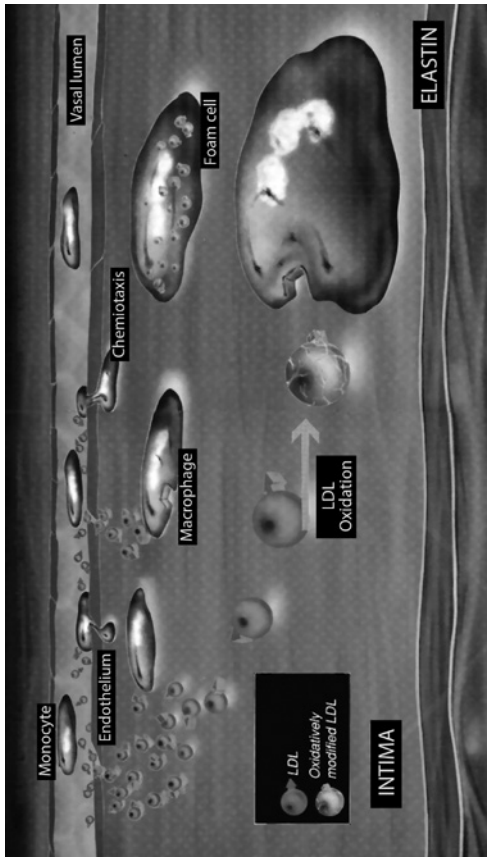


Fig. 10 – Formation of the foam cells

## Relevance of the $CoQ_{10}$ reductive status

The use of very sensitive electrochemical detectors allows to quantify the percentage of $CoQ_{10}$ in the oxidized and in the reduced form and plasma is a very suitable material with which to measure this ratio. The redox status is usually expressed as ubiquinol/total $CoQ_{10}$ (%) or ubiquinone/ total $CoQ_{10}$. In freshly drawn blood $CoQ_{10}$ is almost fully reduced, i.e. almost 95% of the coenzyme is Ubiquinol. Decreased ubiquinone/ total $CoQ_{10}$ ratio is a sensitive marker of an improved antioxidant status. Quite a few years ago we showed that spontaneous oxidation of ubiquinol in plasma and in isolated LDL was considerably slower in $CoQ_{10}$ enriched plasma or LDL. In other words a higher content of $CoQ_{10}$ also protected ubiquinol from oxidation[8]. In the same paper we also showed a very strong correlation between LDL ubiquinol content and resistance of LDL to lipid peroxidation. Also in those years an inverse correlation was found between ubiquinol content and hydroperoxide levels both in seminal plasma and in seminal fluid[9]. Using multiple regression analysis we found a strong correlation among sperm count, motility and ubiquinol-10 content. An inverse correlation between ubiquinol/ ubiquinone ratio and the percentage of sperm cells with abnormal morphology was also found. More recently, these concepts have been validated in vivo. One of our recent studies has shown that supplementation with olive oil enriched with $CoQ_{10}$ leads to a lower ox/total $CoQ_{10}$ ratio, a marker of an improved redox status[10]. The same phenomenon in rather high doses was described a few years ago in a study by Miles *et al.* where ubiquinol was administered to children affected by trysomy-21.

## Relevance of Ubiquinol concentration in plasma

It is known that Indians or South Asians living in different countries are particularly susceptible to coronary heart di-

sease. Hughes *et al.*[11] determined the antioxidant status including $CoQ_{10}$ and various lipid parameters in a random sample of Indians and Chinese from the general population of Singapore. Although no significant differences were found in plasma concentrations of total cholesterol, triglycerides and LDL cholesterol between the two ethnic groups, both Ubiquinol and total $CoQ_{10}$ concentrations in plasma were significantly lower in Indian males than in Chinese males; the ratios of ubiquinol and total $CoQ_{10}$ to triglycerides, total cholesterol and LDL were also lower in the Indian subgroup. There were no significant differences in other antioxidant levels. One might reasonably conclude that the consistently lower values of Ubiquinol in Indian males may contribute to the higher susceptibility of this ethnic group to coronary heart disease.

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# Ubiquinol as a food supplement

As widely discussed in the previous chapters $CoQ_{10}$ is a generic term which refers to both its oxidized and reduced form: the latter can also be called ubiquinol-10 ($CoQ_{10}H_2$) or just Ubiquinol.

In the mitochondrial respiratory chain $CoQ_{10}$ is continuously reduced and then reoxidized by appropriated enzymes. Also in extracellular systems, such as blood plasma and seminal fluid plasma $CoQ_{10}$ is present in the two forms. Therefore also in the extramitochondrial environment there are several enzymatic systems capable of regenerating ubiquinol from ubiquinone, the extra-mitochondrial $CoQ_{10}$ reductases. Speaking about antioxidant effect, ubiquinol is the active form of $CoQ_{10}$. This potent lipid soluble antioxidant is endogenously synthesized and is also, in small quantities, introduced with food. The most common form of $CoQ_{10}$ as a supplement is the oxidized one; in recent years ubiquinol has become available, thanks to Kaneka technology[1,2,3]. Our body is capable of reducing ubiquinone to ubiquinol but, as we will further discuss, administering ubiquinol is more effective in some situations. When discussing superiority of Ubiquinol we must keep in mind the concepts of reducing capacity and of bioavailability.

**$CoQ_{10}$ must first be converted to Ubiquinol in order to be absorbed. This can be achieved through the action of specific enzymes, called "Reductases" This somehow constitutes a burden for our body, as every reduction needs the oxidation of some other molecules. Furthermore, there is evidence, at least in some animal models, that reducing capacity decreases with**

**age. In fact it has been demonstrated that Ubiquinol is better absorbed, compared to $CoQ_{10}$.**

## $CoQ_{10}$-reducing capacity decreases with age

DT- diaphorase (NQO1), is a well- known enzyme, important for detoxification of several compounds with benzene rings. It is also one of the main extramitochondrial enzymatic systems capable of reducing $CoQ_{10}$ to ubiquinol. The reducing equivalents for this reaction come from $NADPH+H^+$. NQO1 specific activity significantly decreases with age in mouse liver and in rat liver. Activity of thioredoxin reductase also showed a marked decrease in kidneys of aged rats. On the other hand also the levels of NADPH, an essential electron donor for NQO1 enzyme, were found to significantly decline with age[5-9].

## Improved bioavailability of ubiquinol

There is experimental evidence that bioavailability of ubiquinol-10, when given orally, is superior to the corresponding bioavailability of oxidized $CoQ_{10}$. Several factors in the formulation affect bioavailability of $CoQ_{10}$, therefore we can make a comparison if, for a certain formulation, the carrier is the same and the only change is that $CoQ_{10}$ is in the oxidized or the reduced state. This observation can be made, for example, if we compare Kaneka $CoQ_{10}$, in the oxidized form, as described in the paper by Ikematsu *et al.* with Kaneka ubiquinol as described by Hosoe *et al.*[2]. **We can see that plasma levels achieved with ubiquinol are close to the corresponding levels after a dose nearly three times as big of ubiquinone.**

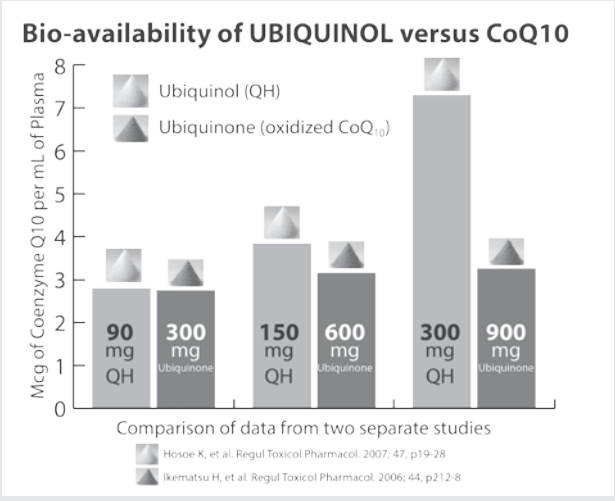


Fig. 11 – Bio-availability of UBIQUINOL versus $CoQ_{10}$

A similar conclusion can be drawn speaking about other formulations as well. Our research group gained direct evidence of this concept having conducted several studies with the oxidized, classical formulation, of $CoQ_{10}$ and with the corresponding formulation containing Ubiquinol. For instance the average level reached with 300 mg/day of oxidized $CoQ_{10}$ in a group of patients affected by ischemic heart disease was 3.2 µg/ ml, and 4 µg when the patients also underwent a program of physical exercise. The same formulation and the same dosage generated levels around 4µg/ml in a group of healthy volunteers. When this group was treated with 300 mg of the corresponding Ubiquinol formulation, an average level close to 6µg/ml was reached[4]. As we will refer below, a group of cardiac patients who had previously been treated with ubiquinone was given the same doses of ubiquinol and higher plasma levels were seen, together with a better clinical outcome.

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2nd PART

# Health benefits from Ubiquinol supplementation

# Coenzyme $Q_{10}$ and Ubiquinol in cardiovascular disease

$CoQ_{10}$ was discovered in the heart, and the heart muscle has indeed a very high concentration in this essential factor. Our studies in the early 1970s, while I was working with Dr Folkers in Austin, were conducted on more than one hundred heart biopsies obtained during surgery: there was a certain degree of $CoQ_{10}$ deficiency in the cardiac muscle of patients in heart failure and the deficiency was more severe in the sickest and most compromised patients[1]. Those results were confirmed later, when the HPLC technique became available. $CoQ_{10}$ deficiency was not the cause of the disease, but we can reasonably hypothesize that it did contribute to the progressive worsening typical of this condition, where the heart is really energy starving. Based on these findings an approach of complementary treatment of Chronic Heart Failure (CHF) with $CoQ_{10}$ was started. In the following years a mild, yet significant effect of $CoQ_{10}$ on high blood pressure was seen. Regarding this last issue we could only speculate that the antioxidant properties of $CoQ_{10}$ might be involved. In the recent years we gained a broader picture of the multifaceted effect of $CoQ_{10}$ and Ubiquinol in cardiovascular disease: these effects are likely based on its well known role in mitochondrial bioenergetics, on antioxidant protection and on counteracting endothelial dysfunction. These concepts will be explored in the following pages.

## Congestive Heart Failure

Congestive heart failure (CHF) is one of the main indications for the use of $CoQ_{10}$ as adjuvant therapy.

CHF is a condition in which the pumping capacity of the heart is seriously compromised. In CHF the myocardial cells

lack the contractility that is necessary to effectively pump blood to the various parts of the body. In the preceding pages the essential role of $CoQ_{10}$ in cellular bioenergetics was highlighted, and optimizing the $CoQ_{10}$ status of the cardiac cells has been shown to improve their functional capacity. Although it is beyond the aim of this book to critically analyze all of the papers dealing with $CoQ_{10}$ and CHF, I will mention some of the studies which help to clarify at which extent $CoQ_{10}$ can influence this condition.

The early studies with $CoQ_{10}$ in cardiology were conducted by Dr Karl Folkers. Also, an original pioneer in this field was Dr Per Langsjoen, a cardiologist in Texas.

The many years of experience of Langsjoen and Folkers are summarized in a paper published in 1990 in the *American Journal of Cardiology*[2]. The authors started a study in 1981 with a pilot trial, double blind with crossover, on a group of 19 patients affected by dilated cardiomyopathy, NYHA class III and IV. In that study $CoQ_{10}$ administration led to an increase of $CoQ_{10}$ blood levels with simultaneous improvement of myocardial function and clinical conditions. In the following 42 months, 126 patients affected by the same disease were enrolled and followed-up for 5 years. 75% of these patients were more than 50 years old with an age distribution comparable between the two genders. Most of the patients were in NYHA class III and IV and stabilized by conventional therapy. The patients started a treatment with 100 mg of $CoQ_{10}$ per day. Mean blood $CoQ_{10}$ levels at start were 0,85 µg/ml, i.e., significantly lower than the corresponding levels in a group of 54 normal control subjects, showing an average of 1.07 µg/ml. These values rose after $CoQ_{10}$ therapy to approximately 2 mg/ml, which were maintained. Mean ejection fraction (EF) was 41% at the beginning of the trial, and rose highly significantly to 59% stable values after 6 months. A detailed analysis of single patients showed that 71% of the patients achieved significant improvement of EF

after 3 months of therapy and 16% within 6 months. On the whole there was a significant EF improvement in 87% of the patients. A total number of 106 patients improved by one or two NYHA classes, with some degree of correlation with the improvement in EF.

A contribution on the effect of $CoQ_{10}$ in the treatment of cardiac failure was presented by Hoffman-Bang, Swedberg *et al*., who set up a double blind, crossover study on 79 patients, 60 of whom in NYHA class III[3]. Analysis of data regarding the quality of life revealed a significant improvement of sensory perception and physical performance. Significant differences were also recorded concerning the EF measured with an induced increase of preload, i.e. with the legs up, and the maximum workload measured by an ergometric test.

Among the numerous Italian studies, we should mention a multicenter, double blind trial coordinated by Trimarco and Condorelli[4]. 33 clinical centers took part in the study, enrolling 641 patients; 319 were assigned to the $CoQ_{10}$ treated group (100-150mg/day) and 322 to the placebo treated, control group. A total of 16 and 21 patients died, respectively, in 12 months, but the difference was not significant. However, the percentile incidence of acute pulmonary edema was significantly lower in the $CoQ_{10}$ treated group, compared with the control group. The episodes of cardiac asthma were also significantly less frequent in the $CoQ_{10}$ treated group; finally, arrhythmias were significantly more frequent in the control group. A very interesting result was that **the percentage of patients requiring one or more hospital admissions during the study was close to 40% for the control group, compared with 20% for the $CoQ_{10}$ treated group (P<0,01).** Variation of NYHA class was also evaluated. In the $CoQ_{10}$ treated group, there was a significant reduction of the NYHA class, which indicates a functional improvement. This reduction was already signifi-

cant after 3 months of $CoQ_{10}$ therapy, and was constant up to 12 months. There was no NYHA class reduction in the patients of the placebo group. Finally, physicians participating in the study and the patients themselves were asked to express an overall clinical pronouncement on the effects of the treatment, using a 1 to 3 score.

The mean score expressed by the patients as well by the physicians throughout the course of the study was unchanged for the placebo control group, whereas progressively increased in the $CoQ_{10}$ treated group.

It is reasonable to inquire whether an increased radical production is present in heart failure. The heart is under the control of the autonomous nervous system and the failing heart undergoes a series of reactive mechanisms which also imply a higher release of catecholamines: this often leads to a worsening of the failure itself. For instance, the increased incidence of arrhythmia in patients affected by heart failure might reasonably be related, at least in part, to an increased stimulation by catecholamine.

In the presence of an excess of catecholamines, one of the two possible catabolic pathways of catecholamines would be enhanced, i.e. oxidation of catecholamines to adrenochrome and adrenolutin. This catabolic pathway involves the intervention of superoxide anion and therefore of other reactive oxygen species. Experimental evidence in different fields also points out that there is an increased consumption of Coenzyme Q in situations characterized by increased levels of oxidative insult. Therapeutic use of $CoQ_{10}$ in congestive heart failure, besides the rationale mentioned at the beginning of this chapter, might also be related to the possible beneficial effects of antioxidants in this clinical syndrome, quite possibly the ubiquinol antioxidant form.

In a double-blind controlled study conducted in Israel patients in NYHA class IV treatment with conventional therapy

plus $CoQ_{10}$ led to significant improvement in dyspnea, NYHA class, nocturia and fatigue[5]. The most significant result was observed in the "six minute walking test". In fact, patients treated with $CoQ_{10}$ increased their distance from 269 to 382 meters, while in the placebo group there was a negative variation, from 254 to 177 meters.

A paper recently published in the International Journal of Cardiology highlights the findings of a 5-year prospective study, led by Dr. Urban Alehagen, conducted among elderly Swedish citizens treated with selenium and coenzyme $Q_{10}$[6].

Selenium is an essential nutrient involved in different vital processes of the body, such as antioxidant defense, oxidative metabolism and immune response. A close connection is known between the selenium content of soil, selenium dietary intake and health. The best- exemplified situation is Keshan disease, an endemic cardiomyopathy found in selenium deficient areas of inland China. Besides this particular situation, there are concerns on whether the daily intake of selenium is sufficient in many Western European countries. Salonen *et al.* observed a 2.9-fold increased risk of cardiovascular death in patients with low selenium levels[7]. On the other hand the involvement of coenzyme $Q_{10}$ in cardiology has been known for many years, starting from the initial observations of low $CoQ_{10}$ myocardial levels and correlating with the extent of myocardial dysfunction. More recent findings indicate lower levels of coenzyme $Q_{10}$ in plasma of heart patients with lower survival, compared to the corresponding levels in patients who survived longer as discussed in the previous pages[8]. Therefore a background on the relevance of both $CoQ_{10}$ and selenium in the management of heart disease already existed.

The Alehagen paper highlights **some biochemical facts which might strengthen the relationship between $CoQ_{10}$ and selenium. One which is closely related to the**

**main topic of this booklet is that selenium is essential for the function of thioredoxin reductase, an enzyme among those which, in our body, reduce ubiquinone to ubiquinol, the active form of $CoQ_{10}$ endowed with antioxidant properties.**

In the study by Alehagen and his group, 443 people from a rural town in South East Sweden, aged 70-88 years, were divided into 2 groups: 221 of them took the active treatment and 222 received the placebo. Active treatment consisted of $CoQ_{10}$, 200 mg/day,and 200 micrograms/day of organic selenium yeast tablets. Participants underwent a clinical examination, ECG and echocardiography; blood was withdrawn for the analysis of BNP, a recognized marker of cardiac muscle deterioration.

The follow-up lasted for about 5 years and 36 participants in the placebo group suffered all-cause mortality compared with 28 participants in the active treatment group: the difference though was not statistically significant. Instead, cardiovascular mortality resulted significantly higher in the placebo group (28/222, 12.6%) compared to the active treatment group (13/221, 5.9%). This was evident when performing a univariate analysis and also when taking into account, in a multivariate regression analysis, other variables which alone led to increased mortality, such as male gender, age, NYHA class III, smoking, diabetes, etc. According to the multivariate regression analysis, treatment with $CoQ_{10}$ and selenium determined an HR (hazard ratio) of 0.46 (P=0.02). From a practical point of view $CoQ_{10}$ plus selenium more than halved the mortality hazard. A significant difference in NT-proBNP plasma concentration levels between the two groups was noted at 24 months, and this was further pronounced at 48 months: the lower levels of proBNP in the active treatment group underline a better status of myocardial tissue. Cardiac systolic function assessed by echocardiography also showed a significantly better score in the active

supplementation group compared to the placebo one. This study, although conducted in a limited number of participants, and belonging to a rather narrow age range, shows a remarkably reduced risk of cardiovascular mortality, upon long-term treatment with $CoQ_{10}$ and selenium versus placebo and this was accompanied by a better cardiac functionality and improved markers of cardiac muscle improvement.

These encouraging results strongly support further supplementation studies on larger populations, with different age groups, especially in conditions where a selenium and/or $CoQ_{10}$ deficiency may be present. In the last 20 years several metanalyses on $CoQ_{10}$ impact in CHF were conducted; the last one, by Fotino *et al.*, was published in 2013[9]. This review included controlled studies that reported EF and NYHA class as primary outcome. Supplementation with $CoQ_{10}$ resulted in a pooled mean net change of 3.67% (95% CI: 1.60%, 5.74%) in the EF and -0.30 (95% CI: -0.66, 0.06) in the NYHA functional class. These changes were significant in some subclasses of studies.

## Q-Symbio

In this double blind, placebo-controlled study, a group of researchers, coordinated by Prf. Svend Aage Mortensen, investigated the effects of $CoQ_{10}$ on patients symptoms, functional capacity and biomarker status (NT-proBNP) and the long-term outcome by monitoring morbidity and mortality. This paper has just been accepted for publication in the Journal of the American College of Cardiology, Heart Failure[12].

Patients affected by Heart Failure (H.F.), in New York Heart Association (NYHA) Class III or IV, who were receiving current pharmacologic therapy were randomly assigned in parallel groups to $CoQ_{10}$ 100 mg three times daily or to placebo. **The primary long-term endpoint was the time to first MACE (major adverse cardiovascular event) including**

**unplanned hospitalization due to worsening of HF, cardiovascular death, urgent cardiac transplantation and mechanical support**, using a time to first event analysis.

A total of 420 patients – $CoQ_{10}$ (N=202), placebo (N=218)– were enrolled with a follow-up time of 2 years. After 3 months there was a trend with a reduced level of NT-proBNP in the $CoQ_{10}$ group. After 2 years there was a significant improvement of the NYHA Class in the $CoQ_{10}$ group. The primary endpoint was reached by 29 patients in the $CoQ_{10}$ group, as compared with 55 patients in the placebo group (14 percent vs. 25 percent). $CoQ_{10}$ treated patients had significantly lower cardiovascular mortality and lower occurrence of hospitalizations for HF. All cause mortality was also lower in the $CoQ_{10}$ group, 18 patients vs. 36 patients in the placebo-group (9 percent vs. 17 percent). There were fewer adverse events in the $CoQ_{10}$ group compared to the placebo group.

The authors conclude that: "Q-SYMBIO is the first double-blind trial in chronic HF addressing whether $CoQ_{10}$ supplementation might improve survival. The $CoQ_{10}$ treated patients had reduced hospital admission rates for worsening HF and lower cardiovascular death both of which may reflect a significant improvement in cardiac function. $CoQ_{10}$ treatment was safe with a reduced all cause mortality rate. Therefore $CoQ_{10}$ should be considered as a part of the maintenance therapy of patients with chronic HF".

**A few years ago the reduced form of coenzyme $Q_{10}$, ubiquinol, became available and several studies showed a superior effect compared to ubiquinone, also in cardiology practice.**

## Ubiquinol in clinical practice

Dr. Peter Langsjoen has gained extensive experience in treating patients affected by congestive heart failure with

$CoQ_{10}$. In the past few years he also started using ubiquinol. According to Dr. Langsjoen, patients with congestive heart failure often fail to achieve therapeutic plasma $CoQ_{10}$ levels even with high doses of ubiquinone and clinical improvement is limited. He postulated that the intestinal edema in these critically ill patients may impair $CoQ_{10}$ absorption. He initially described seven of these patients in whom plasma levels of $CoQ_{10}$ and the clinical outcome significantly improved when the patients switched from ubiquinone to ubiquinol, at about the same doses. The authors hypothesized that the mechanism of $CoQ_{10}$ intestinal absorption is also compromised, because of the very serious edema and, for some still unknown reason, ubiquinol is nonetheless better absorbed. As we mentioned in the previous chapter, it has been demonstrated that, at least in healthy people, ubiquinol has better bioavailability compared to ubiquinone. Later on an additional 23 patients with heart failure, already on maximal medical therapy and on ubiquinone, were switched to ubiquinol. Plasma $CoQ_{10}$ levels on an average 384 mg/day of ubiquinone were 2.9 µg/ml. When the authors replaced ubiquinone with ubiquinol (average 334 mg/day) plasma $CoQ_{10}$ levels rose to 5.3 µg/ ml. This improved $CoQ_{10}$ level was associated with improvement in both systolic and diastolic myocardial function, along with improved NYHA classification[10]. By 2009 the authors were convinced of the superior absorption of ubiquinol in patients with cardiovascular disease and treated approximately another 300 patients.

Dr. Langsjoen also prospectively analyzed patients presenting to his cardiology clinic with signs and symptoms of heart failure that had appeared about six years after statin drug therapy and in whom no other cause of heart failure could be identified[11]. In particular he referred about 83 patients with isolated diastolic dysfunction (present in 88% of the patients) and combined systolic and diastolic dysfunction (in the other 12%). All patients discontinued their statin therapy and began supplementation with ubiquinol at an average of

260 mg/day. Plasma levels of $CoQ_{10}$ rose to an average of 4.6µg/ml. **According to Dr Langsjoen Statin cardiomyopathy (SCM) is a common cause of heart failure.With combined statin discontinuation and Ubiquinol supplementation SCM was reversible to normal in 33 out of 67patients (49%), improved in 18 patients (26%) and unchanged or worse in 14 patients (21%).**

According to the New York Heart Association (NYHA) classification, there are four classes of patients in heart failure.

***Class I*** - No limitation: Ordinary physical activity does not cause undue fatigue, dyspnea, or palpitation.

***Class II*** - Slight limitation of physical activity: Such patients are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or angina.

***Class III*** - Marked limitation of physical activity: Although patients are comfortable at rest, less than ordinary activity will lead to symptoms.

***Class IV*** - Inability to carry out any physical activity without discomfort: Symptoms of congestive failure are present even at rest. With any physical activity, increased discomfort is experienced.

# Ubiquinol and Endothelial function

During the last two decades much research has addressed endothelial dysfunction. The endothelium is the inner lining of our arteries. This fundamental component of our blood vessels acts by releasing several vasoactive factors that are responsible for relaxation and contraction of arteries, inhibition of platelet aggregation (clot prevention) and smooth cell proliferation (also a cause of atheroma), and finally for exerting anti-inflammatory properties. Among the vasorelaxing factors, one of the most studied is nitric oxide (NO·). Endothelial dysfunction reflects an imbalance between release of vasodilator and vasoconstrictor endothelial-derived factors. A decrease in the availability of NO· involves either a decrease in its production by endothelial cells or in increased elimination of NO· itself. It is known that oxidative stress is one of the causes of NO· inactivation and superoxide anion is a molecular species responsible for it. Intercellular matrix, the material that holds endothelial cells together, is also endowed with defensive tools against NO· oxidation. These are mainly represented by extracellular superoxide dismutase (ecSOD), an enzyme which eliminates superoxide anion. Investigating this complex molecular scenario involves sophisticated biochemical methods which are not routinely performable. Endothelial function can be measured indirectly by assessing the vasodilatory response of peripheral arteries to stimuli that increase NO· release. One of the techniques consists in measuring flow-mediated dilation (FMD). Using a blood pressure cuff applied on the upper arm, blood flow is stopped for a few minutes: upon release of the cuff the sheer stress produced by resumed blood flow stimulates NO· production by endothelial cells. Upon NO· release there is an increase in the artery diameter which can be measured by an echograph.

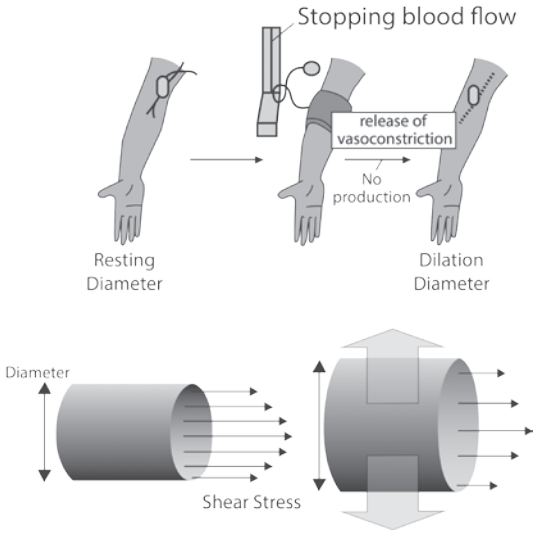


Fig. 12 – Measurement of endothelial function

A decrease below the expected arterial dilation indicates endothelial dysfunction, which is predictive of adverse clinical events. **$CoQ_{10}$ positively affects FMD, mitigating endothelial dysfunction.** The first observation was made by Gerald Watts and his group[13], who documented positive effect of $CoQ_{10}$ in ameliorating FMD, and therefore endothelial dysfunction, in a group of Type II diabetes mellitus patients. In the same years intensive work started in Ancona, in cooperation with Dr. Belardinelli, focused on the effect of $CoQ_{10}$ and physical exercise in patients affected by coronary heart disease. This cooperation led to the publication of two papers, both in the European Heart Journal[14,15]. In the first one we studied the effect of physical exercise alone, $CoQ_{10}$ with and without physical exercise, and placebo. It was already known that physical exercise improves endothelial dysfunction in patients affected by ischemic heart disease: $CoQ_{10}$ supplementation was effective to the same extent

and the combination of $CoQ_{10}$ + exercise was even more impressive. In the second paper we confirmed the positive effect of $CoQ_{10}$ in improving FMD in patients suffering from ischemic heart disease and we also discovered that $CoQ_{10}$ increases the amount of extracellular SOD. This effect was particularly evident in patients with more altered FMD.

**Endothelial function is also influenced by the inflammatory status of these cells, and ubiquinol affects these processes as well.** During ageing, the endothelium undergoes specific modifications characterized by enhanced inflammatory response and compromised NO·-producing activity. Very recently, we were able to study these processes at molecular level using an in vitro model of vascular ageing, i.e. a culture of endothelial cells grown in vitro until they reach senescence. **This enabled us to evaluate the effect of ubiquinol in modulating inflammatory response associated with senescence[16].** This was carried out in basal conditions and in the presence of an acute pro-inflammatory stimulus, i.e. the exposure to bacterial lipopolysaccharide (LPS). In senescent endothelial cells the enhanced release of inflammatory markers is known to be activated by modulation of specific intracellular signalling processes regulated by small non coding RNA defined microRNA (miR). Exposure to ubiquinol of young cells challenged with LPS was able to silence inflammatory-associated signalling, effectively curbing cell release of interleukin-6, the main proinflammatory factor responsible for SASP (Senescence associated secretory phenotype). In older cells intracellular signalling was also quenched although this did not translate in a significant decrease of IL-6 release. This data is probably associated with an overly high inflammatory background in older cells.

**Both resistance of LDL to peroxidation and endothelial function are positively influenced by plasma ubiquinol concentration which is affected to a great extent by exogenous $CoQ_{10}$. It has long been known that after**

a single or prolonged administration of $CoQ_{10}$ small increases can be found in heart, muscle and other organs while remarkably high amounts of $CoQ_{10}$ can be found in liver and plasma and high ubiquinol concentrations in plasma certainly protect the arteries. In this sense, ubiquinol is available as a pre-activated, antioxidant form ready to deploy its benefits without need of conversion from ubiquinone.

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# Physical exercise, free radicals and $CoQ_{10}$/Ubiquinol

It has been calculated that about 3% of the oxygen consumed by our organism is not completely reduced to water, undergoing instead, the monoelectronic reduction, which generates superoxide anion and other reactive oxygen species able to initiate radical reactions and insult. This quota becomes remarkably high when there is an increase in the overall oxygen uptake, such as in the course of physical exercise, especially in endurance sports. High intensity aerobic exercise involves extremely elevated oxygen consumption, which, as in the case of leg muscles, can reach levels one hundred times higher compared to the resting levels. The increased radical production associated with physical effort is also due to hyperthermia typical of high intensity exercise[1]. In fact, it has been demonstrated that temperature increase stimulates the uncoupling of oxidative phosphorylation. Instead of flowing all the way along the respiratory chain to make $H_20$ and ATP, electrons leak through a «short cut» toward oxygen to make superoxide anion.

Numerous experimental studies conducted with laboratory animals as well as on man have demonstrated an enhanced production of free radicals in strenuous as well as in not-so-strenuous exercise[2-4]. Remarkable increases in the concentration of pentane, a final product of lipid peroxidation in expired air, were measured in men during heavy exercise. It was also possible to monitor an increase of plasma malondialdehyde (another product of lipid peroxidation) following physical exercise. In a group of ultramarathon endurance athletes, the increase of malondialdehyde correlated with the release into the blood stream of lactic dehydrogenase and creatine kinase, induced by physical exercise[3]. These studies have not always yielded consistent results. It is reasonable to figure out that the level of strain and the extent of training of tested subjects exerts its influence in

these kinds of experiments. If we consider, for instance, lipid peroxidation, it will depend on the extent of oxidative insult and the endowment in antioxidants of the muscle fiber or of other tissues. This last point is very important since it brings us to consider the effect of acute physical exercise and of training on the mechanisms of antioxidant defense, either enzymatic or non-enzymatic mechanisms.

Several studies showed an increase of "defensive enzymes" i.e.,superoxide dismutase, catalase, glutathione peroxidase in different models of acute and chronic exercise. In one of these studies, conducted on athletes on whom muscle biopsies were performed, it was possible to demonstrate an increase of superoxide dismutase and of catalase correlated with the values of maximal oxygen uptake ($V0_2$ max) of the inquired athletes.

It is well known that training is a powerful stimulus for the biosynthesis of myofibrillar elements as well as of the machinery that generates the ATP necessary to sustain the exercise. It also constitutes a stimulus capable of inducing the biosynthesis of antioxidant systems. In fact, muscles involved in a program of endurance training have a better capability of oxidizing both pyruvate and fatty acids, the fuels which provide the energy for exercise itself. These enhanced oxidative properties stem from an increased number of capillary vessels, increased content of myoglobin, the final oxygen carrier, and, above all, increased volume of single mitochondria and more numerous mitochondria, the organelles where the final harvesting of energy takes place. This change toward a type of muscle with more evident oxidative character is also confirmed in humans, where it is possible to find, upon endurance training, an increase of type I oxidative fibers

It has also been proven that the abundance in Coenzyme Q correlates with the endowment in type I oxidative fibers, the fibers which get most of their energy from the aerobic mitochondrial machinery. This increase in Coenzyme Q may reasonably be interpreted not only as part of the more general increase in mito-

chondria and mitochondrial components induced by endurance training, but also as a potentiation of the cellular environment in molecules capable of counteracting the oxidative insult typical of enhanced aerobic activity. Robert Beyer highlighted, many years ago, that the content of Coenzyme Q in the mitochondrial membrane of normal sedentary 25 month old animals is 30% lower compared to the Coenzyme Q content in 3 month old animals. Aging is associated with a tissue decrease in Coenzyme Q and training is capable of minimizing this effect. A regimen of endurance training was capable to increase the Coenzyme Q concentration of heart submitochondrial particles from such elderly animals by 41% when compared to sedentary elderly animals. It is worthwhile to mention some details of the Beyer paper[6]. The extent of lipid peroxidation in the membranes prepared from these 3 groups (young, old, old but trained) shows small differences, but they possess different antioxidant potentialities. The addition of succinate, which reduces endogenous Coenzyme Q, through the succinate dehydrogenase Coenzyme Q reductase, results in significant inhibition of lipid peroxidation in each case, but is also in direct proportion to the endogenous Coenzyme Q content of the submitochondrial particles, being greatest in submitochondrial particles from 25 month old trained and 3 month old sedentary animals. When tested in vitro the three mitochondrial populations showed the same peroxidation level, but upon addition of succinate, which converts ubiquinone to ubiquinol, the mitochondria containing more ubiquinone became more protected. These data highlight that training counteracts the ubiquinone decrease produced by age, but the conversion to Ubiquinol is necessary in order to gain a better antioxidant protection. It is clear that training stimulates the synthesis of ubiquinone in order to meet the increased metabolic demand and the need for extra protection against oxidative insult. Under these conditions, a disproportion might occur between the availability of enzymatic and apoenzymatic components, i.e., although Coenzyme Q is absolutely increased, there might be a relative deficiency with regard to the increase of the other mitochondrial components and of lipid peroxidation.

To further inquire into this concept, it is useful to know whether the administration of $CoQ_{10}$ is capable of attenuating some biohumoral phenomenon known to be related with the extent of oxidative damage. In an experiment conducted by Shimomura *et al*.[7], a group of trained animals, part of which were on $CoQ_{10}$ treatment, were exercised for 30 min on treadmill, in a downhill position. $CoQ_{10}$ treated animals had higher level of $CoQ_{10}$ in their muscles, and the early rise in creatine kinase and lactic dehydrogenase plasma levels, due to the exercise, was evident at a remarkably significant lower extent, in the treated animals. This is a sign of less muscular damage.

Similar observations were also made in an experiment conducted on athletes. Bargossi *et al.* administered $CoQ_{10}$ to a group of marathon runners and tested these athletes by a cycloergometer submaximal exercise[8]. The elevation of plasma levels of enzymes (a sign of muscle suffering) was lower after $CoQ_{10}$ treatment compared to the baseline control. Already several years ago, research on the effects of $CoQ_{10}$ in physical exercise was focused on two issues: protection from exercise-related damage and improvement of physical performance.On the basis of the concepts expressed in the pages dealing with energy conservation and mitochondrial Coenzyme Q content, several studies were conducted on the bioenergetic role of $CoQ_{10}$ in sports medicine. In one of the early reports we were able to show that administration of $CoQ_{10}$ to a group of young cyclists significantly increased the $VO_2$max. This effect was later confirmed on athletes practicing less aerobic specialties, like volleyball or basketball[9].

Since those early observations, numerous studies have been conducted on the effects of $CoQ_{10}$ on physical exercise capacity and on fatigue. Some studies have highlighted a positive effect while others did not. A study from Cooke *et al.*[10] showed that following a single administration of $CoQ_{10}$, plasma levels significantly correlated with muscle $CoQ_{10}$ levels, maximal oxygen consumption and treadmill time to exhaustion. A trend for increased time to exhaustion was observed following two weeks

of $CoQ_{10}$ supplementation. In another trial, by Mizuno *et al.* (2008)[11] oral administration of $CoQ_{10}$ improved subjective fatigue sensation and physical performance. Kon conducted a double blind study[12] where a group of kendo athletes supplemented with $CoQ_{10}$ showed lower levels of CK, myoglobin (markers of muscular damage) and lipid peroxides (markers of oxidative stress) compared to the corresponding values in the placebo group. Also in 2008 Tauler *et al.* showed that supplementation with a combination of antioxidants including $CoQ_{10}$ was able to prevent plasma oxidative damage induced by soccer[14]. A study by Zheng *et al.* examined the acute effects of $CoQ_{10}$ and placebo on autonomic nervous activity and energy metabolism at rest and during exercise: fat oxidation significantly increased during exercise in the $CoQ_{10}$ group; results suggested that $CoQ_{10}$ increases autonomic nervous activity during low intensity exercise[15]. In a double blind pilot study patients with post-polio syndrome were treated with 200 mg of $CoQ_{10}$/day. Muscle strength, muscle endurance and quality of life increased statistically significantly in all 14 patients but there was no significant difference between the $CoQ_{10}$ and placebo groups (Skough *et al.* 2008)[16].

Studies conducted with $CoQ_{10}$ in the field of physical performance have shown results which are not univocal; in fact sometimes they were inconclusive. A critical analysis of the possible reasons for such disparity of results must take into consideration several issues.

The first observation concerns dosage. Coenzyme $Q_{10}$ in general is poorly absorbed and even for the best formulations we could calculate an intestinal absorption not greater than 4%. Having tested different formulations and different dosages we learned that the amount absorbed (in terms of % of the administered dose) is better for low dosages; moreover, it is important to ingest $CoQ_{10}$ together with a meal. Then, of course, the quality of the formulation is important: with a few exceptions we found that oily formulations, such as soft-gels, are better absorbed than dry formulations. This is not surprising: being $CoQ_{10}$ a fat

it is better absorbed together with fats. In some cases there could be some factors which inhibit dissolution of the tablet and absorption of the coenzyme . Studies with Coenzyme $Q_{10}$ in the field of physical exercise and sport medicine aim at measuring performance, but also mitigation of the muscle and more general damage associated with high intensity training. Many $CoQ_{10}$ users commonly refer to be able to perform a certain amount of work, at a certain training load, in a more natural, less painful way. This is not only a matter of sensations, and perhaps the physiological difference may be biochemically measurable. In a recent study by Diaz–Castro *et al.*[22] it was demonstrated that $CoQ_{10}$ supplementation before strenuous exercise decreases the oxidative stress and modulates the inflammatory signaling, reducing the subsequent muscle damage. Scientific evidence on the effects of $CoQ_{10}$ in sport medicine is also based on the concept of dose and of length of treatment. Appropriateness of dose, quality of the formulation, length of treatment all result in a certain plasma level, which could be related with a specific biological effect. In fact some biochemical, physiological or clinical effects imply attaining a specific plasma level. Several years ago we were able to demonstrate that improvement of endothelial function, in patients with ischemic heart disease, occurred with remarkably higher frequency when Coenzyme $Q_{10}$ intake led to a plasma level of 2.5 µg/ml.

As I mentioned in the previous chapter Ubiquinol has a better bioavailability compared to oxidized coenzyme $Q_{10}$ and this feature prompted its use in two well designed studies in exercise performance. In the study by Bloomer *et al.*[20] seventeen physically active subjects, regularly participating in aerobic and/or anaerobic exercise training, took one single capsule, for breakfast, containing 300 mg of Ubiquinol, per day, for 30 days, or a capsule of placebo. The same subjects, after a 21-day washout period switched to the "other treatment", in a double-blind crossover model. Fifteen subjects completed the study. Treatment with ubiquinol did not significantly improve physical performance, except in some selected individuals. Looking at the data there

was a very significant correlation between increase in Ubiquinol plasma levels and improvement in cycle sprint total work. In fact all 6 subjects in whom there was at least a 200 % change in Ubiquinol plasma level underwent an improvement, while none of the subjects below this threshold did increase their performance. Though the amount of ubiquinol administered (300 mg) to the participants is a considerable amount, it was consumed in a single dose at breakfast. For some of them this single dose was sufficient to reach a plasma level accompanied by a physiological response. The good correlation between % of plasma ubiquinol change and cycle total work make us predict that a significant response would have indeed been attained if plasma levels of Ubiquinol had been a little higher.

Alf, Schmidt and Siebrecht published in 2013 the results of a study where they treated with Ubiquinol, or with placebo, a group of 100 subjects, recruited among the young German athletes training regularly prior to the London Olympic Games[21]. All athletes received 5 capsules every day, each one containing either lactose, with an oily carrier, or 60 mg of Ubiquinol, and treatment lasted 6 weeks. Physical performance was measured, in the different phases of the study, as maximum power output, in terms of Watt/kg of body weight. Both groups increased their physical performance: in the placebo group the increase in those 6 weeks was 8.5%, while in the Ubiquinol group the improvement was 11% and this difference was significant. In conclusion, the positive effect of training was significantly more evident in the athletes who took Ubiquinol. The authors of this paper highlight that, besides the better performance, Ubiquinol was also beneficial for the health of those athletes.

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# $CoQ_{10}$/Ubiquinol and male infertility

A growing body of evidence indicates that damage inflicted on spermatozoa by reactive oxygen species (ROS) plays a key pathogenetic role, implicating oxidative stress as a mediator of sperm dysfunction in the etiology of male infertility[1]. Spermatozoa have a high content of polyunsatured fatty acids (PUFA) within the plasma membrane and a low concentration of cytoplasmic scavenging enzymes, thus being readily susceptible to peroxidation in the presence of elevated levels of ROS in the seminal fluid. Peroxidative membrane damage leads to a modification of permeability with an increased inflow of sodium and calcium and ATP depletion. Consequent activation of Ca-dependent enzymes (proteases, phospholipases) results in a cascade of protein and lipid damage, including also enzyme inactivation and structural DNA alteration and eventually cell death. The source of reactive oxygen species is related to both spermatozoa and the infiltrating leucocytes in semen. We must highlight that ROS also play an important physiological role for sperm functionality, for instance, a certain level of superoxide anion is necessary for the capacitation of sperm cells, the process which allows sperm cells to penetrate the egg membrane. As with other systems and organs, a certain amount of ROS and other radicals has a physiological effect, while an excess creates damage. Spermatozoa and seminal plasma are endowed with a rather low level of antioxidant enzymes, but they also possess chain-breaking antioxidants capable of counteracting oxidant radicals by hampering the propagation of free radical chain reactions.

Both the bioenergetic and the antioxidant roles of $CoQ_{10}$ suggested a possible involvement in male fertility. On one hand, it is known that a large amount of mitochondria are

present in spermatozoa, in which motility requires a high energy expenditure. On the other hand, as shown in the previous paragraphs, the protection of membranes from oxidative stress could play a role in preserving sperm integrity. Moreover, the biosynthetic machinery for CoQ is present at remarkably high levels in rat testis.

The first determination of endogenous $CoQ_{10}$ levels in seminal fluid was performed by our group showing that $CoQ_{10}$ was assayable in total seminal fluid and in seminal plasma[2,5]. Its levels showed a good correlation with sperm count and motility, except in the population of varicocele patients in whom the correlation with sperm motility was completely lacking. Varicocele is an abnormal enlargement of testicular vessels, often associated with impaired fertility. Moreover, in the varicocele patients a significantly higher proportion of total $CoQ_{10}$ was present in seminal plasma when compared with normal subjects or other infertile patients without varicocele (the ratio plasma/seminal fluid $Q_{10}$ was 69 ± 7.1% vs 41. 2 ±. 5.6%,). These data were also confirmed in a larger series of patients.

After surgical repair of the varicocele, a partial correction of the anormality in the seminal plasma-sperm cell distribution of $CoQ_{10}$ was demonstrated[4,6]. All these referred studies consider total $CoQ_{10}$ levels, irrespective of its redox status. The first report on the assay of reduced and oxidized forms of ubiquinone was performed by our group[7]. We showed a significant correlation between the reduced form (ubiquinol) and sperm count in seminal plasma, an inverse correlation between ubiquinol and hydroperoxide levels both in seminal plasma and seminal fluid, a strong correlation – using multiple regression analysis–between sperm count, motility and ubiquinol content in seminal fluid, and, finally, an inverse correlation between ubiquinol/ubiquinone ratio and the percentage of abnormal forms. These results indicate an important role of Ubiquinol in inhibiting hydroperoxide for-

mation. We also found a lower ubiquinol/ubiquinone ratio in sperm cells from idiopathic asthenozoospermic (IDA, an abnormal motility of sperm cells, of unknown origin) patients and in seminal plasma from IDA and varicocele-associated asthenozoospermic (VARA) patients compared to controls[8].

The important conclusion was that the concentration of Ubiquinol and ubiquinol/ubiquinone ratio may be an index of oxidative stress in seminal fluid and a decrease of Ubiquinol can constitute a risk factor for semen quality. Sperm cells characterized by low motility and abnormal morphology, equipped with low $CoQ_{10}$ content, could be less capable in counteracting oxidative stress, which could lead to a reduced ubiquinol/ubiquinone ratio.

## Studies on exogenous $CoQ_{10}$ administration

The effect of $CoQ_{10}$ on sperm motility in vitro had been previously reported by Lewin & Lavon[9]. A significant increase in motility had been observed in sperm obtained from asthenozoospermic men which had been incubated with exogenous $CoQ_{10}$, whereas no significant variation was reported in the motility of sperm cells from normal subjects. The same study also reported the effect of exogenous $CoQ_{10}$ in vivo in a group of patients with low fertilization rates after in vitro fertilization with intracytoplasmatic sperm injection for male factor infertility. No significant changes were reported in most sperm parameters, but a significant improvement was noticed in fertilization rates after a treatment with 60 mg/day for a mean of 103 days.

In an attempt to elucidate a potential therapeutic role, we administered $CoQ_{10}$ to a group of idiopathic asthenozoospermic infertile patients. Twenty-two patients (mean age: 31 years, range: 25-39 years) affected by idiopathic

asthenozoospermia were enrolled in the study[10]. The patients were selected at the Andrology Unit of the Division of Endocrinology, Umberto I Hospital, University of Ancona (Italy). 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 x $10^6$/ml, sperm motility (forward[11] 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 six 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 six months of therapy. A semen analysis was further performed after six months from interruption of therapy (wash-out).

The results showed, for the first time, an increase of $CoQ_{10}$ levels in seminal plasma after treatment. As for blood plasma the increase upon treatment with 200 mg/day of $CoQ_{10}$ was capable of triplicating the basal values. A significant increase of $CoQ_{10}$ content was also detected in sperm cells. Treatment with $CoQ_{10}$ also resulted in an increased content of phospholipids (namely phosphatydilcholine) both in seminal plasma and sperm cells. These phospholipids are essential components of the lipoproteins that transport $CoQ_{10}$ in the seminal fluid.A possible explanation for this finding is that increased $CoQ_{10}$ levels also need an appropriate high concentration of a lipid carrier.

As far as semen features are concerned, a significant difference was found in forward (class a+b) motility of sperm cells after six months of $CoQ_{10}$ dietary implementation (from 9.13+/-2.50 to 16.34+/-3.43%, $p<0.05$). The improvement of motility was also confirmed by means of computer-assisted determination of kinetic parameters. A significant

increase of VCL and VSL was found after treatment. No significant differences were found in sperm cell concentration and morphology.

Interestingly, although a direct correlation was not found, 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 .

Sperm forward motility was significant reduced after six 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 three out of twenty-two patients (13,6 %) achieved spontaneous pregnancy within three months from the discontinuation of therapy (2.4 % pregnancy rate per cycle).

The data of our study show a significant improvement of kinetic features of sperm cells after six 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.

Several years later we were able to confirm these observations in a double blind, placebo controlled study[15], where we also used a dose of 200mg/day.The exogenous administration of coenzyme $Q_{10}$ increased the level of the same and ubiquinol in semen and was effective in improving sperm kinetic features in patients affected by idiopathic asthenozoospermia.Patients with a lower baseline value of motility and lower levels of coenzyme $Q_{10}$ had a statistically significant higher probability to be responders to the treatment.

In conclusion, the administration of $CoQ_{10}$ may play a positive role in treatment of asthenozoospermia, probably rela-

ted both to its function in mitochondrial respiratory chain and to its antioxidant properties.

The issue was also addressed by Prof Safarinejad, in Teheran[12]. A placebo-controlled, randomized clinical trial was used to assess the effects of $Q_{10}$ on three semen parameters – sperm density, sperm motility, and sperm morphology. Study participants (212 infertile men with idiopathic oligoasthenoteratozoospermia (OAT)) were treated for 26 weeks, followed by a 30-week washout period. The trial showed that 300 mg/day coenzyme $Q_{10}$ significantly improved all three semen parameters, whereas they remained unchanged in the placebo group.

It would appear, however, that study outcomes based only on improvement in semen values cannot cover the issue in its entirety. It is likely that a more accurate outcome measure would be the pregnancy rate, since that is the ultimate goal of infertility treatment. Yet while using the pregnancy rate as the principal goal of therapy end points, it is imperative to take into account the spontaneous pregnancy rate in infertile couples on no treatment. In light of these observations, another prospective open label study analyzed the impact of $Q_{10}$ therapy on spontaneous pregnancy rates in couples with idiopathic male factor infertility. This study, also by Prof. Safarinejad[13], achieved a pregnancy rate of 34.1 per cent. The dose of $CoQ_{10}$ was 300 mg twice a day, and the length of treatment 12 months. Compared to the crude 12-month cumulative spontaneous pregnancy rate of 6.4 per cent on the waiting list for male subfertility patients, this is a remarkable increase and might not have been achieved if these patients had been left untreated. The study even showed a trend towards higher pregnancy rates when the $Q_{10}$ administration time was extended beyond the initial 6-month period.

The same author further extended his observations by employing Ubiquinol[14].

In a double-blind, placebo controlled, randomized study, a total of 228 men with unexplained infertility were randomly assigned into one of two groups. The first group received 200 mg Ubiquinol for 26 weeks, while the second group received a similar regimen of placebo. A 12-week off-treatment period followed for both groups. The Ubiquinol supplementation resulted in 81.6 per cent, 31.7 per cent and 24 per cent improvements in sperm density, sperm motility and sperm morphology respectively.

Compared with the previous study, Ubiquinol was more effective than $Q_{10}$ in improving sperm count and motility. Sperm density increased more than 2.5-fold with Ubiquinol compared to conventional $Q_{10}$.

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Besides the topics discussed in this review, it is worthwhile to mention a series of papers dealing with ubiquinol. A List of these references with a brief comment on their content appears in following table.

| Author year | Field | Summary |
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| Kawasaki T 2012 | ALS | Treatment with ubiquinol can provide benefit to patients with ALS. |
| Murata 2008 | ALS | Ubiquinol ratio was decreased in the CSF (cerebrospinal fluid) of sALS patients compared to that in CSF of controls (p<0,005). |
| Isobe 2010b | Alzheimer | A human study with 30 patients with untreated Alzheimer»s disease and 30 age-matched health controls showed that ubiquinol ratio was inversely correlated with concentrations for 8-OHdG in the CSF of AD patients. 8-OHdG can be viewed as a biochemical market of hydroxyl radical-induced injury. The autors conclude that these and earlier results strongly support the view that administration of Ubiquinol (reduced $CoQ_{10}$) may be a novel and effective treatment in AD. |
| Miles 2003 | Analytical | Ubiquinol ratio in plasma is tightly regulated and is about 96% +- 1% in healthy adults |
| Onur 2014 | Anti-aging | % ubiquinol decreases with age indicating higher oxidative stress or rather a decreased anti-oxidative capacity of aged animals. |
| Tian 2013 | Anti-aging | Ubiquinol decelerates senescence and age-associated hearing loss by a mechanism of activation of mitochondrial function through the expression of sirtuin genes (known as long life genes). |

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| Elsayed 2001 | Antioxidant | In a review GlaxoSmithKline researcher it as concluded that smoking was shown to deplete the body of its endogenous antioxidants such as ubiquinol. Antioxidant depletion was shown to increase individual vulnerability to free radicals and other oxidant species produced by cigarette smoking and therefore leads to elevated morbidity, aging, and death. Dietary supplementation with antioxidants can reduce the overall oxidative burden that is increased by cigarette smoking. |
| Safari 2007 | Atherosclerosis | Ubiquinol has a positive effect on the affinity of native LDL to its receptor, which is important because the uptake of LDL by its receptor decreases the modification of LDL and prevents formation of atherosclerosis. |
| Zakova 2007 | Atherosclerosis | Ratio of Ubiquinol/LDL-C is likely to be a risk factor for atherogenesis |
| Gvozdjakova 2012 IQA abstract | Autism | Beneficial effect of ubiquinol supplementation in autistic children |
| Kaneka 2011 press release | Beauty - oral | In this animal study, ubiquinol demonstrated stronger suppressive effects on wrinkle formation than ubiquinone. These results suggest that oral intake of ubiquinol may effectively suppress wrinkle formation (photo-aging) induced by ultraviolet rays by suppressing inflammation and angiogenesis in the skin. |
| Bruge 2013 | Beauty - skin | Ubiquinol, but not $Q_{10}$, can efficiently counteract UVA associated mitochondrial depolarization suggesting a potential role of this molecule in antiageing cosmetological formulations. |

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| Kaya 2012 | Cardiovascular | Ubiquinol ratio was significantly lower in patients with CAD (Coronary Artery Disease) than the controls. Ubiquinol supplementation may show cardioprotective effects in CAD. |
| Ates 2013 | Diabetes | %Ubiquinol is significantly lower in PDRP-diabetic. High levels of %Ubiquinol indicate a protective effect. |
| Mezawa 2012 | Diabetes | Ubiquinol improves glycemic control in type 2 diabetes by improving insulin secretion without any adverse effects. |
| Macunluoglu 2012 | Dialysis | Plasma $CoQ_{10}$ levels are decreased and positively correlated to Coronary Flow Reserve in HemoDialysis patients. The data suggest that abnormalities in serum levels of $CoQ_{10}$ may facilitate the development of Endothelial Dysfunction in HemoDialysis patients and that these levels may prove to be useful biomarkers for subclinical cardiovascular risk assessment in this patient population. |
| Ohkawa 2004 | Dialysis | Maintaining the level of co-antioxidants, especially ubiquinol-10, in LDL seems to be important for inhibiting the pro-oxidative effect of α-tocopherol and for preventing LDL oxidation in hemodialysis patients. |
| Owada 2013 | Dialysis | Ubiquinol improves oxidative stress in hemodialyses patients. |
| Kubo 2008 | Diet | The estimated average daily intakes of ubiquinol-10 and total coenzyme $Q_{10}$ calculated from our results and data on Japanese daily food consumption were 2.07 and 4.48 mg, respectively. Thus, intake of ubiquinol-10 accounted for 46% of the total coenzyme $Q_{10}$ intake. |

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| Passi 2002 | Diet | Ubiquinol ratio in muscle tissue can be useful as an index of fish freshness. |
| Miles 2007 | Down syndrome | The pro-oxidant status in plasma of children with trisomy 21, as assessed by ubiquinol- ratio, may be normalized with ubiquinol-supplementation |
| Fukuda Watanabe 2012 IQA Sevilla POSTER | Fatigue | Dietary intake of ubiquinol improves symptoms in chronic fatigue syndrome |
| Watanabe 2012 Press Release | Fatigue | Ubiquinol improves symptoms of Chronic Fatigue Syndrome and improvements are correlated to plasma $CoQ_{10}$ levels. |
| Watanabe 2013 Press Release | Fatigue | Ubiquinol decreases significantly the frequency of nocturnal awakening and improved significantly the performance of arithmetic tasks in CFS patients. |
| Choy 2012 | Fertility | Expert comments on Safarinejad 2012 paper confirming that Ubiquinol has potential to alter existing practices in treatment of male infertility. |
| Thakur 2012 IQA abstract | Fertility | Human study that shows that 150mg Ubiquinol per day is beneficial for oligospermic patients as well as in cases of testicular abnormality. |
| Miyamae 2013 | Fibromyalgia | Ubiquinol attenuates general fatigue and hypercholesterolemia in juvenile fybromyalgia. |
| Fisher 2012 | Gene expression | Ubiquinol decreases monocytic expression and DNA methylation of the pro-inflammatory CXCL2 gene in humans |

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| Schmelzer 2011 | Gene expression | Ubiquinol affects the expression of genes involved in PPARα signalling and lipid metabolism without changing the promoter DNA methylation status in the liver of mice |
| Hayashi 2012 IQA abstract + POSTER | Immunity | Ubiquinol shows a protective effect on Influenza A virus-infected mice. This effect might be due to the stimulation of the immune function. |
| Kelekci 2012 | Immunity | $CoQ_{10}$ is decreased significantly in patients with H1N1 (Pandemic Influenza) |
| Schmelzer 2009 | inflammation | The consistent in vitro and in vivo data suggest that ubiquinol may fine-tune the inflammatory response via moderate reduction of miR-146a expression. |
| Schmelzer 2009 | inflammation | In THP-1 cells (human monocytic cell line), the researchers found that Ubiquinol reduces significantly the secretion of the pro-inflammatory agents TNF-α, MIP-1α, and RANTES in response to LPS. In general, they continue, Ubiquinol mediates stronger anti-inflammatory effects on the tested pro-inflammatory compounds than PDTC and NAC, two well known radical scavengers mediating its anti-inflammatory properties through a diminished NFkB activation. |
| Schmelzer IQA 2010 abstract | inflammation | Summary of Dr. Schmelzer studies providing evidence in-vitro and in-vivo that ubiquinol reduces inflammatory processes, LDL cholesterol and cell differentiation/ proliferation by modelling NFκB and PPARα signalling pathways |

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| Schmelzer 2011 | LDL | Ubiquinol supplementation in humans mediates distinct reducing effects on LDL cholesterol levels (-12,7%) with a pronounced effect on atherogenic small dense LDL particles |
| Ryo 2011 | Oral health | Orally administered $CoQ_{10}$ (100 mg/day) improves salivary secretory function by improving the decreased ATP production in salivary glands, increasing energy metabolism, and exerting an antioxidant effect in salivary glands damaged by oxidant stress. Ubiquinol was associated with a higher percent increase in salivary secretion compared to conventional $Q_{10}$. |
| Sugano 2013 | Oral health | 150 mg Ubiquinol per day showed significant improvements in plaque adhesion and bleeding versus placebo. Also a tendency to improve saliva antioxidant activity and bad breath could be shown. |
| Cleren 2008 | Parkinson | It was shown that the efficacy of reduced $CoQ_{10}$ (Ubiquinol) to protect against depletion of dopamine was greater than that of $CoQ_{10}$ in an MPTP model of Parkinsonism in mice. |
| Roland 2010 | Pregnancy | A human study with 30 normotensive and 29 preeclamptic patients showed that Ubiquinol ratio was significantly lower in preeclamptic compared to normotensive pregnancies ($p=0.04$). $CoQ_{10}$ is a sensitive marker of oxidative stress in preeclampsia. |
| Deguchi 2008 | Psychological Functions | The findings of this human study suggest that the maintainance of $CoQ_{10}$ in the reduced state is important for QOL improvements. The results showed significant increases in the «vitality» and «mental health» scores in elderly (+80 years) after Ubiquinol supplementation. |

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| Kawaharada 2013 | Psychological Functions | Regular intake of Ubiquinol may reduce physical symptoms due to job stress and thereby enhance work engagement, indicating usefulness for stress management for workers. |
| Shih 2007 | Reduction | Aged rats exhibit 86% significant decrease in NQO1 activity relative to the young group. |
| Ishikawa 2011 | Renal Disease | Ubiquinol, the reduced form of $CoQ_{10}$, effectively ameliorates renal function, probably due to its antioxidant effect. |
| Ishikawa 2012 | Renal Disease | Ubiquinol has potential preventing Cyclosporine nephrotoxicity. |
| Sato 2012 | Renal Disease | Ubiquinol may prevent or minimize cyclosporine nephrotoxicity by an antioxidant effect. |
| Hidaka 2008 | Safety | Overview: Safety assessment of coenzyme $Q_{10}$ ($Q_{10}$ and Ubiquinol) |
| Alf 2013 | Sporting Activity | UBIQUINOL supplementation enhances peak power production in trained athletes: a double-blind, placebo controlled study |
| Kaneka 2011 press release physical activity | Sporting Activity | Ubiquinol improves physical activity and mental health scores in healthy subjects. |
| Kettawan 2007 IQA abstract | Sporting Activity | In a study with 130 rats a significant elongation of swimming duration was observed by 4-week oral administration of $CoQ_{10}$. The researchers suggest that ubiquinol was consumed to protect the body from oxidative stress during excessive aerobic exercise load and defend against cellular damage. |

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| Maruoka 2012 | Sporting Activity | A single consumption of Ubiquinol produced a very significant effect on running time. |
| Vaughan2013 | Statins | Statins may reduce metabolic capacity of skeletal muscle resulting in reduced cellular energy availability which can be rescued with ubiquinol administration |
| Zlatohlavek 2013 | Statins | A six-month administration of Ubiquinol (reduced form of $CoQ_{10}$) decreased muscle pain and sensitivity statistically significantly in statin myopathy. |

# Conclusions

In this booklet I tried to depict the location of Coenzyme $Q_{10}$, its bioenergetic role and antioxidant properties: both crucial cellular roles,the bioenergetic and the defensive one, are actually covered by the same substance. Moreover, we learned that it is also implicated in the field of gene expression. From what I exposed in the preceding pages it should be clear that Ubiquinol is the form endowed with antioxidant potential. The metabolic machinery is capable of reducing $CoQ_{10}$ in our body, but making the already reduced form available constitutes an advantage, especially in conditions, such as aging, when the reducing potential is hampered. A remarkable number of medical benefits, in different fields, has been reported with Coenzyme $Q_{10}$ and it is quite clear that Ubiquinol has the potential to provide even more benefits. Speaking of the effects of Coenzyme $Q_{10}$ , the extent of clinical improvement also depends from the dose and it correlates with the plasma levels reached with a certain dose. The superior bioavailability of Ubiquinol has led many physicians to witness more remarkable progress in their patients. On the other hand, with our in vitro systems, when we incubate our cell lines with Ubiquinol, cellular uptake and biochemical effects are undoubtedly more pronounced than when traditional Coenzyme $Q_{10}$ is used. Moreover, the age-related unavoidable decline of vital functions is something that really involves mitochondrial and extramitochondrial Coenzyme $Q_{10}$. Ubiquinol is a natural product, located in the heart of our metabolism: gaining a better intellectual comprehension of this molecule will stimulate research and extend its biomedical applications.

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